# Antigen presentation in the thymus for positive selection and central tolerance induction

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Abstract | Understanding how thymic selection imparts self-peptide–MHC complex restriction and a high degree of self tolerance on the T cell repertoire requires a detailed description of the parameters that shape the MHC ligand repertoire of distinct thymic antigen-presenting cells and of how these cells communicate with T cells. Several recent discoveries pertaining to cortex-specific pathways of antigen processing, the heterogeneity of thymic dendritic cells and the intercellular transfer of self antigens have uncovered surprising and unique aspects of antigen presentation in the thymic microenvironment. Here, we discuss these new findings in the context of how individual thymic stromal cell types support T cell selection in a cooperative rather than a redundant manner.

Negative selection
(Also known as clonal deletion). The intrathymic elimination of double-positive or single-positive thymocytes that express T cell receptors with high affinity for self antigens.

Positive selection
The process by which immature double-positive thymocytes expressing T cell receptors with intermediate affinity and/or avidity for self-peptide–MHC complexes are induced to differentiate into mature single-positive thymocytes.

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The fact that recognition of 'self' is essential for thymocyte survival and lineage commitment but may also induce cell death (through a process known as negative selection) remains an incompletely resolved issue termed the selection paradox. Building on seminal work from the mid 1990s, the recent discovery that quantifiable variations in the affinity of a T cell receptor (TCR) for a peptide–MHC complex can result in qualitatively different signals and thus determine thymocyte fate is an important advancement[1]. These findings extend the classical affinity model, which suggests that thymocytes expressing TCRs with no or very low affinity for peptide–MHC complexes die by neglect, whereas very high affinity contacts lead to death through negative selection. Only interactions with an intermediate affinity allow positive selection and CD4 or CD8 lineage commitment, such that thymocytes carrying these TCRs ultimately become part of the peripheral T cell repertoire (FIG. 1).

The central assumptions of the affinity model are supported by a considerable body of evidence. In its present form, however, this model neither sufficiently explains mechanisms of central tolerance other than negative selection, such as the deviation of autoreactive thymocytes into the natural $CD4^+CD25^+$ regulatory T ($T_{Reg}$) cell lineage, nor incorporates the spatial and temporal compartmentalization of thymic selection processes. As T cell development proceeds along a well-ordered passage of thymocytes through discrete thymic microenvironments (FIG. 2), it is likely that distinct properties of individual stromal cell types, in conjunction with partly non-overlapping sets of self-antigen-derived epitopes presented by these different antigen-presenting cells (APCs), have a pivotal role in the fate choices of developing T cells. In this Review we discuss recent progress in elucidating the cell biology of the main stromal cell types involved in thymocyte selection, with particular emphasis on the delineation of determinants that shape the repertoire of self-peptide–MHC complexes on the surface of these APCs. For conceptual clarity, we confine this discussion to factors that are likely to impinge on positive selection and tolerance induction in the αβ T cell lineage (but excluding natural killer T cells) and restrict the range of APCs reviewed to cortical thymic epithelial cells (cTECs), medullary TECs (mTECs) and dendritic cells (DCs).

## Antigen presentation in the cortex

The survival and CD4 or CD8 lineage commitment of double-positive thymocytes depend on interactions with self-peptide–MHC complexes displayed by cTECs. Although there is some evidence that cTECs may also contribute to negative selection[2,3] and $T_{Reg}$ cell induction[4,5], we focus our discussion on a series of recent discoveries relating to the self-peptide–MHC complex repertoire of cTECs in the context of positive selection.

***A brief history of positive selection.*** One of the concepts put forward in the mid 1980s, to resolve the apparent paradox that interactions between developing thymocytes and self-peptide–MHC complexes can result in diametrically different cell fate decisions, posed that ligands on

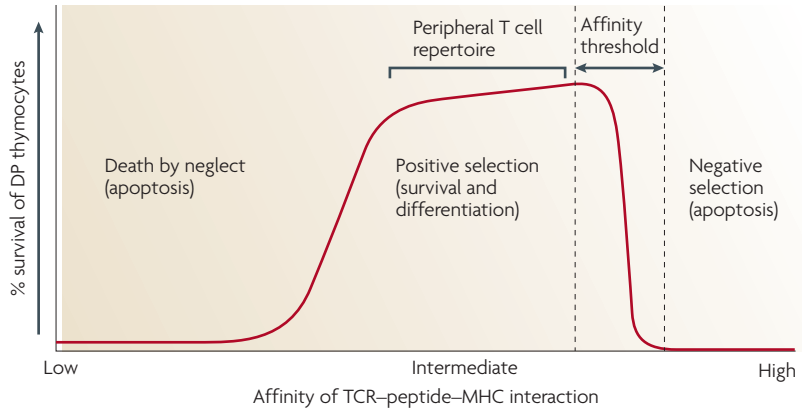



Figure 1 | **The affinity model of thymocyte selection.** According to this model, the affinity of the T cell receptor (TCR)–peptide–MHC interaction is the key determinant of T cell selection. Double-positive (DP) thymocytes expressing TCRs with no or too low an affinity for self-peptide–MHC complexes die by neglect. This mechanism is thought to account for 80–90% of the loss of thymocytes during thymic selection. Thymocytes with intermediate affinity for self-peptide–MHC complexes receive a survival signal (in a process termed positive selection), commit to the CD4 or CD8 T cell lineage and subsequently pass through the thymus medulla to become part of the peripheral T cell pool. High-affinity binding of the TCR to self-peptide–MHC complexes induces cell death by apoptosis, a process that is known as negative selection (or clonal deletion). There is evidence from *in vitro* experiments that a remarkably narrow, quantifiable affinity threshold defines whether a given interaction leads to positive or negative selection[1].

positively selecting cTECs might be different from those on tolerance-inducing APCs in the medulla. In addition to suggesting a spatial and temporal segregation of positive selection and tolerance induction, this 'altered peptide' model suggested that developing thymocytes would engage combinations of peptides and MHC for positive selection that are not encountered anywhere else in the body[6]. However, when the same authors directly tested this hypothesis by sequencing MHC-bound peptides, they found that the main epitopes associated with MHC class II molecules on cTECs were also present in preparations from splenic APCs[7]. Of note, although pooled material equivalent to several hundred mice was analysed, technological limitations at the time allowed the identification of only ~12 of the most abundant peptides that together occupy ~20% of MHC class II molecules. Nevertheless, on the basis of these findings and independent studies showing that pooled splenic or synthetic peptides could mediate positive selection in fetal thymic organ cultures[8,9], the notion that positive selection would require an entirely distinct set of peptides or a unique MHC conformation was largely abandoned.

Two other hypotheses to resolve the selection paradox were based on the avidity or the affinity of the TCR–peptide–MHC interaction. Although the two models are frequently used synonymously, they are based on distinct assumptions. The avidity model predicts that the quantity of a given peptide–MHC complex expressed by cTECs dictates whether a thymocyte expressing an interacting TCR will be positively selected or deleted, whereas the affinity model instead postulates a crucial role of the quality of the individual TCR–peptide–MHC interaction. The observation that agonist peptides that efficiently activated mature T cells could also promote positive selection in fetal thymic organ cultures when present at very low concentrations provided support for the avidity model[10,11]. However, it was subsequently shown that T cells generated in this way were functionally impaired[12]. Related experimental models indicated that positive selection of functional T cells required peptides with antagonist or partial agonist properties[13], thus emphasizing the importance of the quality (affinity) rather than the quantity (avidity) of the TCR–peptide–MHC interaction[14], and to date there is some consensus that peptides that efficiently mediate positive selection *in vitro* are structurally related (although not necessarily in their primary sequence), but not identical, to ligands that can fully activate mature T cells. More recent refinements of the affinity model are based on the evidence that a surprisingly narrow affinity threshold determines whether a given TCR specificity is positively or negatively selected[1] (FIG. 1). The biophysical properties of TCR–peptide–MHC interactions capable of supporting positive selection *in vitro* and the ensuing signalling events have been reviewed elsewhere[15].

Much of our current knowledge regarding peptide–MHC complexes that can drive positive selection of particular TCR specificities derives from *in vitro* models using permutations of cognate epitopes. More recently, 'naturally occurring' peptides capable of inducing positive selection in such *in vitro* systems have been identified[16–19]. However, none of these studies provided direct evidence that the respective peptides (identified by bioinformatic approaches or eluted from peripheral APCs) are present on cTECs or that these peptides contribute to positive selection under natural conditions. Thus, limited information is available regarding the physiological peptide–MHC repertoire expressed by cTECs *in vivo* and the way that these complexes might shape the polyclonal αβ T cell repertoire. Although model systems of limited peptide–MHC diversity still induced the selection of a remarkably large T cell repertoire, it has become clear that the generation of a fully diverse T cell repertoire *in vivo* depends on a matching complexity of selecting ligands (reviewed in REFS 20,21) and that peptides of very low abundance can support the development of most positively selected thymocytes[22]. This suggests that the interaction between TCRs and self peptides during positive selection *in vivo* is highly specific, so that with an enhanced diversity of peptide–MHC complexes on cTECs, the fraction of TCRs in the pre-selection T cell repertoire that encounter one or more interaction partner(s) of matching affinity will increase.

Most of the available studies pertaining to peptide–MHC complexes on cTECs *in vivo* found a positive correlation between the peptide complexity and the diversity of the T cell repertoire. However, our knowledge of the actual nature of the ligands on cTECs that are involved in positive selection *in vivo* is limited, and it should be emphasized that the postulates of the affinity model and the altered peptide hypothesis are clearly not mutually exclusive. Indeed, several recent discoveries indicate that cTECs generate MHC-bound peptides through pathways that are distinct from those used by other thymic or peripheral APCs and are therefore reminiscent of the central postulate of the altered peptide model.

Central tolerance
Self tolerance that is created at the level of the central lymphoid organs. Developing T cells in the thymus, and B cells in the bone marrow, that strongly recognize self antigen face deletion or marked suppression.

$CD4^+CD25^+$ regulatory T ($T_{Reg}$) cells
A subset of lymphocytes that suppress autoreactive T cells that escape negative selection in the thymus.

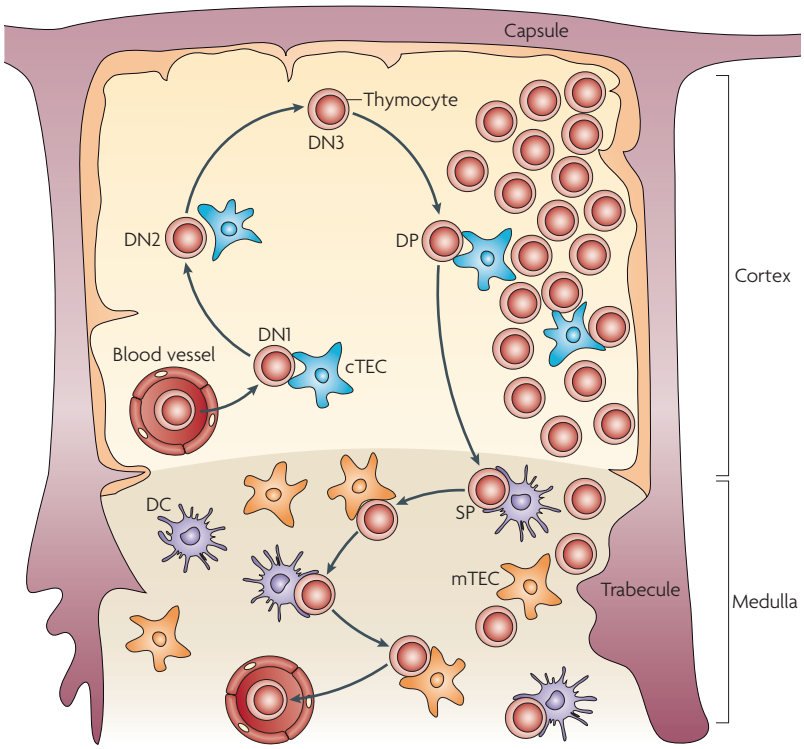



Figure 2 | **Stromal cell interactions along the migratory route of developing T cells.** T cell progenitors enter the thymus through blood vessels near the cortico-medullary junction. Development of sequential stages of double-negative (DN) thymocytes is accompanied by an outward movement of cells towards the sub-capsular zone. After T cell receptor β-selection, double-positive (DP) cells randomly move through the cortex and presumably scan cortical thymic epithelial cells (cTECs) for positively selecting ligands[119–121]. After positive selection and CD4 or CD8 lineage commitment, single-positive (SP) thymocytes rapidly relocate to the medulla, where they scan medullary antigen-presenting cells (mostly dendritic cells (DCs) and medullary TECs (mTECs)), presumably for their entire 4–5 day residency[48,70].

***Cathepsins.*** The first evidence for a distinct proteolytic pathway that shapes the MHC ligand repertoire of cTECs identified a role for cathepsins in CD4$^+$ T cell development. Cathepsins are a class of lysosomal proteases implicated in the degradation of the invariant chain (Ii; also known as H-2 class II histocompatibility antigen γ-chain), which protects against premature loading of MHC class II molecules, and in the generation of antigenic peptides from substrates in the lysosome[22]. Interestingly, cTECs preferentially express cathepsin L (encoded by *Ctsl*), whereas other haematopoietic APCs and mTECs predominantly express cathepsin S. Inactivation of the *Ctsl* gene resulted in a 60–80% reduction of the thymic CD4 single-positive (SP) T cell compartment[23]. The analysis of mice deficient in both cathepsin L and Ii indicated that this was not solely due to inefficient cleavage of Ii but due to, at least in part, changes in the MHC ligand repertoire of cTECs[24]. Supporting this, three transgenic TCR specificities that were efficiently selected in the thymi of wild-type mice were not selected in *Ctsl*$^{-/-}$ mice. Considering the correlation of the diversity of peptide–MHC complexes and the diversity of the T cell repertoire[9,25,26], one plausible explanation for the reduced CD4 SP T cell numbers in *Ctsl*$^{-/-}$ mice could be a reduced complexity of the MHC class II ligand repertoire. However, as arguably much less diverse peptide–MHC complexes — such as those of H-2DM-deficient mice[27] or of mice that supposedly express only a single peptide–MHC complex[28] — support the generation of surprisingly heterogeneous CD4 SP T cell compartments (comparable in size to that of *Ctsl*$^{-/-}$ mice), a mutually non-exclusive explanation also has to be taken into account: when lacking cathepsin L, cTECs may generate MHC class II-bound peptides through the action of residual cathepsin S, thereby rendering the positively selecting MHC class II-bound peptides of cTECs more akin to those of tolerogenic APCs in the medulla (both mTECs and DCs constitutively use cathepsin S). As a consequence, a disproportionately large fraction of CD4 SP cells may be subject to clonal deletion due to re-encounter on mTECs or DCs of the same peptides that promoted their positive selection.

In order to test this hypothesis, one would have to experimentally eliminate the effect of clonal deletion on the CD4 SP T cell repertoire. This can be achieved in bone marrow chimaeras in which haematopoietic cells, but not stromal cells, are MHC class II deficient, so that the positively selected CD4$^+$ T cell repertoire is not censored by clonal deletion (at least to the extent that results from encounters with self-antigen on DCs). Indeed, in chimaeras in which MHC class II-deficient bone marrow cells are transferred to *Ctsl*$^{-/-}$ mice, the size of the CD4 SP T cell compartment was substantially increased and was similar to that in wild-type mice that had received MHC class II-deficient bone marrow cells[24]. This supports the notion that positive selection on MHC class II-bound peptides generated by cathepsin L is not essential for efficient CD4 SP T cell generation *per se,* but rather prevents subsequent excessive loss of positively selected SP T cells.

***Thymus-specific serine protease.*** A recent finding that corroborates the hypothesis that cTECs use distinct pathways to generate positively selecting MHC class II ligands involves thymus-specific serine protease (TSSP; encoded by *Prss16*), which was discovered in a screen for cTEC-specific genes[29,30]. The exact positioning of TSSP in the MHC class II pathway remains elusive; however, its subcellular localization in endosomal and/or lysosomal compartments suggests that TSSP has a role in the proteolytic generation of MHC class II-bound peptides. The phenotype of *Prss16*$^{-/-}$ mice is more subtle than that of *Ctsl*$^{-/-}$ mice: the overall size of the CD4 SP T cell compartment is unaltered[31]. However, positive selection of two MHC class II-restricted transgenic TCR specificities was substantially decreased in *Prss16*$^{-/-}$ mice[32]. Of note, polymorphisms in the *PRSS16* gene are associated with susceptibility to autoimmune diabetes in humans[33], possibly pointing to a link between an altered MHC ligand repertoire expressed by cTECs in the absence of this serine protease and the selection of an incompletely tolerized CD4$^+$ T cell population.

Cathepsins
Proteases that are mostly located in lysosomes and lysosome-like organelles and can be divided into cysteine, aspartate and serine cathepsin subgroups according to their active-site amino acid.

### Box 1 | **Macroautophagy**

Macroautophagy is an evolutionarily conserved catabolic process by which portions of cytoplasm, ~1 μm in diameter, are engulfed by a double-membrane organelle. These macroautophagosomes can contain other organelles, such as mitochondria, as well as fragments of the nucleus. They fuse with endosomes and lysosomes, resulting in proteolytic degradation and recycling of their cargo. Although macroautophagy has traditionally been regarded as a metabolic adaptation to starvation[104], it also has a role in the removal of protein aggregates[105], in developmental processes (discussed in REF. 106) and in the immune system (reviewed in REFS 38,107).

Early work indicated that inhibition of macroautophagy abrogated MHC class II-restricted presentation of a cytoplasmic antigen *in vitro*[108]. Subsequently, these findings were extended to include an endogenous tumour antigen[109] and viral epitopes[110]. Recent experiments argue that a broad range of macroautophagosome-associated cytoplasmic antigens are delivered to MHC class II molecules through the macroautophagy pathway[111,112]. A tissue survey identified the thymus as a unique site of unusually high constitutive macroautophagy[39]. Among thymic stromal cells, the highest activity was detected in cTECs, with 60% of cells classed as macroautophagy positive, whereas ~10% of mature mTECs showed macroautophagic activity[40]. Importantly, constitutive macroautophagy was not detected in thymic dendritic cells.

***Macroautophagy.*** Another indication that the peptide–MHC class II complexes expressed by cTECs are different to those expressed by tolerance-inducing haematopoietic APCs comes from evidence that these cell types sample self antigens from different compartments: intracellular sources and the extracellular space, respectively. Thus, TECs in general and cTECs in particular are inefficient at presenting exogenous proteins from the classical endocytic pathway on MHC class II molecules, despite their high level of surface expression of MHC class II molecules[34–36]. Therefore, it was postulated that TECs use unconventional pathways of MHC class II loading that would lead to the predominant presentation of endogenously derived self antigens[37]. This prediction has recently been confirmed by the demonstration that macroautophagy (BOX 1), a bulk protein degradation process implicated in the delivery of intracellular antigens to the MHC class II pathway[38], shapes the nascent CD4$^+$ T cell compartment. TECs, especially cTECs, are among the few cell types that display an unusually high level of constitutive macroautophagy[39].

The potential contribution of macroautophagy in TECs to T cell selection was tested in mice that lack the essential autophagy gene *Atg5*. It was found that bulk polyclonal thymocyte development seemed unaffected with respect to the relative abundance of CD4 or CD8 SP thymocyte subsets or TCR variable (V) region usage; however, positive selection of some MHC class II-restricted transgenic TCR specificities was altered in *Atg5*$^{-/-}$ thymi[40], consistent with macroautophagy being required for the generation of certain, but not all, peptide–MHC class II complexes for positive selection (reviewed in REF. 41).

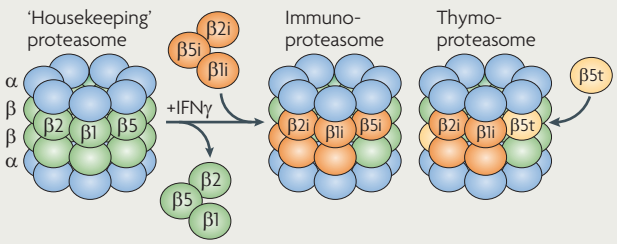
***Thymoproteasome.*** The discovery of the proteasome subunit β5t (encoded by *Psmb11*) revealed that alterations in the biochemistry and cell biology of antigen processing in cTECs also apply to the MHC class I pathway (BOX 2). β5t is exclusively expressed in cTECs, and *Psmb11*$^{-/-}$ mice revealed that β5t deficiency resulted in a severely diminished CD8 SP T cell compartment[42]. Moreover, impaired allo- and virus-specific immune responses in these mice indicated a qualitatively altered CD8$^+$ T cell repertoire[43]. Less efficient positive selection cannot be attributed to reduced expression levels of MHC class I molecules on cTECs as a result of destabilized peptide–MHC complexes because MHC class I expression levels on cTECs are not changed in *Psmb11*$^{-/-}$ mice[42,43]. Thus, it

### Box 2 | **Proteasomes in immunity**

Peptides for MHC class I loading are a by-product of the basal proteolytic degradation of ubiquitylated intracellular proteins by the proteasome. The barrel-shaped proteasome (see the figure) is a molecular machine consisting of a catalytic core (the 20S proteasome) and two 19S regulatory subunits (not depicted). The 20S core is composed of two pairs of staggered heptameric rings containing seven α- or β-subunits. In the 'housekeeping' proteasome, each β-ring has three proteolytically active sites that reside in the subunits β1 (also known as δ), β2 (also known as Z) and β5 (also known as MB1), which have different preferences for protein cleavage after acidic, basic and hydrophobic residues, respectively. Consistent with a role for the chymotrypsin-like activity of β5 in the generation of epitopes bound to MHC class I molecules, the carboxyl termini of peptides that have been experimentally isolated from MHC class I molecules often contain hydrophobic anchor residues.

Although the housekeeping proteasome is the predominant species in steady-state tissue cells, exposure to interferon-γ (IFNγ) results in the incorporation of the inducible catalytic subunits β1i (also known as LMP2), β2i (also known as MECL1) and β5i (also known as LMP7) into newly assembled immunoproteasomes[113]. The immunoproteasome is constitutively expressed in professional antigen-presenting cells and medullary thymic epithelial cells. Its proteolytic activity increases the prevalence of peptides with hydrophobic or basic C termini, thereby possibly increasing the production of peptides that neatly fit into MHC class I molecules.

In cortical thymic epithelial cells, the specific subunit β5t is incorporated into the proteasome instead of β5 or β5i to form the thymoproteasome[44]. The thymoproteasome displays diminished chymotrypsin-like activity, resulting in a reduced production of peptides with a hydrophobic residue at the C terminus. Because hydrophobic C termini are preferred by MHC class I molecules, it is possible that the thymoproteasome generates peptides with lower MHC affinity and thus less stable peptide–MHC complexes.




Endocytic pathway
A trafficking pathway used by all cells for the internalization of endocytosed molecules from the plasma membrane to lysosomes.

Macroautophagy
(Also known as autophagy). The generally nonspecific autophagic sequestration of cytoplasm into a double- or multiple-membrane-delimited compartment (a macroautophagosome) of non-lysosomal origin. Certain proteins, organelles and pathogens may be selectively degraded by macroautophagy.

Proteasome
A giant multicatalytic protease resident in the cytoplasm and the nucleus. In addition to having a crucial role in protein turnover, the proteasome is thought to carry out the first catalytic step in the MHC class I-restricted processing of most, if not all, antigens.

seems likely that an altered composition of MHC class I ligands is responsible for the aberrant CD8$^+$ T cell selection in *Psmb11*$^{-/-}$ mice (reviewed in REF. 44), a supposition that is supported by impaired positive selection of several MHC class I-restricted transgenic TCRs in these animals[43].

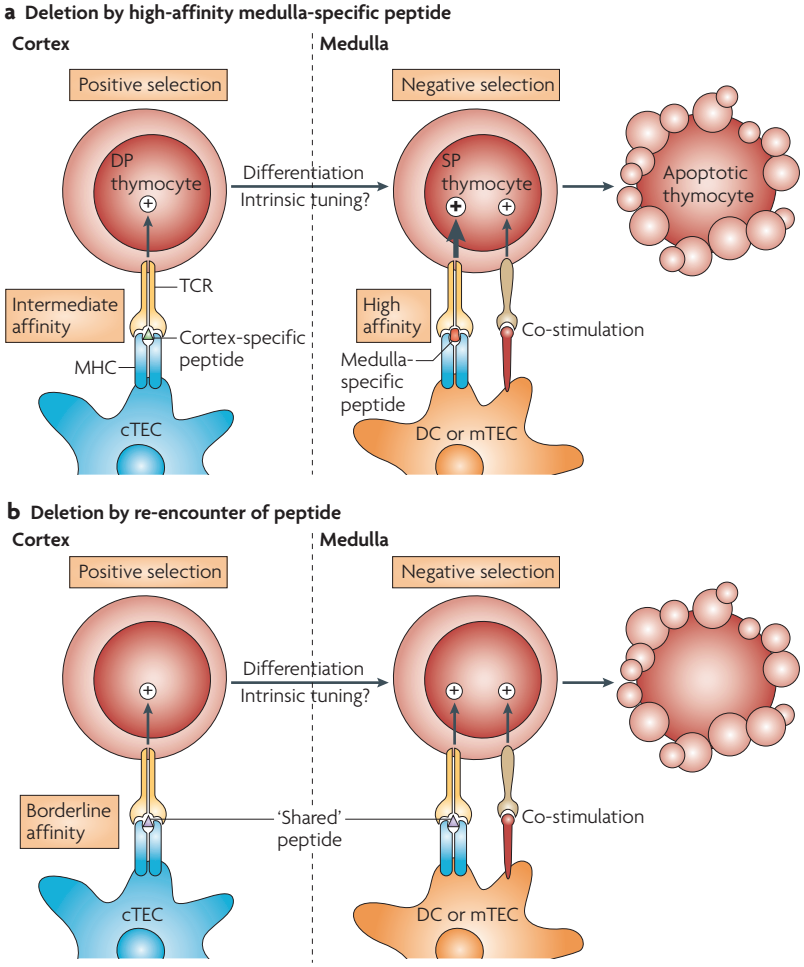



Figure 3 | **Unique peptide–MHC complexes on cortical thymic epithelial cells may be necessary to prevent excessive loss of positively selected T cells.** The deletion of single-positive (SP) thymocytes in the medulla may not only occur as a result of high-affinity interactions with self peptides selectively presented by medullary antigen-presenting cells (APCs) (including tissue-restricted antigen (TRA)-derived epitopes) (**a**), but may also result from the re-encounter of MHC ligands present on both cortical thymic epithelial cells (cTECs) and medullary APCs, including medullary TECs (mTECs) and dendritic cells (DCs) (**b**). Such 'shared' peptides, in particular those with an affinity that is close to the threshold between positive and negative selection of double-positive (DP) thymocytes (FIG. 1), might initially deliver a survival signal for thymocytes in the cortex but may lead to the negative selection of immature SP T cells in the medulla when encountered in the context of co-stimulatory molecules that potentiate the TCR signal[122,123]. Furthermore, it is possible that subsequent to positive selection, differentiating SP thymocytes enter an intrinsically regulated, transient phase of susceptibility to negative selection[124]. Enhancement of the signal intensity by the co-stimulatory properties of medullary APCs would increase the proportional loss of T cells that have been positively selected on 'shared' peptides. We propose that this deletion pathway resulted in an evolutionary pressure to select for cTEC-specific antigen processing pathways that generate unique peptides and thus minimize the abundance of 'shared' peptides. Note that the affinity of the interaction of the TCR with the peptide–MHC complex is assumed to be different in the cortex and medulla in part **a** but not in part **b**.

It is possible that the reduced size of the CD8$^+$ T cell pool in *Psmb11*$^{-/-}$ mice reflects a diminished complexity of MHC class I ligands on cTECs. However, *Psmb11*$^{-/-}$ cTECs may assemble proteasomes that instead contain the β5i subunit, termed immunoproteasomes (BOX 2) (indeed, β5i is also expressed in cTECs, albeit at lower levels than β5t[42]), so that their MHC class I ligand repertoire may still be of considerable diversity but be similar to that of the tolerance-inducing mTECs and DCs (which constitutively express the immunoproteasome). Therefore (in analogy to what has been argued with respect to cathepsin L-deficient mice) rather than reflecting less efficient positive selection, the diminished CD8 SP T cell compartment in *Psmb11*$^{-/-}$ mice may result from a disproportionately high level of clonal deletion of CD8$^+$ T cells owing to the same peptide–MHC class I complexes being expressed on positively selecting cTECs as on negatively selecting APCs in the medulla (FIG. 3).

***A renaissance of altered peptides?*** Direct evidence that the thymoproteasome, TSSP or macroautophagy alters the MHC ligand repertoire of cTECs can only be obtained by an analysis of all MHC-bound peptides. As we currently lack such information, it remains possible that these proteolytic enzymes or pathways (summarized in FIG. 4) have substrates other than MHC ligands that could also influence selection. Nonetheless, perhaps the most plausible scenario is that cTECs indeed generate a partly (but not entirely) unique set of MHC-bound peptides. So, it seems that the altered peptide hypothesis may have been dismissed prematurely.

It is not immediately evident how such a crucial role for cTEC-specific MHC ligands can be reconciled with the hypothesis that the ligands that promote positive selection may also be essential for the peripheral homeostasis of mature T cells[18,45] and/or could act as co-agonists in the context of immune responses to foreign antigen[16,18,46,47]. According to this view, positive selection selects for TCRs within an affinity window that ensures that re-encounter of positively selecting self ligands in the periphery does not lead to overt activation, but would provide a 'sub-threshold' signal that promotes survival and full responsiveness. It remains to be seen whether and how this 'reinterpretation' of positive selection and the newly discovered cTEC-specific proteolytic pathways can be incorporated into a unifying conceptual framework.

## Antigen presentation in the medulla

Subsequent to positive selection and CD4 or CD8 lineage commitment, thymocytes translocate to the medulla, where they reside for ~4–5 days before receiving their 'exit permit'[48]. Numerous experimental observations highlight that the quality control of developing T cells through interactions with peptide–MHC complexes on medullary APCs is indispensable for central tolerance. For example, genetic lesions that impede the entry of positively selected thymocytes into the medulla or result in premature egress of thymocytes[49,50], disorganization

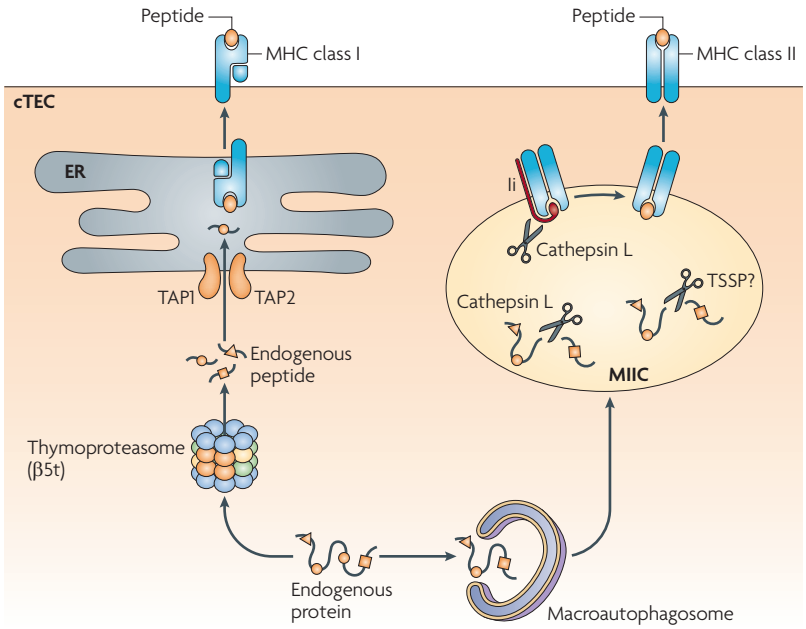



Figure 4 | **Cortical thymic epithelial cells generate MHC-bound peptides through unique pathways.** MHC class I-bound peptides on the surface of cortical thymic epithelial cells (cTECs) seem to be primarily generated by the thymoproteasome. Regarding the production of MHC class II-bound peptides, cTECs display a notable inefficacy in classical exogenous MHC class II loading. Instead, they seem to focus their MHC class II-bound peptides on endogenously derived antigens by shuttling cytoplasmic material into the MHC class II compartment (MIIC) through macroautophagy. The proteolytic degradation of lysosomal substrates in cTECs is, at least in part, executed by enzymes specifically expressed by these cells, namely cathepsin L and thymus-specific serine protease (TSSP). Whereas biochemical data support a role for cathepsin L in both the processing of the invariant chain (Ii) and the generation of MHC class II-bound peptides, a role for TSSP in the proteolytic degradation of lysosomal substrates has so far only been inferred from the knock-out phenotype. ER, endoplasmic reticulum; TAP, transporter associated with antigen processing.

of the medullary architecture[51] or disrupted development of mTECs[52–56] result in severe manifestations of systemic autoimmunity.

Here, the discussion of self-peptide presentation by stromal cells in the medulla is focused on mTECs and DCs. We elaborate on factors that are likely to shape the MHC ligand repertoire of either cell type and address the intricacies of intercellular transfer of self peptides.

***Medullary thymic epithelial cells.*** With the development of sensitive PCR methods in the late 1980s, allowing detection of minute amounts of a given mRNA species, the notion arose that 'transcriptional noise' may allow the low-level transcription of any gene in any cell[57,58]. Intrigued by this new perspective, speculation arose that the thymus might be a 'patchwork quilt' of ectopic gene expression, offering an explanation as to how developing thymocytes might be exposed to the antigenic diversity of peripheral tissues[59]. We now know that the ectopic transcription of hundreds, if not thousands, of tissue-restricted antigens (TRAs) in the thymus, controlled in part by the autoimmune regulator (*Aire*) gene, is an essential cornerstone of T cell tolerance[60] (BOX 3). However, rather than reflecting an inherent leakiness of transcriptional control, this phenomenon, termed promiscuous gene expression, is a distinct property of mTECs[61,62]. The cellular and molecular control of promiscuous gene expression, its relevance for the prevention of autoimmunity and the role of *Aire* have been exhaustively reviewed elsewhere[60,63,64]. In the context of this discussion, it is sufficient to highlight the stochastic nature of promiscuous gene expression, reflected by the low frequency (1–3%) of mTECs that express a particular TRA[65], and that, contrary to previous assumptions, mature mTECs are replaced every 1 to 2 weeks, thus continuously changing the topology of antigen expression within the medulla[66,67].

To reconcile the astounding efficacy of tolerance induction towards TRAs with the dispersion of few mTECs expressing a given antigen throughout the medulla, it has been suggested that thymocytes may use their lengthy medullary residency to scan numerous APCs and/or that transfer of mTEC-derived TRAs to neighbouring DCs would increase the probability of such antigens being encountered by developing thymocytes[35,68]. Both concepts recently received experimental support: real-time imaging of SP thymocyte motility revealed that these cells — in a similar manner to mature T cells in the secondary lymphoid tissue[69] — engage in multiple sequential and short-lived interactions with APCs in the medulla[70]. Of note, this study focused on CD8 SP T cell–DC interactions, and it remains to be seen whether the behaviour of CD4 SP T cells and the interactions of thymocytes with mTECs follow similar rules. Assuming this to be the case, such a highly dynamic scanning process may in fact allow 'saturating' encounters with medullary APCs during the 4–5 day residency of SP T cells in the medulla[71]. However, evidence for intercellular antigen transfer from mTECs to DCs has also been obtained and is discussed below.

The observations outlined so far are consistent with a model whereby mTECs may not by themselves function as tolerogenic APCs of endogenously expressed self antigens, but instead primarily serve as suppliers of TRAs that eventually spread to and are presented by neighbouring DCs. However, such a mandatory division of labour between mTECs and DCs is clearly not the rule. For example, mTECs were found to autonomously — that is, independently of antigen transfer to and presentation by DCs — induce clonal deletion of $CD8^+$ T cells specific for a model antigen expressed in a manner reflecting promiscuous gene expression[72]. Furthermore, an autonomous tolerogenic function of mTECs as APCs is suggested by the fact that during their progressive differentiation, the onset of AIRE and TRA expression is linked to the acquisition of several hallmarks of full APC competence (for example, expression of CD30L (also known as TNFSF8), CD80, CD86 and intercellular adhesion molecule 1 (ICAM1))[65,73].

Considering the established rules of MHC class I loading, it may not be unexpected that epitopes derived from intracellular antigens are presented on MHC class I molecules of mTECs and thereby induce

Box 3 | **Promiscuous gene expression in mTECs and the role of AIRE**

In the late 1980s, seminal work on the intrathymic expression of pancreas-specific genes challenged the concept of a clear demarcation between the transcriptomes of peripheral tissues and the thymus[114,115]. Various experimental models suggested that expression of tissue-restricted antigens (TRAs) by rare stromal cells in the medulla was sufficient to induce $CD4^+$ T cell tolerance[35,116]. The analysis of highly purified thymic stromal cell preparations unambiguously identified mTECs as the principal intrathymic source of promiscuous gene expression[62]. The scope and interspecies conservation of promiscuous gene expression have been characterized in great detail. Intrathymically expressed genes encode functionally and structurally diverse antigens representing essentially all organs (reviewed in REF. 60).

In 1997, two groups reported that mutations in the human autoimmune regulator (*AIRE*) gene cause the monogenically transmitted autoimmune disease autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED)[117,118]. Targeted disruption of the *Aire* gene in mice reduces promiscuous expression of numerous genes in mTECs and elicits various organ-specific autoimmune manifestations[61]. The mechanisms by which AIRE controls promiscuous gene expression remain unclear. Structural motifs and proteomic analyses of interacting proteins indicate that AIRE may activate transcription through binding to inactive histone marks and/or may facilitate splicing or transcript maturation (reviewed in REFS 63,64). Single-cell analyses have revealed variations in promiscuous gene expression between individual mTECs, suggesting that the regulation of promiscuous transcription by AIRE involves stochastic components[65].

$CD8^+$ T cell tolerance. However, as discussed above, mTECs (which, together with their cortical counterparts, are the only non-haematopoietic cells that constitutively express high levels of MHC class II molecules) contribute to $CD4^+$ T cell tolerance by shuttling endogenous self antigens onto MHC class II molecules. For instance, negative selection of $CD4^+$ T cells specific for a liver-associated antigen that was ectopically expressed by mTECs was independent of antigen presentation by haematopoietic APCs[35]. In another system, targeting of a model antigen to mTECs resulted in the deviation of specific $CD4^+$ T cells into the $T_{Reg}$ cell lineage, which again did not require a contribution of DCs, but was autonomously mediated by antigen-presenting mTECs themselves[74]. Importantly, we recently could show that mTECs also present non-transgenic, physiologically expressed endogenous TRAs to $CD4^+$ T cells[75].

Like cTECs, mTECs exhibit a remarkably poor efficacy in MHC class II-restricted presentation of extracellular antigens[35], suggesting the preferential use of unconventional, endogenous MHC class II loading pathways to sample the different sub-cellular compartments (such as the cytoplasm, nucleus and mitochondria) in which TRAs are localized. Among several potential mechanisms (reviewed in REF. 41), the unusual occurrence of a high rate of constitutive macroautophagy in a subset of mature mTECs rendered this pathway a particularly attractive candidate that may favour the delivery of endogenous self antigens into the MHC class II pathway for the induction of $CD4^+$ T cell tolerance (BOX 1). Indeed, mice with disrupted autophagy in TECs (generated by the transplantation of embryonic *Atg5*$^{-/-}$ thymi into athymic nude mice) show perturbed positive selection of $CD4^+$ T cells and signs of organ-specific autoimmunity[40].

These experiments are limited because currently they cannot distinguish between a specific requirement for macroautophagy in cTECs versus mTECs. The effect of disrupted macroautophagy in TECs on positive selection can almost certainly be attributed to alterations in the composition of MHC class II complexes on cTECs. However, future work will show whether the lack of self tolerance caused by the absence of macroautophagy in all TECs truly reflects the escape of otherwise 'censored' (that is, clonally deleted or $T_{Reg}$ cell-deviated) T cell specificities from central tolerance owing to incomplete presentation of endogenous self antigens on MHC class II molecules of mTECs.

***Dendritic cells.*** Peripheral DCs have been known for a long time to be heterogeneous with regard to lineage derivation, migratory behaviour, antigen processing (for example, cross-presentation) and effector function[76]; however, a similar phenotypic and functional heterogeneity of thymic DCs has only recently been shown. Accordingly, thymic DCs are now subdivided into three major subsets: two subtypes of $CD11c^{hi}$ conventional DCs (cDCs) that are either $CD11b^-CD8\alpha^+CD172a^-$ or $CD11b^+CD8\alpha^{-/low}CD172a^+$ and $CD11c^{mid}CD45RA^+$ plasmacytoid DCs (pDCs) (reviewed in REF. 77). Because the function of pDCs in the thymus, despite constituting ~30% of all thymic DCs, remains elusive, we focus here on cDCs and highlight features that are of particular relevance for central tolerance.

Thymic DCs were thought to arise intrathymically from a common progenitor of T cells and DCs[78]. More recently, it has become clear that only $CD11b^-CD8\alpha^+CD172a^-$ cDCs, which make up approximately two thirds of cDCs in the thymus, are of intrathymic origin, whereas $CD11b^+CD8\alpha^{-/low}CD172a^+$ cDCs (and all pDCs) are immigrants from peripheral sites[79–81]. Depending on their site of differentiation, we refer here to these cDC subsets as autochthonous (intrathymic origin) and migratory cDCs, respectively. It has been contested whether autochthonous cDCs truly derive from a common T cell and DC progenitor[82,83]. Instead, new cell lineage tracing experiments suggest a branching point between T cells and autochthonous cDCs that precedes the immigration of progenitor cells into the thymus (H.-R. Rodewald, personal communication).

Several studies have attempted to define the parameters guiding the translocation of migratory cDCs from peripheral sites to the thymus. Following the intravenous injection of numerous steady-state splenic DCs, all three major subsets of peripheral cDCs (that is $CD11b^-CD8\alpha^+$ 'lymphoid' DCs, $CD11b^+CD8\alpha^-$ 'myeloid' DCs and $CD11b^-CD8\alpha^-$ DCs) displayed a similar ability of homing to the thymus[84]. The marker CD172a (also known as SHPS1) was not used in this study, but other studies have shown that it is expressed by peripheral $CD11b^+CD8\alpha^-$ myeloid cDCs[85]. The apparent discrepancy of the observation that all peripheral cDC subsets can migrate to the thymus with the observation that under physiological conditions almost all migratory thymic cDCs

Cross-presentation
The presentation of exogenous antigen that has been re-routed to the MHC class I pathway of antigen presentation by APCs to $CD8^+$ T cells.

Plasmacytoid DCs
(Plasmacytoid dendritic cells). Immature DCs with a plasmacytoid morphology, which produce type I interferons in response to viral infection.

(that is, $CD11b^+CD8\alpha^{-/low}CD172a^+$ cDCs) have myeloid characteristics may result from the artificial mobilization (by intravenous injection) of DC subsets into the circulation[84] as opposed to physiological migration patterns. Consistent with this, most circulating cDCs in the blood are $CD172a^+$, thus resembling migratory cDCs in the thymus[80]. Accordingly, the fact that nearly all peripherally derived migratory cDCs in the thymus have a myeloid phenotype may reflect their relative abundance in the circulation rather than unique features, for example a currently unknown combination of chemokine receptors and/or adhesion molecules that enable their migration into the thymus.

Shortly after entering the thymus, migratory $CD172a^+$ cDCs undergo further maturation, including upregulation of MHC class II and co-stimulatory molecules[80]. Immigrant cDCs preferentially locate to the medulla and intermingle with autochthonous cDCs in a seemingly random fashion, whereas few migratory cDCs localize to the cortex[80,84]. Interestingly, some migratory cDCs undergo one or two cell divisions on entering the thymus. This is reminiscent of what has been shown for DCs in the spleen, where daughter DCs continually presented antigens captured by their progenitors, hence potentially prolonging the duration of antigen presentation[86].

Functional evidence for the tolerogenic capacity of migratory cDCs has been obtained in several experimental systems; for example, antigen-pulsed or superantigen-expressing DCs that were intravenously injected into mice were found to induce negative selection of specific thymocytes[84,87]. Interestingly, pretreatment of adoptively transferred DCs with lipopolysaccharide impaired their homing to the thymus, suggesting a mechanism whereby under physiological conditions unwanted central tolerance induction towards pathogen-derived antigens taken up by peripheral DCs in an inflammatory context would be prevented[84].

The tolerogenic consequences of DC trafficking to the thymus under steady-state conditions have been addressed in two models that did not involve the experimental transfer of DCs. In the first, clonal deletion of MHC class II-restricted TCR transgenic (OT-II) thymocytes (which recognize ovalbumin (OVA)) in mice that expressed OVA specifically in cardiomyocytes (and supposedly not in mTECs) was reduced by injection of a monoclonal antibody specific for α4 integrin, which is a component of very late antigen 4 (VLA4). As DC homing to the thymus is vascular cell adhesion molecule 1 (VCAM1)–VLA4 dependent, this suggests that migratory DCs that had taken up OVA in the periphery contributed to the induction of central tolerance[84]. In a second study, thymi from OT-II TCR-transgenic mice were grafted into host mice that expressed OVA under the control of a DC-specific promoter[88]. It was found that before the emergence of autochthonous DCs (the development of which occurs after the influx of migratory DCs), migratory cDCs not only led to a partial deletion of OVA-specific CD4 SP T cells but also induced a slight increase in the number of $OT\text{-}II^+$ $T_{Reg}$ cells.

The observation that an increase in $T_{Reg}$ cell development was induced by migratory cDCs is related to the question of whether the capacity for intrathymic induction of $T_{Reg}$ cells is a specific attribute of one or several thymic APC subset(s). This issue applies not only to the general distinction between epithelial and haematopoietic APCs but also, within the respective lineages, to the role of cTECs versus mTECs and autochthonous versus migratory DCs. Of note, all thymic DC subsets, as well as mTECs, have proved similarly effective in converting immature thymocytes, but not peripheral naive $CD4^+$ T cells, into $T_{Reg}$ cells *in vitro*[89]. Therefore, it seems that $T_{Reg}$ cell induction is under T cell-intrinsic developmental control rather than dependent on particular features of a dedicated APC type. Nevertheless, although these findings reveal a certain redundancy in the ability of various APC types to induce $T_{Reg}$ cells, it remains possible that in the *in vivo* microenvironment, distinct thymic APC subsets differ in their specific contribution to the composition of the polyclonal $T_{Reg}$ cell repertoire.

Related to their role in deletional versus dominant tolerance is the issue of whether autochthonous and migratory DCs present discrete ranges of MHC ligands. Intuitively, their distinct anatomical origin would suggest that they sample different self-antigen pools. Furthermore, it is conceivable that differences in the principal pathways of antigen sampling and processing render the MHC-bound peptides they present partly unique; however, current experimental evidence addressing this question is ambiguous. Thus, an intravenously administered protein antigen was taken up equally well by autochthonous and migratory cDCs *in vivo*[80], and MHC class II-restricted presentation of cell-associated antigen *in vitro* resulting from uptake of apoptotic cells was indistinguishable[81]. By contrast, particulate high-molecular weight antigen in the serum could be detected only in the migratory subset of thymic cDCs, presumably after acquisition in the periphery[80]. By inference, the finding that only migratory DCs could sample serum antigen indicates that certain peripheral self antigens may indeed be selectively presented by migratory DCs. Conversely, it is conceivable that autochthonous cDCs tap into self-antigen reservoirs that are less accessible to the migratory subset. Consistent with this, autochthonous cDCs efficiently cross-presented antigens derived from apoptotic cells to $CD8^+$ T cells *in vitro*, whereas migratory DCs were much less capable of doing so[81]. Future studies will show whether this feature predisposes autochthonous cDCs to cross-present mTEC-derived self antigen in the *in vivo* environment.

Taken together, it may be assumed that the heterogeneity and the distinct origin of thymic DC subsets broaden the range of self antigens displayed in the thymus (FIG. 5). Migratory cDCs are likely to present peripheral self antigens that are not included in the array of TRAs promiscuously expressed by mTECs or are not present in the circulation at tolerogenic levels. It is also possible that they contribute to the maintenance of tolerance to innocuous foreign antigens derived from the commensal gut flora or food.

**Superantigen**
A protein that binds to and activates all T cells that express a particular set of Vβ T cell receptor genes.

**Lipopolysaccharide**
(Also known as endotoxin). A constituent of the cell walls of Gram-negative bacteria that is important for eliciting the immune response to Gram-negative bacterial infection.

**Dominant tolerance**
The active suppression of an autoimmune response, *in vitro* or *in vivo*, by suppressor cells including $T_{Reg}$ cells. By contrast, deletional tolerance and induction of anergy are types of passive tolerance. Dominant tolerance is transferable to naive recipients, whereas passive tolerance is not.

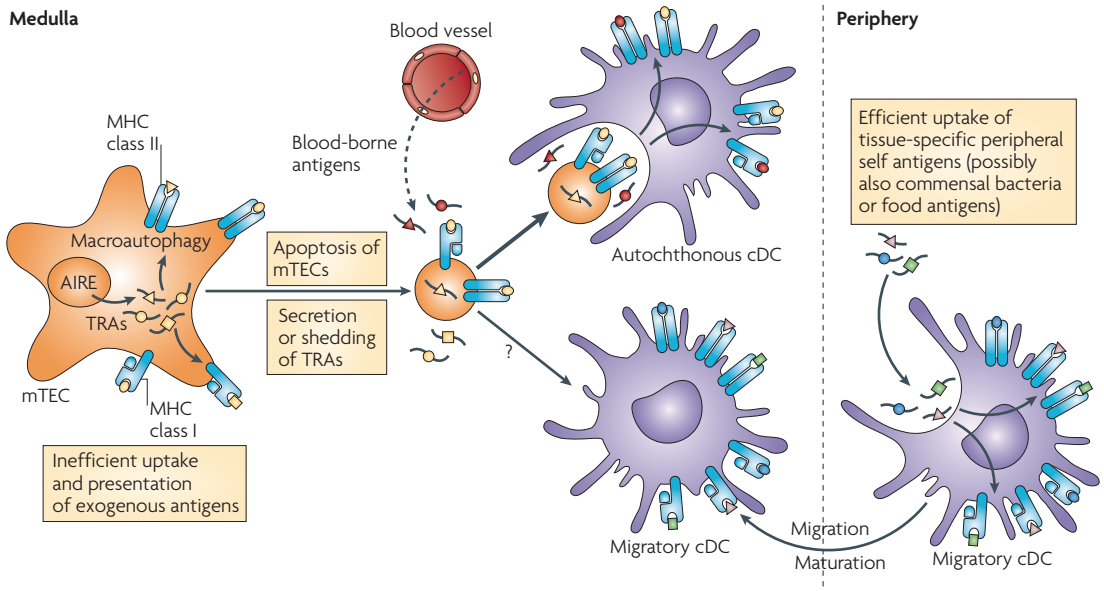



Figure 5 | **Determinants that shape the peptide–MHC complex repertoires of medullary thymic epithelial cells and thymic conventional dendritic cells.** Promiscuously expressed tissue-restricted antigens (TRAs) gain access to both the MHC class I and the MHC class II pathways of medullary thymic epithelial cells (mTECs), whereby macroautophagy may enhance the loading of endogenous antigens onto MHC class II molecules. At the same time, mTECs are inefficient at presenting exogenous antigens. mTEC-derived antigens can also be transferred to and presented by conventional dendritic cells (cDCs) in the thymus. Depending on the nature of the respective self antigen, it is conceivable that TRAs are released or shed in soluble form to be subsequently captured and processed by cDCs for presentation on MHC class I or MHC class II molecules. In addition, apoptosis of terminally differentiated mTECs may lead to the release of apoptotic fragments that can also transfer mTEC-derived self antigens to cDCs for cross-presentation, a process presumably enhanced by the pro-apoptotic function of autoimmune regulator (AIRE). In addition, functional peptide–MHC complexes are unidirectionally translocated from mTECs to cDCs; however, the mechanistic details remain unclear. In contrast to mTECs, thymic cDCs efficiently present blood-borne antigens that reach the thymus through the circulation. It is assumed that autochthonous CD172a$^-$ cDCs (that is, cDCs of intrathymic origin) are the preferred recipients of mTEC-derived antigens, as depicted here. We think this is likely because migratory CD172a$^+$ cDCs undergo further maturation on reaching the thymic microenvironment, suggesting that, akin to what has been described for mature, tissue-derived DCs in secondary lymphoid organs, they may 'freeze' their peripherally imprinted cargo of MHC ligands. In addition to tissue-specific peripheral self antigens, the antigens captured and processed by migratory cDCs before their relocation to the medulla may also encompass non-self antigens derived from innocuous foreign sources such as commensal bacteria or food.

***Intercellular antigen transfer: where, how and why?*** As outlined above, various studies have shown that mTECs have an autonomous role as presenters of endogenously expressed self antigens in the context of both MHC class I and MHC class II molecules. A second route by which mTEC-derived antigens are presented to developing CD4$^+$ and CD8$^+$ T cells is by transfer to and presentation by thymic DCs. Early studies in 1994, using a monoclonal antibody that recognizes a peptide derived from the α-chain of the MHC class II allele I-E$^d$ (amino acids 52–68; E$\alpha_{52-68}$) when bound to I-A$^b$ MHC class II molecules[90], showed the *in vivo* transfer of the E$\alpha_{52-68}$ peptide from TECs onto MHC class II molecules on thymic DCs, and it was appreciated that this may be a mechanism that enhances the efficacy of tolerance induction by spreading self antigens through the thymic microenvironment[91]. Of note, the intercellular transfer of the E$\alpha_{52-68}$ peptide was unidirectional, consistent with the differential efficacies of TECs and DCs at presenting exogenous antigen on MHC class II molecules through the classical endocytic pathway[91,92]. Subsequent experiments involving OVA-specific TCR-transgenic mice showed that antigen transfer to and presentation by thymic DCs similarly applies to MHC class I-restricted epitopes, as it resulted in the deletion of both MHC class I- and MHC class II-restricted OVA-specific thymocytes when OVA was expressed exclusively by mTECs[72]. In this particular system, which was devised to mimic promiscuous gene expression, deletion of CD8$^+$ T cells — but not CD4$^+$ T cells — also occurred when this route of antigen transfer was abolished; that is, when only mTECs could potentially present endogenously expressed OVA. This shows that, for CD4$^+$ T cell tolerance to certain antigens expressed by mTECs, cooperation with DCs is necessary. In another model system, two variants of hen egg lysozyme (HEL) were expressed by mTECs. The soluble form of HEL was more efficient at intrathymic deletion of specific CD4$^+$ T cells than the membrane-bound variant[93]. Here, facilitated presentation of soluble as opposed to cell-bound TEC-derived antigen by DCs is a plausible explanation; however, the study did not explore whether antigen transfer from mTECs to DCs actually occurred.

Recently, the unidirectional, steady-state transfer of physiologically expressed, native TRAs, such as the tumour rejection antigen P1A and the myelin component proteolipid protein, from mTECs to DCs has been shown to occur *in vivo*[75]. Importantly, such presentation of mTEC-derived self antigens by DCs under physiological conditions is not restricted to secreted molecules but covers all major subcellular compartments, as it also includes membrane, cytoplasmic and nuclear proteins[75]. Although the mechanistic details of this directional antigen transfer are unknown, it is conceivable that antigens released from mTECs in soluble form or in vesicles (for example, apoptotic bodies) are likely to gain access to MHC class I and class II molecules on DCs by means of established pathways of processing and presentation of exogenous material (cross-presentation in the case of MHC class I-restricted presentation). These transfer routes might be facilitated by the surprisingly rapid turnover of mTECs[66,67], so that dying mTECs, possibly enhanced by a pro-apoptotic function of the AIRE protein[67], may constitute a permanent source of TRAs for thymic DCs.

Recently, an additional and less conventional mechanism to spread self antigens in the thymic microenvironment has been proposed that involves the exchange of intact membrane domains that include functional peptide–MHC complexes[75,94]. Remarkably, this phenomenon also seems to be unidirectional from mTECs to DCs (or may occur between mTECs, but not from DCs to mTECs). Such a transfer of preformed peptide–MHC complexes from mTECs to DCs is a formal caveat for the interpretation of results in bone marrow chimaeras designed to abrogate antigen presentation on DCs and thereby reveal the autonomous role of mTECs in central tolerance. It follows that proof of the contribution of mTECs to central tolerance by autonomous presentation of TRAs will require the physical ablation of thymic DCs rather than the genetic elimination of MHC genes in the haematopoietic system. New tools are at hand to make this goal attainable in the near future. For example, diphtheria toxin-mediated ablation of all of the main DC subsets has been achieved[95,96], and other mutant mouse strains are available that selectively lack $CD8^+$ DCs[97] or pDCs[98].

In summary, the antigen transfer between thymic APCs is an obvious means by which to increase the probability of autoreactive T cells encountering rare antigens. How this transfer, which is possibly unique to the thymic medulla, proceeds and how its unidirectionality is regulated remain poorly understood. It is conceivable that conventional routes such as the passive diffusion of material released from apoptotic mTECs — but also more exotic mechanisms, for example endocytosis of mTEC-derived exosomes by DCs[99], membrane nibbling[100,101], antigen transfer through gap junctions[102] or tunnelling nanotubes[94] — are involved. Obtaining definitive proof for the involvement of these mechanisms in central tolerance will depend on the development of methods to selectively manipulate these processes *in vivo*.

Exosome
A small lipid-bilayer vesicle that is released from dendritic cells and other cells. They are composed of cell membranes or are derived from the membranes of intracellular vesicles. They might contain antigen–MHC complexes and interact with antigen-specific lymphocytes directly, or they might be taken up by other antigen-presenting cells.

## Conclusion and perspectives

In the context of positive selection, we feel that the discovery of distinct pathways for the generation of the MHC ligand repertoire of cTECs warrants a reconsideration of the altered peptide hypothesis in a modified form. Although the central tenet of the original hypothesis — that cTECs display an entirely unique set of MHC ligands — has not proved to be correct, it is an intriguing perspective that components of the proteolytic machineries that generate MHC-bound peptides, such as the cTEC-specific proteasomal subunit β5t, may have arisen during evolution to have an exclusive role in T cell selection (reviewed in REF. 103). One possible explanation is that positive selection of a fully diverse T cell repertoire might require peptides with unique structural properties, although presently there is little evidence for this. Alternatively, the evolutionary selection of cTEC-specific proteolytic pathways of peptide generation may have been driven by the necessity to limit the overlap between the peptide–MHC complexes presented by cTECs in the thymic cortex and APCs in the thymic medulla (and presumably also in secondary lymphoid organs), as re-encounter of positively selecting MHC ligands in a context that is optimized for tolerance induction may unduly limit the T cell repertoire. It remains to be shown how this idea can be reconciled with the hypothesis that peptide–MHC complexes identical to those that mediate positive selection may be required for peripheral T cell homeostasis and/or may act as co-agonists during T cell activation. Ultimately, a refined synthesis of the altered peptide theory and the affinity model will hinge on delineating truly physiological peptides on cTECs that are necessary for the selection of a given TCR specificity *in vivo*; this goal remains the 'Holy Grail' in the field of positive selection.

Concerning the tolerogenic function of medullary APCs, we have discussed determinants that are likely to shape the peptide–MHC repertoires of mTECs and DCs and outlined recent evidence for autonomous and cooperative functions of these APCs. It has become clear that mTECs are not only unique in their ability to promiscuously express TRAs, but they also have adapted their cell biology to focus their MHC class II-bound peptides on this endogenous antigen pool, thus fulfilling an autonomous APC function not only in $CD8^+$ but in $CD4^+$ T cell tolerance. Because mTEC-derived self antigens may also be transferred to and presented by DCs, it will be challenging to separate this dual contribution of mTECs to central tolerance — as a 'TRA expresser' and as a 'TRA presenter'. Furthermore, we are just beginning to appreciate the biological implications of the heterogeneity of thymic DCs and the way in which the various DC subsets may differentially contribute to the intrathymic representation of peripheral tissues. Similarly, it remains to be established whether individual thymic DC subsets differ in their cooperation with mTECs, for example whether they are equivalent recipients of mTEC-derived antigens. We would also like to understand whether and how the heterogeneity of medullary APCs and their peptide–MHC complexes are functionally related to distinct modes of tolerance: $T_{Reg}$ cell induction versus negative selection.

1. Daniels, M. A. *et al.* Thymic selection threshold defined by compartmentalization of Ras/MAPK signalling. *Nature* **444**, 724–729 (2006).
2. Ahn, S. *et al.* TSCOT$^+$ thymic epithelial cell-mediated sensitive CD4 tolerance by direct presentation. *PLoS Biol.* **6**, e191 (2008).
3. McCaughtry, T. M., Baldwin, T. A., Wilken, M. S. & Hogquist, K. A. Clonal deletion of thymocytes can occur in the cortex with no involvement of the medulla. *J. Exp. Med.* **205**, 2575–2584 (2008).
4. Bensinger, S. J., Bandeira, A., Jordan, M. S., Caton, A. J. & Laufer, T. M. Major histocompatibility complex class II-positive cortical epithelium mediates the selection of CD4$^+$25$^+$ immunoregulatory T cells. *J. Exp. Med.* **194**, 427–438 (2001).
5. Liston, A. *et al.* Differentiation of regulatory Foxp3$^+$ T cells in the thymic cortex. *Proc. Natl Acad. Sci. USA* **105**, 11903–11908 (2008).
6. Marrack, P. & Kappler, J. The T cell receptor. *Science* **238**, 1073–1079 (1987).
7. Marrack, P., Ignatowicz, L., Kappler, J. W., Boymel, J. & Freed, J. H. Comparison of peptides bound to spleen and thymus class II. *J. Exp. Med.* **178**, 2173–2183 (1993).
8. Ashton-Rickardt, P. G., Van Kaer, L., Schumacher, T. N., Ploegh, H. L. & Tonegawa, S. Peptide contributes to the specificity of positive selection of CD8$^+$ T cells in the thymus. *Cell* **73**, 1041–1049 (1993).
9. Hogquist, K. A., Gavin, M. A. & Bevan, M. J. Positive selection of CD8$^+$ T cells induced by major histocompatibility complex binding peptides in fetal thymic organ culture. *J. Exp. Med.* **177**, 1469–1473 (1993).
10. Ashton-Rickardt, P. G. *et al.* Evidence for a differential avidity model of T cell selection in the thymus. *Cell* **76**, 651–663 (1994).
11. Sebzda, E. *et al.* Positive and negative thymocyte selection induced by different concentrations of a single peptide. *Science* **263**, 1615–1618 (1994).
12. Sebzda, E. *et al.* Mature T cell reactivity altered by peptide agonist that induces positive selection. *J. Exp. Med.* **183**, 1093–1104 (1996).
13. Hogquist, K. A. *et al.* T cell receptor antagonist peptides induce positive selection. *Cell* **76**, 17–27 (1994).
14. Alam, S. M. *et al.* T-cell-receptor affinity and thymocyte positive selection. *Nature* **381**, 616–620 (1996).
15. Palmer, E. & Naeher, D. Affinity threshold for thymic selection through a T-cell receptor-co-receptor zipper. *Nature Rev. Immunol.* **9**, 207–213 (2009).
16. Ebert, P. J., Jiang, S., Xie, J., Li, Q. J. & Davis, M. M. An endogenous positively selecting peptide enhances mature T cell responses and becomes an autoantigen in the absence of microRNA miR-181a. *Nature Immunol.* **10**, 1162–1169 (2009).
17. Hogquist, K. A. *et al.* Identification of a naturally occurring ligand for thymic positive selection. *Immunity* **6**, 389–399 (1997).
18. Lo, W. L. *et al.* An endogenous peptide positively selects and augments the activation and survival of peripheral CD4$^+$ T cells. *Nature Immunol.* **10**, 1155–1161 (2009).
**References 16 and 18 report that specific peptides present on peripheral APCs can promote the positive selection of particular MHC class II-restricted TCRs when added to fetal thymic organ cultures. Importantly, these peptides also function as co-agonists for mature T cells of the same specificity.**
19. Santori, F. R. *et al.* Rare, structurally homologous self-peptides promote thymocyte positive selection. *Immunity* **17**, 131–142 (2002).
20. Goldrath, A. W. & Bevan, M. J. Selecting and maintaining a diverse T-cell repertoire. *Nature* **402**, 255–262 (1999).
21. Starr, T. K., Jameson, S. C. & Hogquist, K. A. Positive and negative selection of T cells. *Annu. Rev. Immunol.* **21**, 139–176 (2003).
22. Honey, K. & Rudensky, A. Y. Lysosomal cysteine proteases regulate antigen presentation. *Nature Rev. Immunol.* **3**, 472–482 (2003).
23. Nakagawa, T. *et al.* Cathepsin L: critical role in Ii degradation and CD4 T cell selection in the thymus. *Science* **280**, 450–453 (1998).
**This is the first report describing a cTEC-specific pathway for the generation of peptide–MHC class II complexes.**
24. 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.* **195**, 1349–1358 (2002).
25. Barton, G. M. & Rudensky, A. Y. Requirement for diverse, low-abundance peptides in positive selection of T cells. *Science* **283**, 67–70 (1999).
26. Sant'Angelo, D. B. *et al.* The imprint of intrathymic self-peptides on the mature T cell receptor repertoire. *Immunity* **7**, 517–524 (1997).
27. Tourne, S. *et al.* Selection of a broad repertoire of CD4$^+$ T cells in H-2Ma$^{0/0}$ mice. *Immunity* **7**, 187–195 (1997).
28. Ignatowicz, L., Kappler, J. & Marrack, P. The repertoire of T cells shaped by a single MHC/peptide ligand. *Cell* **84**, 521–529 (1996).
29. Bowlus, C. L., Ahn, J., Chu, T. & Gruen, J. R. Cloning of a novel MHC-encoded serine peptidase highly expressed by cortical epithelial cells of the thymus. *Cell. Immunol.* **196**, 80–86 (1999).
30. Carrier, A. *et al.* Differential gene expression in CD3ε- and RAG1-deficient thymuses: definition of a set of genes potentially involved in thymocyte maturation. *Immunogenetics* **50**, 255–270 (1999).
31. Cheunsuk, S. *et al. Prss16* is not required for T-cell development. *Mol. Cell Biol.* **25**, 789–796 (2005).
32. Gommeaux, J. *et al.* Thymus-specific serine protease regulates positive selection of a subset of CD4$^+$ thymocytes. *Eur. J. Immunol.* **39**, 956–964 (2009).
**This report shows altered positive selection of two MHC class II-restricted transgenic TCR specificities in mice with a targeted disruption of the cTEC-specific *Tssp* gene.**
33. Viken, M. K. *et al.* Reproducible association with type 1 diabetes in the extended class I region of the major histocompatibility complex. *Genes Immun.* **10**, 323–333 (2009).
34. Kasai, M. *et al.* Difference in antigen presentation pathways between cortical and medullary thymic epithelial cells. *Eur. J. Immunol.* **26**, 2101–2107 (1996).
35. Klein, L., Roettinger, B. & Kyewski, B. Sampling of complementing self-antigen pools by thymic stromal cells maximizes the scope of central T cell tolerance. *Eur. J. Immunol.* **31**, 2476–2486 (2001).
36. Oukka, M. *et al.* Selectivity of the major histocompatibility complex class II presentation pathway of cortical thymic epithelial cell lines. *Eur. J. Immunol.* **27**, 855–859 (1997).
37. Klein, L. & Kyewski, B. Self-antigen presentation by thymic stromal cells: a subtle division of labor. *Curr. Opin. Immunol.* **12**, 179–186 (2000).
38. Munz, C. Enhancing immunity through autophagy. *Annu. Rev. Immunol.* **27**, 423–449 (2009).
39. Mizushima, N., Yamamoto, A., Matsui, M., Yoshimori, T. & Ohsumi, Y. *In vivo* analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. *Mol. Biol. Cell* **15**, 1101–1111 (2004).
40. 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* **455**, 396–400 (2008).
**Using transplantation of *Atg5*$^{-/-}$ thymi, this report shows that a macroautophagy deficiency in TECs leads to altered selection of some MHC class II-restricted TCRs and systemic autoimmunity, presumably as a consequence of diminished endogenous MHC class II loading in TECs.**
41. Nedjic, J., Aichinger, M., Mizushima, N. & Klein, L. Macroautophagy, endogenous MHC II loading and T cell selection: the benefits of breaking the rules. *Curr. Opin. Immunol.* **21**, 92–97 (2009).
42. Murata, S. *et al.* Regulation of CD8$^+$ T cell development by thymus-specific proteasomes. *Science* **316**, 1349–1353 (2007).
**This paper identified and characterized the cTEC-specific proteasomal subunit β5t. Targeted disruption of the gene encoding β5t is shown to result in a dramatically decreased size of the CD8$^+$ T cell compartment.**
43. Nitta, T. *et al.* Thymoproteasome shapes immunocompetent repertoire of CD8 T cells. *Immunity* (in the press).
44. Murata, S., Takahama, Y. & Tanaka, K. Thymoproteasome: probable role in generating positively selecting peptides. *Curr. Opin. Immunol.* **20**, 192–196 (2008).
45. Ernst, B., Lee, D. S., Chang, J. M., Sprent, J. & Surh, C. D. The peptide ligands mediating positive selection in the thymus control T cell survival and homeostatic proliferation in the periphery. *Immunity* **11**, 173–181 (1999).
46. Davis, M. M. *et al.* T cells as a self-referential, sensory organ. *Annu. Rev. Immunol.* **25**, 681–695 (2007).
47. Stefanova, I., Dorfman, J. R. & Germain, R. N. Self-recognition promotes the foreign antigen sensitivity of naive T lymphocytes. *Nature* **420**, 429–434 (2002).
48. McCaughtry, T. M., Wilken, M. S. & Hogquist, K. A. Thymic emigration revisited. *J. Exp. Med.* **204**, 2513–2520 (2007).
49. Kurobe, H. *et al.* CCR7-dependent cortex-to-medulla migration of positively selected thymocytes is essential for establishing central tolerance. *Immunity* **24**, 165–177 (2006).
50. Srivatsan, S. & Peng, S. L. Cutting edge: Foxj1 protects against autoimmunity and inhibits thymocyte egress. *J. Immunol.* **175**, 7805–7809 (2005).
51. Boehm, T., Scheu, S., Pfeffer, K. & Bleul, C. C. Thymic medullary epithelial cell differentiation, thymocyte emigration, and the control of autoimmunity require lympho-epithelial cross talk via LTβR. *J. Exp. Med.* **198**, 757–769 (2003).
52. Akiyama, T. *et al.* Dependence of self-tolerance on TRAF6-directed development of thymic stroma. *Science* **308**, 248–251 (2005).
53. Akiyama, T. *et al.* The tumour necrosis factor family receptors RANK and CD40 cooperatively establish the thymic medullary microenvironment and self-tolerance. *Immunity* **29**, 423–437 (2008).
54. Hikosaka, Y. *et al.* The cytokine RANKL produced by positively selected thymocytes fosters medullary thymic epithelial cells that express autoimmune regulator. *Immunity* **29**, 438–450 (2008).
55. Irla, M. *et al.* Autoantigen-specific interactions with CD4$^+$ thymocytes control mature medullary thymic epithelial cell cellularity. *Immunity* **29**, 451–463 (2008).
56. Rossi, S. W. *et al.* RANK signals from CD4$^+$3$^-$ inducer cells regulate development of Aire-expressing epithelial cells in the thymic medulla. *J. Exp. Med.* **204**, 1267–1272 (2007).
57. Chelly, J., Concordet, J. P., Kaplan, J. C. & Kahn, A. Illegitimate transcription: transcription of any gene in any cell type. *Proc. Natl Acad. Sci. USA* **86**, 2617–2621 (1989).
58. Sarkar, G. & Sommer, S. S. Access to a messenger RNA sequence or its protein product is not limited by tissue or species specificity. *Science* **244**, 331–334 (1989).
59. Linsk, R., Gottesman, M. & Pernis, B. Are tissues a patch quilt of ectopic gene expression? *Science* **246**, 261 (1989).
60. Kyewski, B. & Klein, L. A central role for central tolerance. *Annu. Rev. Immunol.* **24**, 571–606 (2006).
61. Anderson, M. S. *et al.* Projection of an immunological self shadow within the thymus by the aire protein. *Science* **298**, 1395–1401 (2002).
62. Derbinski, J., Schulte, A., Kyewski, B. & Klein, L. Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. *Nature Immunol.* **2**, 1032–1039 (2001).
63. Mathis, D. & Benoist, C. Aire. *Annu. Rev. Immunol.* **27**, 287–312 (2009).
64. Peterson, P., Org., T. & Rebane, A. Transcriptional regulation by AIRE: molecular mechanisms of central tolerance. *Nature Rev. Immunol.* **8**, 948–957 (2008).
65. Derbinski, J., Pinto, S., Rosch, S., Hexel, K. & Kyewski, B. Promiscuous gene expression patterns in single medullary thymic epithelial cells argue for a stochastic mechanism. *Proc. Natl Acad. Sci. USA* **105**, 657–662 (2008).
66. Gabler, J., Arnold, J. & Kyewski, B. Promiscuous gene expression and the developmental dynamics of medullary thymic epithelial cells. *Eur. J. Immunol.* **37**, 3363–3372 (2007).
67. Gray, D., Abramson, J., Benoist, C. & Mathis, D. Proliferative arrest and rapid turnover of thymic epithelial cells expressing Aire. *J. Exp. Med.* **204**, 2521–2528 (2007).
68. Zehn, D. & Bevan, M. J. T cells with low avidity for a tissue-restricted antigen routinely evade central and peripheral tolerance and cause autoimmunity. *Immunity* **25**, 261–270 (2006).
69. Miller, M. J., Hejazi, A. S., Wei, S. H., Cahalan, M. D. & Parker, I. T cell repertoire scanning is promoted by dynamic dendritic cell behaviour and random T cell motility in the lymph node. *Proc. Natl Acad. Sci. USA* **101**, 998–1003 (2004).
70. Le Borgne, M. *et al.* The impact of negative selection on thymocyte migration in the medulla. *Nature Immunol.* **10**, 823–830 (2009).
**This paper provides the first data on how thymocytes scan the medullary microenvironment and thus maximize the chances of self-antigen encounter.**
71. Klein, L. Dead man walking: how thymocytes scan the medulla. *Nature Immunol.* **10**, 809–811 (2009).

72. Gallegos, A. M. & Bevan, M. J. Central tolerance to tissue-specific antigens mediated by direct and indirect antigen presentation. *J. Exp. Med.* **200**, 1039–1049 (2004).
73. Derbinski, J. *et al.* Promiscuous gene expression in thymic epithelial cells is regulated at multiple levels. *J. Exp. Med.* **202**, 33–45 (2005).
74. Aschenbrenner, K. *et al.* Selection of Foxp3$^+$ regulatory T cells specific for self antigen expressed and presented by Aire$^+$ medullary thymic epithelial cells. *Nature Immunol.* **8**, 351–358 (2007).
75. Koble, C. & Kyewski, B. The thymic medulla: a unique microenvironment for intercellular self-antigen transfer. *J. Exp. Med.* **206**, 1505–1513 (2009).
**A systematic analysis of the directional transfer of mTEC-derived antigens to thymic DCs and the functional consequences. Importantly, unidirectional transfer is also shown for native endogenous self antigens.**
76. Shortman, K. & Naik, S. H. Steady-state and inflammatory dendritic-cell development. *Nature Rev. Immunol.* **7**, 19–30 (2007).
77. Wu, L. & Shortman, K. Heterogeneity of thymic dendritic cells. *Semin. Immunol.* **17**, 304–312 (2005).
78. Ardavin, C., Wu, L., Li, C. L. & Shortman, K. Thymic dendritic cells and T cells develop simultaneously in the thymus from a common precursor population. *Nature* **362**, 761–763 (1993).
79. Donskoy, E. & Goldschneider, I. Two developmentally distinct populations of dendritic cells inhabit the adult mouse thymus: demonstration by differential importation of haematogenous precursors under steady state conditions. *J. Immunol.* **170**, 3514–3521 (2003).
80. 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.* **206**, 607–622 (2009).
**This report describes the phenotype, frequency and characteristics of autochthonous and migratory DCs in the mouse thymus.**
81. Proietto, A. I., Lahoud, M. H. & Wu, L. Distinct functional capacities of mouse thymic and splenic dendritic cell populations. *Immunol. Cell Biol.* **86**, 700–708 (2008).
**A systematic comparison of the cytokine and chemokine production, Toll-like receptor expression and antigen presentation capacity of autochthonous and migratory thymic cDCs.**
82. Radtke, F. *et al.* Notch1 deficiency dissociates the intrathymic development of dendritic cells and T cells. *J. Exp. Med.* **191**, 1085–1094 (2000).
83. Rodewald, H. R., Brocker, T. & Haller, C. Developmental dissociation of thymic dendritic cell and thymocyte lineages revealed in growth factor receptor mutant mice. *Proc. Natl Acad. Sci. USA* **96**, 15068–15073 (1999).
84. Bonasio, R. *et al.* Clonal deletion of thymocytes by circulating dendritic cells homing to the thymus. *Nature Immunol.* **7**, 1092–1100 (2006).
**The first evidence that physiological immigration of peripheral DCs into the thymus can induce negative selection of CD4$^+$ T cells that recognize a peripheral model antigen.**
85. Lahoud, M. H. *et al.* Signal regulatory protein molecules are differentially expressed by CD8$^-$ dendritic cells. *J. Immunol.* **177**, 372–382 (2006).
86. Liu, K. *et al.* Origin of dendritic cells in peripheral lymphoid organs of mice. *Nature Immunol.* **8**, 578–583 (2007).
87. Duncan, S. R., Capetanakis, N. G., Lawson, B. R. & Theofilopoulos, A. N. Thymic dendritic cells traffic to thymi of allogeneic recipients and prolong graft survival. *J. Clin. Invest.* **109**, 755–764 (2002).
88. Proietto, A. I. *et al.* Dendritic cells in the thymus contribute to T-regulatory cell induction. *Proc. Natl Acad. Sci. USA* **105**, 19869–19874 (2008).
**Together with reference 80, this paper identifies the peripheral origin of CD172a$^+$ cDCs. Their suggested specialized role in $T_{Reg}$ cell induction remains controversial (as shown in reference 89).**
89. Wirnsberger, G., Mair, F. & Klein, L. Regulatory T cell differentiation of thymocytes does not require a dedicated antigen-presenting cell but is under T cell-intrinsic developmental control. *Proc. Natl Acad. Sci. USA* **106**, 10278–10283 (2009).
90. Rudensky, A., Rath, S., Preston-Hurlburt, P., Murphy, D. B. & Janeway, C. A. Jr. On the complexity of self. *Nature* **353**, 660–662 (1991).
91. Humblet, C., Rudensky, A. & Kyewski, B. Presentation and intercellular transfer of self antigen within the thymic microenvironment: expression of the Eα peptide-I-A$^b$ complex by isolated thymic stromal cells. *Int. Immunol.* **6**, 1949–1958 (1994).
92. Viret, C., Barlow, A. K. & Janeway, C. A., Jr. On the intrathymic intercellular transfer of self-determinants. *Immunol. Today* **20**, 8–10 (1999).
93. Zhang, M. *et al.* T cell tolerance to a neo-self antigen expressed by thymic epithelial cells: the soluble form is more effective than the membrane-bound form. *J. Immunol.* **170**, 3954–3962 (2003).
94. Millet, V., Naquet, P. & Guinamard, R. R. Intercellular MHC transfer between thymic epithelial and dendritic cells. *Eur. J. Immunol.* **38**, 1257–1263 (2008).
**Using somatic and bone marrow chimaeras, this study elegantly shows that intact peptide–MHC complexes are transferred from TECs to DCs and also between TECs.**
95. Birnberg, T. *et al.* Lack of conventional dendritic cells is compatible with normal development and T cell homeostasis, but causes myeloid proliferative syndrome. *Immunity* **29**, 986–997 (2008).
96. Ohnmacht, C. *et al.* Constitutive ablation of dendritic cells breaks self-tolerance of CD4 T cells and results in spontaneous fatal autoimmunity. *J. Exp. Med.* **206**, 549–559 (2009).
97. Hildner, K. *et al. Batf3* deficiency reveals a critical role for CD8α$^+$ dendritic cells in cytotoxic T cell immunity. *Science* **322**, 1097–1100 (2008).
98. Cisse, B. *et al.* Transcription factor E2-2 is an essential and specific regulator of plasmacytoid dendritic cell development. *Cell* **135**, 37–48 (2008).
99. Thery, C., Zitvogel, L. & Amigorena, S. Exosomes: composition, biogenesis and function. *Nature Rev. Immunol.* **2**, 569–579 (2002).
100. Harshyne, L. A., Watkins, S. C., Gambotto, A. & Barratt-Boyes, S. M. Dendritic cells acquire antigens from live cells for cross-presentation to CTL. *J. Immunol.* **166**, 3717–3723 (2001).
101. Russo, V. *et al.* Acquisition of intact allogeneic human leukocyte antigen molecules by human dendritic cells. *Blood* **95**, 3473–3477 (2000).
102. Neijssen, J. *et al.* Cross-presentation by intercellular peptide transfer through gap junctions. *Nature* **434**, 83–88 (2005).
103. Boehm, T. The adaptive phenotype of cortical thymic epithelial cells. *Eur. J. Immunol.* **39**, 944–947 (2009).
104. Mizushima, N. & Klionsky, D. J. Protein turnover via autophagy: implications for metabolism. *Annu. Rev. Nutr.* **27**, 19–40 (2007).
105. Hara, T. *et al.* Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature* **441**, 885–889 (2006).
106. Cecconi, F. & Levine, B. The role of autophagy in mammalian development: cell makeover rather than cell death. *Dev. Cell* **15**, 344–357 (2008).
107. Virgin, H. W. & Levine, B. Autophagy genes in immunity. *Nature Immunol.* **10**, 461–470 (2009).
108. Brazil, M. I., Weiss, S. & Stockinger, B. Excessive degradation of intracellular protein in macrophages prevents presentation in the context of major histocompatibility complex class II molecules. *Eur. J. Immunol.* **27**, 1506–1514 (1997).
109. Nimmerjahn, F. *et al.* Major histocompatibility complex class II-restricted presentation of a cytosolic antigen by autophagy. *Eur. J. Immunol.* **33**, 1250–1259 (2003).
110. Paludan, C. *et al.* Endogenous MHC class II processing of a viral nuclear antigen after autophagy. *Science* **307**, 593–596 (2005).
111. Dengjel, J. *et al.* Autophagy promotes MHC class II presentation of peptides from intracellular source proteins. *Proc. Natl Acad. Sci. USA* **102**, 7922–7927 (2005).
112. Schmid, D., Pypaert, M. & Munz, C. Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. *Immunity* **26**, 79–92 (2007).
113. Kloetzel, P. M. & Ossendorp, F. Proteasome and peptidase function in MHC-class-I-mediated antigen presentation. *Curr. Opin. Immunol.* **16**, 76–81 (2004).
114. Geenen, V. *et al.* The neuroendocrine thymus: coexistence of oxytocin and neurophysin in the human thymus. *Science* **232**, 508–511 (1986).
115. Jolicoeur, C., Hanahan, D. & Smith, K. M. T-cell tolerance toward a transgenic beta-cell antigen and transcription of endogenous pancreatic genes in thymus. *Proc. Natl Acad. Sci. USA* **91**, 6707–6711 (1994).
116. Smith, K. M., Olson, D. C., Hirose, R. & Hanahan, D. Pancreatic gene expression in rare cells of thymic medulla: evidence for functional contribution to T cell tolerance. *Int. Immunol.* **9**, 1355–1365 (1997).
117. Aaltonen, J. *et al.* An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. *Nature Genet.* **17**, 399–403 (1997).
118. Nagamine, K. *et al.* Positional cloning of the APECED gene. *Nature Genet.* **17**, 393–398 (1997).
119. Bhakta, N. R., Oh, D. Y. & Lewis, R. S. Calcium oscillations regulate thymocyte motility during positive selection in the three-dimensional thymic environment. *Nature Immunol.* **6**, 143–151 (2005).
120. Bousso, P., Bhakta, N. R., Lewis, R. S. & Robey, E. Dynamics of thymocyte-stromal cell interactions visualized by two-photon microscopy. *Science* **296**, 1876–1880 (2002).
121. Ebert, P. J., Ehrlich, L. I. & Davis, M. M. Low ligand requirement for deletion and lack of synapses in positive selection enforce the gauntlet of thymic T cell maturation. *Immunity* **29**, 734–745 (2008).
122. Kishimoto, H. & Sprent, J. Several different cell surface molecules control negative selection of medullary thymocytes. *J. Exp. Med.* **190**, 65–73 (1999).
123. Page, D. M. Cutting edge: thymic selection and autoreactivity are regulated by multiple co-receptors involved in T cell activation. *J. Immunol.* **163**, 3577–3581 (1999).
124. Kishimoto, H. & Sprent, J. Negative selection in the thymus includes semimature T cells. *J. Exp. Med.* **185**, 263–271 (1997).



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### FURTHER INFORMATION

**Ludger Klein's homepage:** http://immuno.web.med.uni-muenchen.de/research/ag_klein/index.html
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