Thymus machinery for T-cell selection

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Abstract

An immunocompetent and self-tolerant pool of naive T cells is formed in the thymus through the process of repertoire selection. T cells that are potentially capable of responding to foreign antigens are positively selected in the thymic cortex and are further selected in the thymic medulla to help prevent self-reactivity. The affinity between T-cell antigen receptors expressed by newly generated T cells and self-peptide–major histocompatibility complexes displayed in the thymic microenvironments plays a key role in determining the fate of developing T cells during thymic selection. Recent advances in our knowledge of the biology of thymic epithelial cells have revealed unique machinery that contributes to positive and negative selection in the thymus. In this article, we summarize recent findings on thymic T-cell selection, focusing on the machinery unique to thymic epithelial cells.

Keywords: central tolerance, cortical thymic epithelial cell, medullary thymic epithelial cell, positive selection

Introduction

The thymus produces T cells and selects newly produced T cells to generate a functionally competent and self-tolerant T-cell pool. After migrating into the microenvironment of the thymic cortex, hematopoietic lymphoid progenitors are induced to develop into T-cell antigen receptor (TCR)-expressing CD4⁺CD8⁺ double-positive (DP) thymocytes. The V(D)J rearrangement of TCR α and TCR β genomic loci in early thymocytes allows the formation of great diversity in the pre-selected repertoire of TCR recognition specificities in newly generated cortical DP thymocytes, which are individually selected for their fate according to their TCR recognition specificities. DP thymocytes that express TCRs interacting at low affinity with self-peptide-associated major histocompatibility complex class I (MHC-I) or MHC class II (MHC-II) molecules displayed in the thymic cortex are rescued from cell death to survive and differentiate into CD4⁺CD8[−] or CD4[−]CD8⁺ single-positive (SP) thymocytes, a process called positive selection in the thymus (1–3).

Positively selected thymocytes begin expressing the chemokine receptor CCR7 and migrate to the thymic medulla, where a subpopulation of medullary thymic epithelial cells (mTECs) produce CCR7 ligands, including CCL21 (4, 5), thereby attracting the positively selected thymocytes. In the thymic medulla, a distinct subpopulation of mTECs produces a wide range of self-molecules, including ‘tissue-specific’ self-proteins (i.e. proteins that are otherwise only found in specific peripheral tissues) and displays the vast majority of self-antigens, in cooperation with dendritic cells (DCs) (6–8).

Medullary thymocytes that express TCRs with high affinity for self-peptide–MHC (self-pMHC) complexes displayed in the medullary microenvironment are either deleted by apoptosis or destined to become regulatory T cells and other agonist-selected ‘unconventional’ T-cell populations (3, 9, 10). Negative selection in the thymus conventionally refers to the process of self-antigen-mediated deletion of developing thymocytes through apoptosis, including deletion of the high-affinity self-reactive thymocytes in the medulla. The lineage deviation in the medulla, which gives rise to regulatory and other ‘unconventional’ T cells by high-affinity TCR interactions, could be viewed as a form of positive selection of unconventional T cells, but could alternatively be viewed as a form of negative selection of thymocytes to exclude self-reactive cells from the ‘conventional’ effector T-cell pool.

Here, we start by discussing the importance of TCR affinity thresholds in thymocyte selection. Focusing on recent advances, we then detail the machinery that is utilized in the cortex and in the medulla to screen developing T cells and enable the optimal repertoire to be available for peripheral immune responses.

TCR affinity

The affinity between TCRs and their peptide–MHC ligands determines the fate of developing thymocytes. Early studies showed that differences in ligand concentration and ligating TCR valency as well as differences among agonist

and antagonist peptide ligands affect life-or-death determination in immature thymocytes (11–14). The findings that agonist peptide ligation promotes differentiation into unconventional T-cell lineages, including regulatory T cells, pointed to the view that the quality of TCR signals triggered in developing thymocytes not only determines life-or-death selection in generating a conventional T-cell pool but also contributes to determining a way to direct cells towards unconventional T-cell subpopulations (15, 16).

It is now understood that the affinity between TCRs expressed by developing thymocytes and self-pMHC complexes displayed in the thymus is the major parameter that determines the fate of newly generated T cells. The TCR–ligand affinity determines life or death (i.e. positive and negative selection) as well as lineage direction to become functionally distinct cells (e.g. conventional CD4⁺ helper and CD8⁺ killer T cells). A low-affinity interaction between TCR and pMHC promotes thymocyte maturation to give rise to functionally competent T cells (i.e. positive selection), whereas a high-affinity interaction causes the deletion of self-reactive T cells (i.e. negative selection) or the generation of unconventional T cells (1, 17).

The narrow TCR–pMHC affinity range sets the threshold for life or death in developing thymocytes, contributing to the enrichment of functionally potent and self-protective T cells while excluding potentially harmful self-reactive T cells from the mature T-cell pool (18, 19). It is considered that the affinity threshold determines the dependence on intra-thymocyte signals through *Themis*, *Tespa1*, a voltage-gated sodium-ion channel and *miR-181a* (a non-coding small RNA), all of which are expressed in pre-selected DP thymocytes and are important for promoting positive selection rather than negative selection (20–24).

Within the TCR–ligand affinity range that promotes positive selection, the ligand affinity during positive selection influences later functional capability in positively selected T cells (25–28), as was recently discussed elsewhere (29). Recent studies have indicated that CD8⁺ T cells differ in functional capability depending on their ontogeny during the fetal, neonatal and adult periods (30) and that the TCR–ligand affinity during positive selection appears higher in neonatal mice than in adult mice (31). These observations suggest that the magnitude of TCR–ligand affinity optimal for thymic positive selection, which affects subsequent antigen responsiveness in T cells, diminishes through postnatal ontogeny and ageing.

Cortex machinery

In the thymic cortex, cortical TECs (cTECs) architecturally and functionally provide the microenvironment for T-lymphoid progenitor cells to become T cells, for example by supplying T-lineage-inducing molecules including DLL4 and IL-7 (32, 33). The cTECs also provide a unique microenvironment, for example by expressing MHC-I and MHC-II molecules, for newly generated T cells to undergo positive selection (34, 35). Recent studies have revealed that cTECs are heterogeneous in mediating these functions (35, 36) and are capable of optimizing T-cell positive selection by further providing unique machinery for protein degradation (Fig. 1).

MHC-II-associated self-peptide production in cTECs

Peptides associated with MHC-II molecules are generated by proteolysis within the lumen of the endosomal–lysosomal system. Cathepsin L, a lysosomal endopeptidase abundant in cTECs, is involved in the processing of MHC-II invariant chain (Ii) and in the generation of MHC-II-associated self-peptides that promote cTEC-mediated positive selection of CD4⁺ T

Fig. 1. Cortical protein degradation and medullary gene expression in the thymus.

cells (37–39). Another endosomal peptidase, thymus-specific serine protease (TSSP, also known as Prss16), is also abundant in cTECs. Studies using MHC-II-restricted TCR transgenic mice showed that TSSP contributes to the production of positively selecting MHC-II-associated self-peptides (40–42). TSSP is also reported to play a role in the production of negatively selecting MHC-II-associated self-peptides, influencing the severity of autoimmune inflammation in type 1 diabetes in the NOD mouse strain (43–45).

Both cathepsin L and TSSP are abundant in cTECs but are also expressed in various cell types other than cTECs. Their co-abundance in combination with other proteases may create a unique endosomal-lysosomal proteolytic microenvironment in cTECs, which may contribute to the production of unique MHC-II-associated self-peptides that optimize positive selection of CD4⁺ T cells.

Unlike most cells, in which autophagy is activated by nutrient starvation, cTECs are constitutively active in autophagy-mediated degradation of cytoplasmic proteins (46, 47). Autophagy in cTECs contributes to the production of MHC-II-associated self-peptides, including cytoplasmic protein-derived peptides, thereby optimizing the repertoire selection of CD4⁺ T cells (47, 48).

Stabilization of surface MHC-II in cTECs

March family E3 ubiquitin ligases regulate the degradation and turnover of MHC-II molecules. The cTECs predominantly express March8, the activity of which is attenuated by the immunoglobulin superfamily cell surface protein CD83, which is also expressed in cTECs. CD83 stabilizes surface MHC-II molecules in cTECs by slowing March8-mediated MHC-II turnover (49, 50). CD83-deficient mice are defective in MHC-II stabilization in cTECs and in CD4⁺ T-cell generation in the thymus (49–51), whereas these defects are rescued by a deficiency in March8 (49, 50). CD83- and March8-mediated stabilization of surface MHC-II molecules in cTECs may contribute to maintaining persistent TCR engagement, which is important for positive selection of CD4⁺ T cells in the thymus (2, 52).

MHC-I-associated self-peptide production in cTECs

Peptides associated with MHC-I molecules are generated through proteasome-mediated degradation of cytoplasmic proteins. The cTECs express a unique form of proteasome, termed the thymoproteasome, which is characterized by the inclusion of the catalytic subunit $\beta 5t$, also known as Psmb11 (53, 54). The expression of $\beta 5t$ and $\beta 5t$ -containing thymoproteasomes is highly specific to cTECs (55, 56). In comparison with other forms of proteasomes, $\beta 5t$ -containing thymoproteasomes exhibit altered proteolytic specificity, producing a unique set of MHC-I-associated peptides (57, 58). In $\beta 5t$ -deficient mice, CD8⁺ T cells are reduced in numbers, altered in TCR repertoire and defective in fine-tuning TCR responsiveness (28, 59–61). Thus, thymoproteasome-dependent positive selection in the thymic cortex is important for promoting positive selection of CD8⁺ T cells. The possible mechanisms underlying how $\beta 5t$ -containing thymoproteasome works in the positive selection of CD8⁺ T cells are discussed elsewhere (62–64).

Human genome variations in $\beta 5t$

In humans, $\beta 5t$ is also specifically expressed in cTECs (65). More than 150 kinds of genomic variations, including single nucleotide polymorphisms in the $\beta 5t$ -coding sequence, have been registered in the National Center for Biotechnology Information (NCBI) public database. Among those variants, the rs34457782 single nucleotide polymorphism that alters the 49th amino acid of the human $\beta 5t$ pro-protein from glycine to serine (G49S) is detected at an appreciable allele frequency in various human populations (66). The 49th amino acid is located at the carboxyl terminus of the pro-peptide that is cleaved to generate catalytically active $\beta 5t$ protein, the amino terminus of which begins at the 50th threonine.

The G49S variant in either human $\beta 5t$ or mouse $\beta 5t$ has inefficient processing of the pro-peptide to generate mature $\beta 5t$ protein (66). In mice that are manipulated to carry this variation in the genome, heterozygotes show reduced $\beta 5t$ expression in cTECs and homozygotes further exhibit reduction in the numbers of CD8⁺ T cells in the thymus and the periphery (66). In cohort studies in humans, however, no severe health problems have been noticed in many heterozygous and a small number of homozygous human individuals (66). A study has reported that homozygosity in this variation is associated with an elevated risk of Sjögren's syndrome, an autoimmune disease (67). Further analysis of the health of humans with this genomic variation, particularly of the homozygotes, would help improve our understanding of the thymoproteasome-dependent positive selection of CD8⁺ T cells in humans.

Medulla machinery

It is the privilege of positively selected thymocytes to migrate to the thymic medulla (68, 69). In the medullary microenvironment, the thymocytes must interact with a variety of antigen-presenting cells, including mTECs and hematopoietic DCs, in order to establish self-tolerance in T cells (Fig. 1). Only cells that survive medullary selection may be released from the thymus to the circulation as mature naive T cells.

Promiscuous gene expression in mTECs

More than 85% of all genes encoded in the genome, including many tissue-restricted self-antigen genes, are detectable in mTECs (70–73). This characteristic, termed promiscuous gene expression (pGE), in mTECs contributes to the display of self-antigens, including tissue-restricted self-antigens, to newly generated T cells and therefore the establishment of self-tolerance in T cells.

The nuclear protein Aire is an epigenetic regulator that magnifies pGE in mTECs through interactions with multiple partner proteins (74–78). It has been shown that Aire enhances gene transcription by releasing RNA polymerase II at silenced loci, promoting promiscuousness in gene expression (79). It has also been shown that Aire limits transcription by repressing Brg1-mediated chromatin accessibility, which is implicated as a mechanism to avoid the deleterious effects of ectopically expressed genes (80). The development and function of mTECs, including pGE, is regulated by signals mediated via tumor necrosis factor superfamily members RANK, CD40

and LT β R (81–83)—the ligands of which are provided by positively selected thymocytes and by other thymic hematopoietic cells (84–86)—and by transcription factors RelB, NF κ B2, Fezf2 and Blimp1 (87–92).

Hematopoietic antigen-presenting cells

The thymic medulla is enriched in hematopoietic antigen-presenting cells, including DCs and B cells, which contribute to the establishment of self-tolerance in T cells (93–97). Thymic DCs are classified into CD8 α ⁺Sirp α ⁺ conventional DCs (cDC1s), CD8 α ⁺Sirp α ⁺ cDCs (cDC2s) and plasmacytoid DCs (pDCs). It has been shown that cDC1s are intrathymically generated, whereas cDC2s and pDCs are derived from the peripheral circulation into the thymus (98–100).

The cDC1s efficiently mediate the cross-presentation of mTEC-derived pGE antigens and efficiently promote the generation of regulatory T cells (101). The scavenger receptor CD36 expressed by cDC1s facilitates the transfer of mTEC-derived self-antigens to cDC1s (102). The chemokine CCL21Ser, which is produced by an LT β R-dependent mTEC subpopulation (103), promotes the recruitment of cDC1 progenitors to the thymus (104). The chemokine XCL1, which is produced by a distinct Aire-dependent mTEC subpopulation, supports the interplay between mTECs and DCs to promote the generation of regulatory T cells (105).

Thymic tuft cells

The mTEC population is functionally and morphologically heterogeneous, including cystic ciliated cells, neurosecretory cells, concentric Hassall's corpuscles and cholinergic chemosensory cells (106–108). Recent studies have highlighted a subpopulation of mTECs that resemble tuft cells of the small intestine (109, 110). Like tuft cells in the gut, the thymic tuft cells are dependent on the transcription factor Pou2f3 for their generation, and express chemosensory receptors and the pro-inflammatory cytokine IL-25 (109, 110). Unlike intestinal tuft cells, however, thymic tuft cells are generated as a post-Aire mTEC subpopulation and express both MHC-I and MHC-II molecules (109). The functions of thymic tuft cells are not clear, although reports suggest their roles in supporting type 2 invariant natural killer T cells (iNKT2s) to form an

IL-4-rich microenvironment and/or in controlling type 2 innate lymphoid cells (ILC2s) in the thymic medulla.

Medullary thymocyte traffic

Multiple chemokines expressed by mTECs and other cells in the thymic medulla are important for regulating the traffic of developing thymocytes. Positively selected thymocytes, which are generated in the thymic cortex, begin expressing CCR7 and migrate to the thymic medulla in response to CCR7 ligands produced by mTECs (68, 69, 111, 112). A recent study has pointed to the non-redundant importance of CCL21Ser among several different CCR7 ligands (113). The CCR7–CCL21Ser chemokine axis essentially contributes to the establishment of self-tolerance in T cells in the thymic medulla (113). The medullary migration of positively selected thymocytes is additionally regulated by chemokines CXCL12 and CCL25 as well as Sema3E–PlexinD1 guidance proteins (114–117). Chemokines CCL17 and CCL22 and their receptor CCR4 are also suggested to contribute to the medullary migration of positively selected thymocytes (118, 119).

The egress of mature thymocytes from the thymic medulla to the circulation is regulated by the interaction between sphingosine-1-phosphate receptor 1 (S1P1) expressed by mature thymocytes and S1P, which is abundant in the circulation (120). The role of CCR7 chemokine signals in thymic emigration during the newborn period has also been described (121). A recent study has suggested that CCR7 expression on iNKT cells defines iNKT precursor cells that emigrate from the thymus (122). Another study has pointed to a role for type 2 IL-4 receptor-mediated signals in mTECs in the thymic egress of mature thymocytes (123).

Conclusions

The cortical and medullary microenvironments in the thymus coordinate to sequentially support the formation of a self-protective T-cell pool, by providing distinct yet unique machinery in cTECs and mTECs (Fig. 1). The thymic cortex offers a unique protein degradation machinery both in the cytoplasm and in the endosomal–lysosomal lumen of cTECs to promote the display of a unique set of self-peptides that are important for positive selection of functionally competent T cells. In

Fig. 2. Multiple tasks coordinated by cortex and medulla in the thymus.

contrast, the thymic medulla is equipped with unique gene expression machinery in the nuclei of mTECs, enabling the display of an unusually promiscuous set of self-peptides that contribute to the establishment of self-tolerance in newly generated T cells.

These machineries in cTECs and mTECs appear mechanistically and anatomically distinct from each other, in protein degradation and in gene expression, respectively. However, these sets of machinery share a common importance in displaying the body's unique self to developing thymocytes for the formation of a functionally competent yet self-tolerant T-cell pool. The coordination of these sets of machinery for cTEC-dependent positive selection and mTEC-dependent self-tolerance is central to the development of the functional immune system.

As summarized in this article, recent advances in TEC biology have revealed new mechanisms that contribute to T-cell repertoire formation in the thymus. In parallel, studies have uncovered tremendous diversity in TECs (Fig. 2). Still, these findings have been largely achieved by the analysis of cTECs and mTECs isolated from the thymus by enzymatic digestion method, which can yield only a small fraction of cTECs and mTECs (124, 125). The biology in the majority of TECs has yet to be examined. Further advances in thymus biology may accelerate our understanding of T-cell repertoire formation and immune system development.

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References

- Starr, T. K., Jameson, S. C. and Hogquist, K. A. 2003. Positive and negative selection of T cells. *Annu. Rev. Immunol.* 21:139.
- Singer, A., Adoro, S. and Park, J. H. 2008. Lineage fate and intense debate: myths, models and mechanisms of CD4- versus CD8-lineage choice. *Nat. Rev. Immunol.* 8:788.
- Klein, L., Kyewski, B., Allen, P. M. and Hogquist, K. A. 2014. Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). *Nat. Rev. Immunol.* 14:377.
- Takahama, Y. 2006. Journey through the thymus: stromal guides for T-cell development and selection. *Nat. Rev. Immunol.* 6:127.
- Love, P. E. and Bhandardola, A. 2011. Signal integration and crosstalk during thymocyte migration and emigration. *Nat. Rev. Immunol.* 11:469.
- Kyewski, B. and Klein, L. 2006. A central role for central tolerance. *Annu. Rev. Immunol.* 24:571.
- Anderson, G., Lane, P. J. and Jenkinson, E. J. 2007. Generating intrathymic microenvironments to establish T-cell tolerance. *Nat. Rev. Immunol.* 7:954.
- Derbinski, J. and Kyewski, B. 2010. How thymic antigen presenting cells sample the body's self-antigens. *Curr. Opin. Immunol.* 22:592.
- Sprent, J. and Kishimoto, H. 2002. The thymus and negative selection. *Immunol. Rev.* 185:126.
- Sakaguchi, S., Yamaguchi, T., Nomura, T. and Ono, M. 2008. Regulatory T cells and immune tolerance. *Cell* 133:775.
- Ashton-Rickardt, P. G., Bandeira, A., Delaney, J. R. *et al.* 1994. Evidence for a differential avidity model of T cell selection in the thymus. *Cell* 76:651.
- Sebzda, E., Wallace, V. A., Mayer, J., Yeung, R. S., Mak, T. W. and Ohashi, P. S. 1994. Positive and negative thymocyte selection induced by different concentrations of a single peptide. *Science* 263:1615.
- Takahama, Y., Suzuki, H., Katz, K. S., Grusby, M. J. and Singer, A. 1994. Positive selection of CD4⁺ T cells by TCR ligation without aggregation even in the absence of MHC. *Nature* 371:67.
- Hogquist, K. A., Jameson, S. C. and Bevan, M. J. 1995. Strong agonist ligands for the T cell receptor do not mediate positive selection of functional CD8⁺ T cells. *Immunity* 3:79.
- von Boehmer, H. and Melchers, F. 2010. Checkpoints in lymphocyte development and autoimmune disease. *Nat. Immunol.* 11:14.
- Stritesky, G. L., Jameson, S. C. and Hogquist, K. A. 2012. Selection of self-reactive T cells in the thymus. *Annu. Rev. Immunol.* 30:95.
- Alam, S. M., Travers, P. J., Wung, J. L. *et al.* 1996. T-cell-receptor affinity and thymocyte positive selection. *Nature* 381:616.
- Daniels, M. A., Teixeira, E., Gill, J. *et al.* 2006. Thymic selection threshold defined by compartmentalization of Ras/MAPK signaling. *Nature* 444:724.
- Palmer, E. and Naeher, D. 2009. Affinity threshold for thymic selection through a T-cell receptor-co-receptor zipper. *Nat. Rev. Immunol.* 9:207.
- Fu, G., Casas, J., Rigaud, S. *et al.* 2013. Themis sets the signal threshold for positive and negative selection in T-cell development. *Nature* 504:441.
- Choi, S., Warzecha, C., Zvezdova, E. *et al.* 2017. THEMIS enhances TCR signaling and enables positive selection by selective inhibition of the phosphatase SHP-1. *Nat. Immunol.* 18:433.
- Wang, D., Zheng, M., Lei, L. *et al.* 2012. Tespa1 is involved in late thymocyte development through the regulation of TCR-mediated signaling. *Nat. Immunol.* 13:560.
- Lo, W. L., Donermeyer, D. L. and Allen, P. M. 2012. A voltage-gated sodium channel is essential for the positive selection of CD4(+) T cells. *Nat. Immunol.* 13:880.
- Li, Q. J., Chau, J., Ebert, P. J. *et al.* 2007. miR-181a is an intrinsic modulator of T cell sensitivity and selection. *Cell* 129:147.
- Mandl, J. N., Monteiro, J. P., Vrisekoop, N. and Germain, R. N. 2013. T cell-positive selection uses self-ligand binding strength to optimize repertoire recognition of foreign antigens. *Immunity* 38:263.
- Persaud, S. P., Parker, C. R., Lo, W. L., Weber, K. S. and Allen, P. M. 2014. Intrinsic CD4⁺ T cell sensitivity and response to a pathogen are set and sustained by avidity for thymic and peripheral complexes of self peptide and MHC. *Nat. Immunol.* 15:266.
- Fulton, R. B., Hamilton, S. E., Xing, Y. *et al.* 2015. The TCR's sensitivity to self peptide-MHC dictates the ability of naive CD8(+) T cells to respond to foreign antigens. *Nat. Immunol.* 16:107.
- Takada, K., Van Laethem, F., Xing, Y. *et al.* 2015. TCR affinity for thymoproteasome-dependent positively selecting peptides conditions antigen responsiveness in CD8(+) T cells. *Nat. Immunol.* 16:1069.
- Kondo, K., Takada, K. and Takahama, Y. 2017. Antigen processing and presentation in the thymus: implications for T cell repertoire selection. *Curr. Opin. Immunol.* 46:53.
- Smith, N. L., Patel, R. K., Reynaldi, A. *et al.* 2018. Developmental origin governs CD8⁺ T cell fate decisions during infection. *Cell* 174:117.e14.
- Dong, M., Artusa, P., Kelly, S. A. *et al.* 2017. Alterations in the thymic selection threshold skew the self-reactivity of the TCR repertoire in neonates. *J. Immunol.* 199:965.
- Mohtashami, M., Shah, D. K., Nakase, H., Kianizad, K., Petrie, H. T. and Zúñiga-Pflücker, J. C. 2010. Direct comparison of Dll1- and Dll4-mediated Notch activation levels shows differential lymphoid lineage commitment outcomes. *J. Immunol.* 185:867.
- Shitara, S., Hara, T., Liang, B. *et al.* 2013. IL-7 produced by thymic epithelial cells plays a major role in the development of thymocytes and TCR $\gamma\delta$ intraepithelial lymphocytes. *J. Immunol.* 190:6173.
- Anderson, G. and Takahama, Y. 2012. Thymic epithelial cells: working class heroes for T cell development and repertoire selection. *Trends Immunol.* 33:256.

- 35 Nakagawa, Y., Ohigashi, I., Nitta, T. *et al.* 2012. Thymic nurse cells provide microenvironment for secondary T cell receptor α rearrangement in cortical thymocytes. *Proc. Natl Acad. Sci. USA* 109:20572.
- 36 Takahama, Y., Ohigashi, I., Baik, S. and Anderson, G. 2017. Generation of diversity in thymic epithelial cells. *Nat. Rev. Immunol.* 17:295.
- 37 Nakagawa, T., Roth, W., Wong, P. *et al.* 1998. Cathepsin L: critical role in li degradation and CD4 T cell selection in the thymus. *Science* 280:450.
- 38 Honey, K., Nakagawa, T., Peters, C. and Rudensky, A. 2002. 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.
- 39 Takada, K. and Takahama, Y. 2015. Positive-selection-inducing self-peptides displayed by cortical thymic epithelial cells. *Adv. Immunol.* 125:87.
- 40 Gommeaux, J., Grégoire, C., Nguessan, P. *et al.* 2009. Thymus-specific serine protease regulates positive selection of a subset of CD4⁺ thymocytes. *Eur. J. Immunol.* 39:956.
- 41 Viret, C., Lamare, C., Guiraud, M. *et al.* 2011. Thymus-specific serine protease contributes to the diversification of the functional endogenous CD4 T cell receptor repertoire. *J. Exp. Med.* 208:3.
- 42 Guerder, S., Hassel, C. and Carrier, A. 2018. Thymus-specific serine protease, a protease that shapes the CD4 T cell repertoire. *Immunogenetics*. In press. doi:10.1007/s00251-018-1078-y
- 43 Viret, C., Leung-Theung-Long, S., Serre, L. *et al.* 2011. Thymus-specific serine protease limits autoreactive CD4 T cell development and autoimmune diabetes in mice. *J. Clin. Invest.* 121:1810.
- 44 Viret, C., Mahiddine, K., Baker, R. L., Haskins, K. and Guerder, S. 2015. The T cell repertoire-diversifying enzyme TSSP contributes to thymic selection of diabetogenic CD4 T cell specificities reactive to ChgA and IAPP autoantigens. *J. Immunol.* 195:1964.
- 45 Serre, L., Girard, M., Ramadan, A. *et al.* 2017. Thymic-specific serine protease limits central tolerance and exacerbates experimental autoimmune encephalomyelitis. *J. Immunol.* 199:3748.
- 46 Kuma, A., Hatano, M., Matsui, M. *et al.* 2004. The role of autophagy during the early neonatal starvation period. *Nature* 432:1032.
- 47 Nedjic, J., Aichinger, M., Emmerich, J., Mizushima, N. and Klein, L. 2008. Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance. *Nature* 455:396.
- 48 Aichinger, M., Wu, C., Nedjic, J. and Klein, L. 2013. Macroautophagy substrates are loaded onto MHC class II of medullary thymic epithelial cells for central tolerance. *J. Exp. Med.* 210:287.
- 49 von Rohrscheidt, J., Petrozziello, E., Nedjic, J. *et al.* 2016. Thymic CD4 T cell selection requires attenuation of March8-mediated MHCII turnover in cortical epithelial cells through CD83. *J. Exp. Med.* 213:1685.
- 50 Liu, H., Jain, R., Guan, J. *et al.* 2016. Ubiquitin ligase MARCH 8 cooperates with CD83 to control surface MHC II expression in thymic epithelium and CD4 T cell selection. *J. Exp. Med.* 213:1695.
- 51 Fujimoto, Y., Tu, L., Miller, A. S. *et al.* 2002. CD83 expression influences CD4⁺ T cell development in the thymus. *Cell* 108:755.
- 52 Brugnera, E., Bhandoola, A., Cibotti, R. *et al.* 2000. Coreceptor reversal in the thymus: signaled CD4⁺8⁺ thymocytes initially terminate CD8 transcription even when differentiating into CD8⁺ T cells. *Immunity* 13:59.
- 53 Murata, S., Sasaki, K., Kishimoto, T. *et al.* 2007. Regulation of CD8⁺ T cell development by thymus-specific proteasomes. *Science* 316:1349.
- 54 Murata, S., Takahama, Y., Kasahara, M. and Tanaka, K. 2018. The immunoproteasome and thymoproteasome: functions, evolution and human disease. *Nat. Immunol.* 19:923.
- 55 Ripen, A. M., Nitta, T., Murata, S., Tanaka, K. and Takahama, Y. 2011. Ontogeny of thymic cortical epithelial cells expressing the thymoproteasome subunit $\beta 5t$. *Eur. J. Immunol.* 41:1278.
- 56 Ohigashi, I., Zuklys, S., Sakata, M. *et al.* 2013. Aire-expressing thymic medullary epithelial cells originate from $\beta 5t$ -expressing progenitor cells. *Proc. Natl Acad. Sci. USA* 110:9885.
- 57 Sasaki, K., Takada, K., Ohte, Y. *et al.* 2015. Thymoproteasomes produce unique peptide motifs for positive selection of CD8(+) T cells. *Nat. Commun.* 6:7484.
- 58 Florea, B. I., Verdoes, M., Li, N. *et al.* 2010. Activity-based profiling reveals reactivity of the murine thymoproteasome-specific subunit $\beta 5t$. *Chem. Biol.* 17:795.
- 59 Nitta, T., Murata, S., Sasaki, K. *et al.* 2010. Thymoproteasome shapes immunocompetent repertoire of CD8⁺ T cells. *Immunity* 32:29.
- 60 Xing, Y., Jameson, S. C. and Hogquist, K. A. 2013. Thymoproteasome subunit- $\beta 5t$ generates peptide-MHC complexes specialized for positive selection. *Proc. Natl Acad. Sci. USA* 110:6979.
- 61 Takada, K., Kondo, K. and Takahama, Y. 2017. Generation of peptides that promote positive selection in the thymus. *J. Immunol.* 198:2215.
- 62 Murata, S., Takahama, Y. and Tanaka, K. 2008. Thymoproteasome: probable role in generating positively selecting peptides. *Curr. Opin. Immunol.* 20:192.
- 63 Takahama, Y., Takada, K., Murata, S. and Tanaka, K. 2012. $\beta 5t$ -containing thymoproteasome: specific expression in thymic cortical epithelial cells and role in positive selection of CD8⁺ T cells. *Curr. Opin. Immunol.* 24:92.
- 64 Takahama, Y., Ohigashi, I., Murata, S. and Tanaka, K. 2018. Thymoproteasome and peptidic self. *Immunogenetics*. In press. doi:10.1007/s00251-018-1081-3
- 65 Tomaru, U., Ishizu, A., Murata, S. *et al.* 2009. Exclusive expression of proteasome subunit $\beta 5t$ in the human thymic cortex. *Blood* 113:5186.
- 66 Ohigashi, I., Ohte, Y., Setoh, K. *et al.* 2017. A human *PSMB11* variant affects thymoproteasome processing and CD8⁺ T cell production. *JCI Insight* 2:e93664.
- 67 Nitta, T., Kochi, Y., Muro, R. *et al.* 2017. Human thymoproteasome variations influence CD8 T cell selection. *Sci Immunol.* 2:eaan5165.
- 68 Ueno, T., Saito, F., Gray, D. H. *et al.* 2004. CCR7 signals are essential for cortex-medulla migration of developing thymocytes. *J. Exp. Med.* 200:493.
- 69 Kwan, J. and Killeen, N. 2004. CCR7 directs the migration of thymocytes into the thymic medulla. *J. Immunol.* 172:3999.
- 70 Sansom, S. N., Shikama-Dorn, N., Zhanybekova, S. *et al.* 2014. Population and single-cell genomics reveal the Aire dependency, relief from Polycomb silencing, and distribution of self-antigen expression in thymic epithelia. *Genome Res.* 24:1918.
- 71 Koble, C. and Kyewski, B. 2009. The thymic medulla: a unique microenvironment for intercellular self-antigen transfer. *J. Exp. Med.* 206:1505.
- 72 Danan-Gothold, M., Guyon, C., Giraud, M., Levanon, E. Y. and Abramson, J. 2016. Extensive RNA editing and splicing increase immune self-representation diversity in medullary thymic epithelial cells. *Genome Biol.* 17:219.
- 73 Abramson, J. and Anderson, G. 2017. Thymic epithelial cells. *Annu. Rev. Immunol.* 35:85.
- 74 Anderson, M. S., Venanzi, E. S., Klein, L. *et al.* 2002. Projection of an immunological self shadow within the thymus by the aire protein. *Science* 298:1395.
- 75 Org, T., Rebane, A., Kisand, K. *et al.* 2009. AIRE activated tissue specific genes have histone modifications associated with inactive chromatin. *Hum. Mol. Genet.* 18:4699.
- 76 Abramson, J., Giraud, M., Benoist, C. and Mathis, D. 2010. Aire's partners in the molecular control of immunological tolerance. *Cell* 140:123.
- 77 Koh, A. S., Kingston, R. E., Benoist, C. and Mathis, D. 2010. Global relevance of Aire binding to hypomethylated lysine-4 of histone-3. *Proc. Natl Acad. Sci. USA* 107:13016.
- 78 Meredith, M., Zemmour, D., Mathis, D. and Benoist, C. 2015. Aire controls gene expression in the thymic epithelium with ordered stochasticity. *Nat. Immunol.* 16:942.
- 79 Giraud, M., Yoshida, H., Abramson, J. *et al.* 2012. Aire unleashes stalled RNA polymerase to induce ectopic gene expression in thymic epithelial cells. *Proc. Natl Acad. Sci. USA* 109:535.
- 80 Koh, A. S., Miller, E. L., Buenrostro, J. D. *et al.* 2018. Rapid chromatin repression by Aire provides precise control of immune tolerance. *Nat. Immunol.* 19:162.

- 81 Boehm, T., Scheu, S., Pfeffer, K. and Bleul, C. C. 2003. Thymic medullary epithelial cell differentiation, thymocyte emigration, and the control of autoimmunity require lympho-epithelial cross talk via LTbetaR. *J. Exp. Med.* 198:757.
- 82 Rossi, S. W., Kim, M. Y., Leibbrandt, A. *et al.* 2007. RANK signals from CD4(+)J3(-) inducer cells regulate development of Aire-expressing epithelial cells in the thymic medulla. *J. Exp. Med.* 204:1267.
- 83 Akiyama, T., Shimo, Y., Yanai, H. *et al.* 2008. The tumor necrosis factor family receptors RANK and CD40 cooperatively establish the thymic medullary microenvironment and self-tolerance. *Immunity* 29:423.
- 84 Hikosaka, Y., Nitta, T., Ohigashi, I. *et al.* 2008. The cytokine RANKL produced by positively selected thymocytes fosters medullary thymic epithelial cells that express autoimmune regulator. *Immunity* 29:438.
- 85 White, A. J., Nakamura, K., Jenkinson, W. E. *et al.* 2010. Lymphotoxin signals from positively selected thymocytes regulate the terminal differentiation of medullary thymic epithelial cells. *J. Immunol.* 185:4769.
- 86 Roberts, N. A., White, A. J., Jenkinson, W. E. *et al.* 2012. Rank signaling links the development of invariant $\gamma\delta$ T cell progenitors and Aire(+) medullary epithelium. *Immunity* 36:427.
- 87 Burkly, L., Hession, C., Ogata, L. *et al.* 1995. Expression of relB is required for the development of thymic medulla and dendritic cells. *Nature* 373:531.
- 88 Weih, F., Carrasco, D., Durham, S. K. *et al.* 1995. Multiorgan inflammation and hematopoietic abnormalities in mice with a targeted disruption of RelB, a member of the NF-kappa B/Rel family. *Cell* 80:331.
- 89 Zhu, M., Chin, R. K., Christiansen, P. A. *et al.* 2006. NF-kappaB2 is required for the establishment of central tolerance through an Aire-dependent pathway. *J. Clin. Invest.* 116:2964.
- 90 Takaba, H., Morishita, Y., Tomofuji, Y. *et al.* 2015. Fezf2 orchestrates a thymic program of self-antigen expression for immune tolerance. *Cell* 163:975.
- 91 Roberts, N. A., Adams, B. D., McCarthy, N. I. *et al.* 2017. Prdm1 regulates thymic epithelial function to prevent autoimmunity. *J. Immunol.* 199:1250.
- 92 Cosway, E. J., Lucas, B., James, K. D. *et al.* 2017. Redefining thymus medulla specialization for central tolerance. *J. Exp. Med.* 214:3183.
- 93 Gallegos, A. M. and Bevan, M. J. 2004. Central tolerance to tissue-specific antigens mediated by direct and indirect antigen presentation. *J. Exp. Med.* 200:1039.
- 94 Hubert, F. X., Kinkel, S. A., Davey, G. M. *et al.* 2011. Aire regulates the transfer of antigen from mTECs to dendritic cells for induction of thymic tolerance. *Blood* 118:2462.
- 95 Perera, J., Meng, L., Meng, F. and Huang, H. 2013. Autoreactive thymic B cells are efficient antigen-presenting cells of cognate self-antigens for T cell negative selection. *Proc. Natl Acad. Sci. USA* 110:17011.
- 96 Yamano, T., Nedjic, J., Hinterberger, M. *et al.* 2015. Thymic B cells are licensed to present self antigens for central T cell tolerance induction. *Immunity* 42:1048.
- 97 Perry, J. S. and Hsieh, C. S. 2016. Development of T-cell tolerance utilizes both cell-autonomous and cooperative presentation of self-antigen. *Immunol. Rev.* 271:141.
- 98 Shortman, K., Vremec, D., Corcoran, L. M., Georgopoulos, K., Lucas, K. and Wu, L. 1998. The linkage between T-cell and dendritic cell development in the mouse thymus. *Immunol. Rev.* 165:39.
- 99 Wu, L. and Shortman, K. 2005. Heterogeneity of thymic dendritic cells. *Semin. Immunol.* 17:304.
- 100 Bonasio, R., Scimone, M. L., Schaeferli, P., Grabie, N., Lichtman, A. H. and von Andrian, U. H. 2006. Clonal deletion of thymocytes by circulating dendritic cells homing to the thymus. *Nat. Immunol.* 7:1092.
- 101 Perry, J. S. A., Lio, C. J., Kau, A. L. *et al.* 2014. Distinct contributions of Aire and antigen-presenting-cell subsets to the generation of self-tolerance in the thymus. *Immunity* 41:414.
- 102 Perry, J. S. A., Russler-Germain, E. V., Zhou, Y. W. *et al.* 2018. CD36 mediates cell-surface antigens to promote thymic development of the regulatory T cell receptor repertoire and allo-tolerance. *Immunity* 48:923.e4.
- 103 Lkhagvasuren, E., Sakata, M., Ohigashi, I. and Takahama, Y. 2013. Lymphotoxin β receptor regulates the development of CCL21-expressing subset of postnatal medullary thymic epithelial cells. *J. Immunol.* 190:5110.
- 104 Cosway, E. J., Ohigashi, I., Schauble, K. *et al.* 2018. Formation of the intrathymic dendritic cell pool requires CCL21-mediated recruitment of CCR7⁺ progenitors to the thymus. *J. Immunol.* 201:516.
- 105 Lei, Y., Ripen, A. M., Ishimaru, N. *et al.* 2011. Aire-dependent production of XCL1 mediates medullary accumulation of thymic dendritic cells and contributes to regulatory T cell development. *J. Exp. Med.* 208:383.
- 106 Farr, A., Nelson, A., Hosier, S. and Kim, A. 1993. A novel cytokine-responsive cell surface glycoprotein defines a subset of medullary thymic epithelium *in situ*. *J. Immunol.* 150:1160.
- 107 Farr, A. G. and Rudensky, A. 1998. Medullary thymic epithelium: a mosaic of epithelial "self"? *J. Exp. Med.* 188:1.
- 108 Panneck, A. R., Rafiq, A., Schütz, B. *et al.* 2014. Cholinergic epithelial cell with chemosensory traits in murine thymic medulla. *Cell Tissue Res.* 358:737.
- 109 Miller, C. N., Proekt, I., von Moltke, J. *et al.* 2018. Thymic tuft cells promote an IL-4-enriched medulla and shape thymocyte development. *Nature* 559:627.
- 110 Bornstein, C., Nevo, S., Giladi, A. *et al.* 2018. Single-cell mapping of the thymic stroma identifies IL-25-producing tuft epithelial cells. *Nature* 559:622.
- 111 Kurobe, H., Liu, C., Ueno, T. *et al.* 2006. CCR7-dependent cortex-to-medulla migration of positively selected thymocytes is essential for establishing central tolerance. *Immunity* 24:165.
- 112 Nitta, T., Nitta, S., Lei, Y., Lipp, M. and Takahama, Y. 2009. CCR7-mediated migration of developing thymocytes to the medulla is essential for negative selection to tissue-restricted antigens. *Proc. Natl Acad. Sci. USA* 106:17129.
- 113 Kozai, M., Kubo, Y., Kataikai, T. *et al.* 2017. Essential role of CCL21 in establishment of central self-tolerance in T cells. *J. Exp. Med.* 214:1925.
- 114 Suzuki, G., Sawa, H., Kobayashi, Y. *et al.* 1999. Pertussis toxin-sensitive signal controls the trafficking of thymocytes across the corticomedullary junction in the thymus. *J. Immunol.* 162:5981.
- 115 Suzuki, G., Nakata, Y., Dan, Y. *et al.* 1998. Loss of SDF-1 receptor expression during positive selection in the thymus. *Int. Immunol.* 10:1049.
- 116 Plotkin, J., Prockop, S. E., Lepique, A. and Petrie, H. T. 2003. Critical role for CXCR4 signaling in progenitor localization and T cell differentiation in the postnatal thymus. *J. Immunol.* 171:4521.
- 117 Choi, Y. I., Duke-Cohan, J. S., Ahmed, W. B. *et al.* 2008. PlexinD1 glycoprotein controls migration of positively selected thymocytes into the medulla. *Immunity* 29:888.
- 118 Hu, Z., Lancaster, J. N., Sasipongpanan, C. and Ehrlich, L. I. 2015. CCR4 promotes medullary entry and thymocyte-dendritic cell interactions required for central tolerance. *J. Exp. Med.* 212:1947.
- 119 Cowan, J. E., McCarthy, N. I., Parnell, S. M. *et al.* 2014. Differential requirement for CCR4 and CCR7 during the development of innate and adaptive $\alpha\beta$ T cells in the adult thymus. *J. Immunol.* 193:1204.
- 120 Matloubian, M., Lo, C. G., Cinamon, G. *et al.* 2004. Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. *Nature* 427:355.
- 121 Ueno, T., Hara, K., Willis, M. S. *et al.* 2002. Role for CCR7 ligands in the emigration of newly generated T lymphocytes from the neonatal thymus. *Immunity* 16:205.
- 122 Wang, H. and Hogquist, K. A. 2018. CCR7 defines a precursor for murine iNKT cells in thymus and periphery. *ELife* 7: e34793.
- 123 White, A. J., Baik, S., Parnell, S. M. *et al.* 2017. A type 2 cytokine axis for thymus emigration. *J. Exp. Med.* 214:2205.
- 124 Sakata, M., Ohigashi, I. and Takahama, Y. 2018. Cellularity of thymic epithelial cells in the postnatal mouse. *J. Immunol.* 200:1382.
- 125 Hirakawa, M., Nagakubo, D., Kanzler, B. *et al.* 2018. Fundamental parameters of the developing thymic epithelium in the mouse. *Sci. Rep.* 8:11095.