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Review article

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A guide to thymic selection of T cells

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Abstract

The thymus is an evolutionarily conserved organ that supports the development of T cells. Not only does the thymic environment support the rearrangement and expression of diverse T cell receptors but also provides a unique niche for the selection of appropriate T cell clones. Thymic selection ensures that the repertoire of available T cells is both useful (being MHC-restricted) and safe (being self-tolerant). The unique antigen-presentation features of the thymus ensure that the display of self-antigens is optimal to induce tolerance to all types of self-tissue. MHC class-specific functions of $CD4^+$ T helper cells, $CD8^+$ killer T cells and $CD4^+$ regulatory T cells are also established in the thymus. Finally, the thymus provides signals for the development of several minor T cell subsets that promote immune and tissue homeostasis. This Review provides an introductory-level overview of our current understanding of the sophisticated thymic selection mechanisms that ensure T cells are useful and safe.

Sections

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Introduction

An early mystery of immunology concerned how the immune system could produce immune responses (particularly antibodies) with exquisite specificity for a given pathogen. Was this specificity generated in response to infection? Or did immune cells harbour the ability to produce pathogen-specific antibodies before infection? And if the latter were true, how was specificity to the wide variety of potential pathogens generated in the absence of any microbial 'template'? In 1956, Frank Macfarlane Burnet[1] proposed that immune cells might undergo a process of 'randomization' during their development, generating a diverse repertoire of cells, each with unique pathogen specificities before infection (Fig. 1a). Furthermore, Burnet predicted that in response to infection, the rare immune cells with specificity for the invading pathogen would produce daughter cells capable of secreting pathogen-specific antibodies relevant to the infection. But Burnet's theory had an important caveat — if such a process of randomization did occur, it would require a selection process to eliminate cells of unwanted specificities, such as those that recognize self-antigens[1].

Burnet's 'clonal selection theory' turned out to be strikingly accurate for B cells. It did not, however, anticipate all of the requirements for generating a safe and functional repertoire of T cells. This is because, unlike B cell receptors that recognize antigen directly, T cell receptors (TCRs) recognize a combinatorial ligand composed of an MHC molecule 'presenting' a small peptide (referred to as peptide–MHC (pMHC)) (Fig. 1b). This is known as MHC restriction. Furthermore, because MHC molecules are highly polymorphic, each individual needs to generate TCRs that recognize their own unique MHC molecules (Fig. 1c). Thus, in addition to the elimination of self-reactive cells (known as negative selection), T cells require a positive selection process to ensure that they recognize the MHC molecules expressed by that individual. This may partly explain why T cells, unlike B cells, have their own dedicated organ for development and selection.

The thymus is an organ specialized to support the development of T cells. Although all species that have T cells have a thymus[2], the thymus was the last major organ to be understood in functional terms. It was described first by the physician Galen in the second century[3], but it was not until the pioneering work of Jacques Miller and Bob Good in the 1950s, who studied thymectomized mice and children with thymic defects, that the thymus was understood as the site of T cell development[4,5] (Fig. 2). After MHC restriction was discovered by Rolf Zinkernagel and Peter Doherty in 1974 (ref. 6), the pioneering work of Mike Bevan showed that in order for T cells to react to antigen-presenting cells (APCs), the APCs had to be of the identical MHC type to that present in the thymus[7], thus introducing the concept that the thymus positively selects for MHC-restricted T cells. Confirmation of the process of positive selection was obtained when the first TCR-transgenic mice were created in the 1980s (Box 1). The group of Harald von Boehmer, for example, showed that thymocytes expressing TCRs that recognized a foreign antigen presented by the MHC class I (MHC-I) molecule H2-D^b developed to a mature stage in the thymus only when H2-D^b was present[8]. Around the same time, other work showed that self-reactive T cell clones were eliminated (negatively selected) in the thymus[9,10], and this was also confirmed by studies of TCR-transgenic mice. For example, T cell progenitors (thymocytes) expressing TCRs that recognized a self-antigen presented by H2-D^b were eliminated when both H2-D^b and the self-antigen were present in the thymus[11]. Over the following 30 years, there has been an explosion in our understanding of the molecular mechanisms of positive and negative selection (collectively, thymic selection). In particular, the field has begun to uncover the complex roles of TCR signalling and the transcription factors autoimmune regulator (AIRE)[12] and forkhead box protein 3 (FOXP3)[13] in this process, as we discuss here.

Stages of T cell selection

The stages of T cell development in the thymus are typically distinguished by expression of the surface co-receptor molecules CD4 and CD8 (Fig. 3), progressing from double-negative (DN) cells to double-positive (DP) cells to single-positive (SP) cells (CD4$^+$ or CD8$^+$). These stages of development occur in distinct locations of the thymus and require the movement of thymocytes between the outer thymus cortex and inner thymus medulla[14] (Fig. 4). The cortex and medulla are distinguished primarily by distinct populations of epithelial cells known as cortical thymic epithelial cells (cTECs) and medullary thymic epithelial cells (mTECs)[15]. TECs are uniquely equipped to support the processes of positive and negative selection by presenting distinct self-antigens to developing thymocytes as they migrate between the cortex and medulla. cTECs and mTECs also express distinct chemokines that guide thymocyte migration between these zones[14].

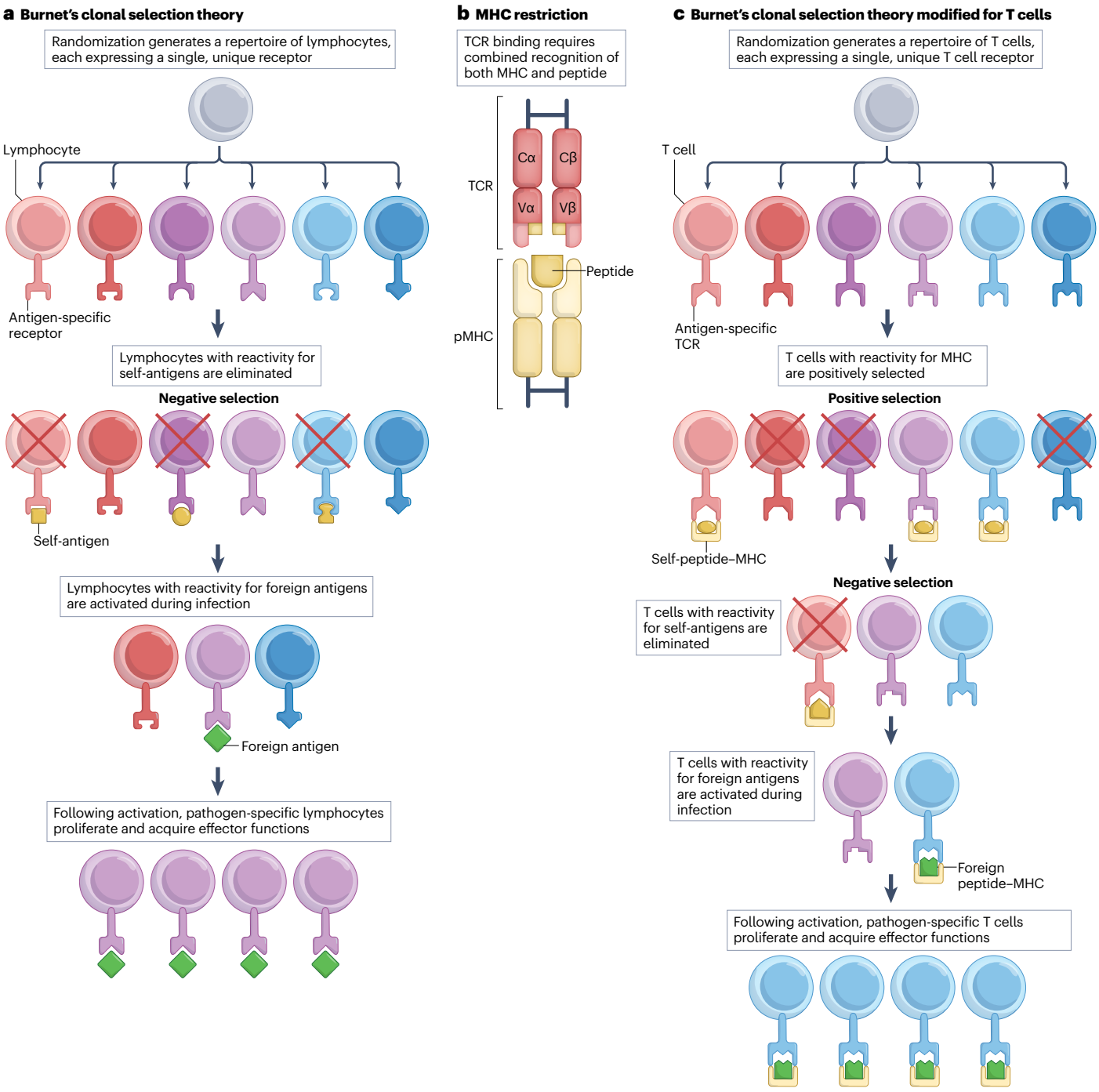
Early development

T cells develop from the same bone-marrow-derived haematopoietic stem cells that give rise to B cells. T cell development is initiated when these multipotent progenitor cells from the bone marrow enter the thymus through blood vessels at the cortical–medullary junction (CMJ) and encounter Notch ligands that drive commitment to a T cell fate[16] (Fig. 4). Thymic entry at the CMJ is facilitated by several

Fig. 1 | T cell development requires positive selection. a, The 'clonal selection theory' first proposed by Frank Macfarlane Burnet in 1956 predicts that each lymphocyte expresses a single, unique, antigen-specific receptor. Burnet further hypothesized that these unique receptors are generated by a process of randomization during lymphocyte development, resulting in a repertoire of cells expressing receptors with different antigen specificities. Following this 'randomization' process, lymphocytes are subject to a negative selection process to eliminate cells of unwanted specificity, such as those that recognize self-antigens. Upon infection, cells with specificity for the pathogen are activated through ligation of their randomly generated receptor. This activation drives proliferation and acquisition of effector functions, such as the production of pathogen-specific antibodies. For the most part, Burnet's theory accurately describes B cell development, during which B cell progenitors undergo V(D)J recombination to generate unique B cell receptors and are subject to negative selection (apoptosis) dependent on reactivity for self-antigens. However, T cells require an additional selection step in their development, owing to their MHC restriction, that was not appreciated until later. **b**, A schematic of a T cell receptor (TCR) interacting with a combinatorial ligand of peptide bound to MHC (pMHC). Unlike B cell receptors, TCRs require combined recognition of peptide and MHC for activation, known as MHC restriction. This is facilitated by physical contacts between the outward-facing antigen-recognition site of the TCR and both peptide and MHC. **c**, A modified version of Burnet's theory, adapted on the basis of what we now know of T cell development. As MHC molecules are highly polymorphic, T cell development requires an additional positive selection step to ensure that T cells recognize the MHC molecules expressed by that individual. Positive selection of T cells is followed by negative selection, which, similar to the negative selection of B cells, eliminates cells with reactivity for self-antigens.

key receptor–ligand interactions; P-selectin glycoprotein ligand 1 (PSGL1) expressed by haematopoietic progenitors binds P-selectin expressed on endothelial cells, and the chemokine receptors CCR9 and CCR7 expressed by haematopoietic progenitors recognize the chemokines CCL25 and CCL19 or CCL21, respectively, produced by the thymic stroma[17]. Following entry at the CMJ, progenitor cells upregulate expression of chemokine receptor CXCR4, resulting in their retention in the cortex where the CXCR4 ligand CXCL12 is expressed by cTECs.

T cell-committed progenitors undergo early stages of development in the cortex as DN thymocytes[16] and begin to rearrange the genes of the TCR locus[18] to generate a unique TCR (Fig. 3). TCRs are heterodimeric receptors, thus requiring the separate rearrangement of two distinct TCR chains. Thymocytes generate either a γδ TCR or an αβ TCR depending on which TCR genes randomly recombine during development. The genes that generate a γδ TCR complete rearrangement at the DN stage, resulting in the selection of mature γδ T cells (Fig. 3).



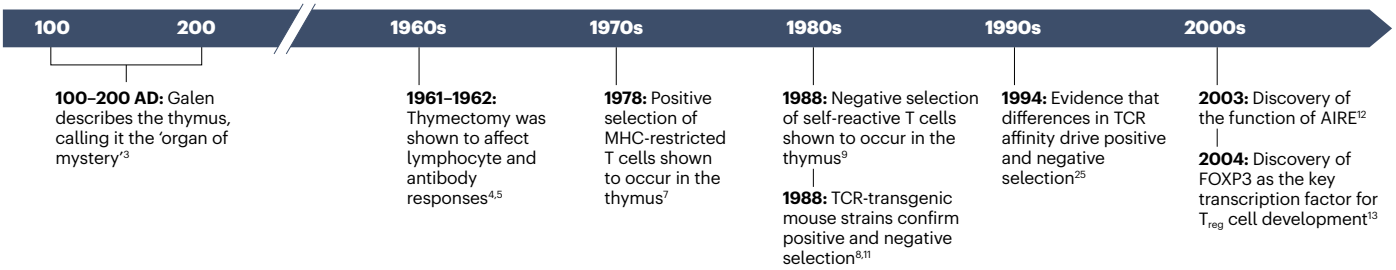


Fig. 2 | A timeline of major discoveries concerning T cell selection in the thymus. AIRE, autoimmune regulator; FOXP3, forkhead box protein 3; TCR, T cell receptor; T_{reg} cell, regulatory T cell.

Although the selection of γδ T cells is interesting and complex[19], this Review focuses on the more abundant αβ T cells, which develop from progenitors that rearrange an intact TCRβ chain during the DN stage. TCRβ rearrangement is followed by a developmental step known as β-selection, which selects for cells that have rearranged a functional TCRβ chain and excludes those cells with out-of-frame rearrangements or rearrangements with premature stop codons. Thymocytes that survive β-selection upregulate CD4 and CD8 co-receptors, becoming DP thymocytes. At this stage, the TCRα locus becomes available for recombination, and the recombination process continues until the thymocyte expresses a functional αβ TCR (Fig. 3).

Thymic selection

Once a rearranged αβ TCR is expressed at the cell surface, all subsequent development events are exquisitely dependent on the specificity of that TCR and the strength of TCR signalling that results from interactions with self-ligands in the thymus. A crucial step is positive selection, which occurs in the thymus cortex (Figs. 3 and 4). Positive selection functions to select T cells that recognize the MHC molecules of that individual. Positive selection also initiates lineage commitment, through which thymocytes are assigned to either the $CD4^+$ T helper cell or $CD8^+$ killer T cell lineages. Upon successful positive selection, $CD4^+$ or $CD8^+$ SP thymocytes upregulate expression of CCR4 and CCR7, which facilitates their migration towards CCL17 and/or CCL22 and CCL19 and/or CCL21 on mTECs, respectively, and entry into the medulla[20,21] (Fig. 4). The medulla is a crucial region for negative selection, which functions to remove or control self-reactive T cells. During negative selection, T cells that recognize self-antigens either undergo programmed cell death (clonal deletion) or are diverted into the immunosuppressive regulatory T cell (T_{reg} cell) lineage (Fig. 3). Fully mature thymocytes that have survived both positive selection and negative selection upregulate homing molecules and exit the thymus at the CMJ[22].

Thymic selection ensures that the unique TCRs expressed by the mature T cells that exit the thymus are functional and safe for the host. Importantly, the thymus selects for self-reactive T cells as a part of this process. This can be observed in *Nur77*^{GFP} reporter mice (Box 1), in which a clear accumulation of TCR-signalled cells is seen in the thymus medulla as a result of positive selection. It is therefore important to ensure that positively selected T cells are not self-reactive in a dangerous way. This is achieved through the elimination of the most dangerous self-reactive clones from the conventional T cell repertoire by negative selection.

The result of thymic selection is the generation and export of two major T cell subsets — $CD8^+$ killer T cells and $CD4^+$ T helper cells — and several minor T cell subsets (γδ T cells, invariant natural killer T cells (iNKT cells), mucosal associated invariant T cells (MAIT cells), intraepithelial lymphocytes (IELs) and T_{reg} cells), discussed later. These T cell lineages have distinct and complementary functions, arming the immune system with an array of T cells specialized for infection control, immune regulation and tissue homeostasis.

Selecting T cells that are functional

Two hallmark properties of T cells are established by positive selection in the thymus. The first is MHC restriction and the second is lineage-specific effector function (helper cells versus killer cells).

Positive selection

Positive selection ensues when a TCR on a DP thymocyte interacts with any of the MHC complexes expressed by cTECs[23]. This survival step is essential to enrich for the (approximately 5% of) thymocyte clones that express TCRs that can recognize antigens in the context of MHC molecules of that individual — these are the useful or 'MHC-restricted' clones[24]. An entirely different set of clones will be selected in another individual, who has a different set of MHC molecules. A DP thymocyte is programmed to die within 3–4 days if it is not triggered by interaction with self-pMHC, a process referred to as 'death by neglect'. Each thymocyte can audition for positive selection with several different TCRs, owing to the unique architecture of the TCRα gene locus, which contains multiple variable (V) and joining (J) gene segments[18]. A thymocyte can edit out 'useless', non-MHC-restricted TCRα VJ genes and express a new TCR using different V and J segments from the TCRα locus in a process referred to as 'processive rearrangement'[18]. This has to occur within the 3–4-day window to rescue a thymocyte from death by neglect.

Distinguishing positive and negative selection

DP thymocytes are also subject to negative selection and can undergo programmed cell death following TCR signalling, as discussed in detail in the next section. Thus, an important question in the field has been how some TCR signals lead to thymocyte survival and others lead to thymocyte death. It is now clear that several mechanisms are involved. A major factor is that the TCR can respond differently to ligands of low affinity versus high affinity. Low-affinity interactions tend to favour positive selection, whereas high-affinity interactions tend to favour negative selection[23]. The affinity model of thymic selection (Fig. 5a) suggests that thymocytes need a 'just right' intermediate signal to avoid death by neglect (to be positively selected) and death by clonal deletion (not to be negatively selected). One of the early experiments to demonstrate this concept used fetal thymic organ culture from TCR-transgenic mice expressing a single TCR of known specificity

(Box 1). When the 'cognate' peptide (the peptide known to be recognized by the transgenic TCR) was added to the fetal thymic organ culture, clonal deletion ensued, but when slight variants of that peptide were added, positive selection ensued[25]. Importantly, these peptide variants were shown to have decreased affinity[26] of pMHC–TCR binding compared with the cognate peptide, which supports the idea that weaker affinity interactions favour positive selection. Another factor to consider is signalling through the costimulatory molecule CD28 on thymocytes, which enhances TCR signalling. In vitro and in vivo models have shown that CD28 is not required for positive selection but enhances negative selection[23,27–29]. However, CD28 costimulation is required for the selection of T_{reg} cells[30,31] and iNKT cells[32] (discussed later), highlighting its complex, and not fully understood, role in thymic selection[33].

cTECs facilitate positive selection

T cells do not develop to maturity in MHC-deficient mice, which underscores the absolute requirement for MHC molecules for the positive selection of T cells. However, as MHC molecules are broadly expressed, it has not always been clear which APCs mediate this crucial step in T cell selection. One of the early experiments to test this used reciprocal bone marrow chimaeras (Box 1), in which MHC-deficient mice

Box 1

Experimental systems for studying thymic selection of T cells

T cell receptor-specific studies

T cell receptor-transgenic mice. Mice that are are genetically engineered so that all T cells express the same rearranged T cell receptor (TCR), typically of known specificity — such as the OT-I TCR, which recognizes an ovalbumin-derived peptide bound to H2-K^b (MHC class I). In the past decade, TCR-retrogenic mice have emerged as a useful alternative for generating thymocytes expressing the same TCR without the need for germline gene editing. TCR-retrogenic mice receive transfers of T cell precursors (often bone marrow or double-negative thymocytes) transduced with a retroviral vector encoding a TCR of interest[101].

Bone marrow chimaeric mice. Mice that receive bone marrow transfer following lethal irradiation to repopulate the haematopoietic stem cell population (which gives rise to thymocytes) with cells of a different genotype. These mice have been particularly useful for distinguishing cell-intrinsic and cell-extrinsic requirements for T cell development because lethal irradiation oblates haematopoietic T cell progenitors but leaves stromal cells (such as thymic epithelial cells) intact. Mixed bone marrow chimaeras can be generated by reconstituting recipients with bone marrow from two (or more) donors of distinct genotypes; TCR-transgenic or TCR-retrogenic bone marrow is also often used in mixed bone marrow chimaeras to generate mice with a desired frequency of T cells expressing a select TCR[63].

TCR repertoire analysis. Deep sequencing of TCR gene expression and comparison between different T cell populations, or mice of different genotypes, to identify patterns indicative of selection (such as the enrichment or loss of particular TCR sequences). TCR repertoire analysis is complicated by the random nature of TCR rearrangement, which generates a unique TCR repertoire even between inbred laboratory mice of the same genotype[59]. Thus, to distinguish bona fide selection patterns from TCR repertoire differences owing to chance, multiple biological replicates must be sequenced and data are often filtered to restrict analysis to high confidence, commonly recurring (or 'public') TCRs[60,102,103].

Fluorescent reporter systems

***Nur77*^{GFP} mice.** Mice that express green fluorescent protein (GFP) under control of the *Nur77* promoter, which is upregulated early following TCR signalling and decays with the cessation of TCR signalling. GFP expression level in *Nur77*^{GFP} mice correlates with TCR signal strength.

***Rag2*^{GFP} mice.** Mice that express GFP under control of the *Rag2* promoter, which encodes one of the RAG enzymes responsible for TCR locus rearrangement[90]. In *Rag2*^{GFP} mice, GFP expression is high in all thymocytes at the double-positive stage but is strongly repressed when a thymocyte undergoes positive selection. However, owing to the stability of the GFP protein, thymocytes continue to be GFP-positive for some time after they have stopped expressing *Rag2*. Thus, the level of GFP expression reflects the relative age of a thymocyte or T cell.

In vitro models

Fetal thymus organ culture. The culture of embryonic thymus lobes to observe the selection and development of T cell progenitors in vitro. Within a week, selection of endogenous thymocytes (present in the thymus lobes on isolation) can be detected in culture. Although fetal thymus organ culture does not generate large numbers of mature T cells, it allows for other reagents to be added to the culture to determine their effects on particular selection events.

Reaggregate culture. The co-culture of thymocytes with stromal cells isolated from an embryonic thymus[104], allowing for the roles of different cell types in thymocyte maturation and selection to be assessed. Importantly, monolayer cultures of thymic stroma and T cell progenitors do not support their full maturation in vitro; cells must be cultured as a 3D reaggregate[40].

Thymic slice culture. The co-culture of thymocytes with thin thymic slices to observe the migration and selection of thymocytes in vitro[105]. Thymic slices can support positive and negative selection. Perhaps most importantly, thymocytes migrate to correct anatomical regions of the thymus when added to these cultures, allowing for observation of thymocyte migration and co-localization with antigen-presenting cells under a microscope.

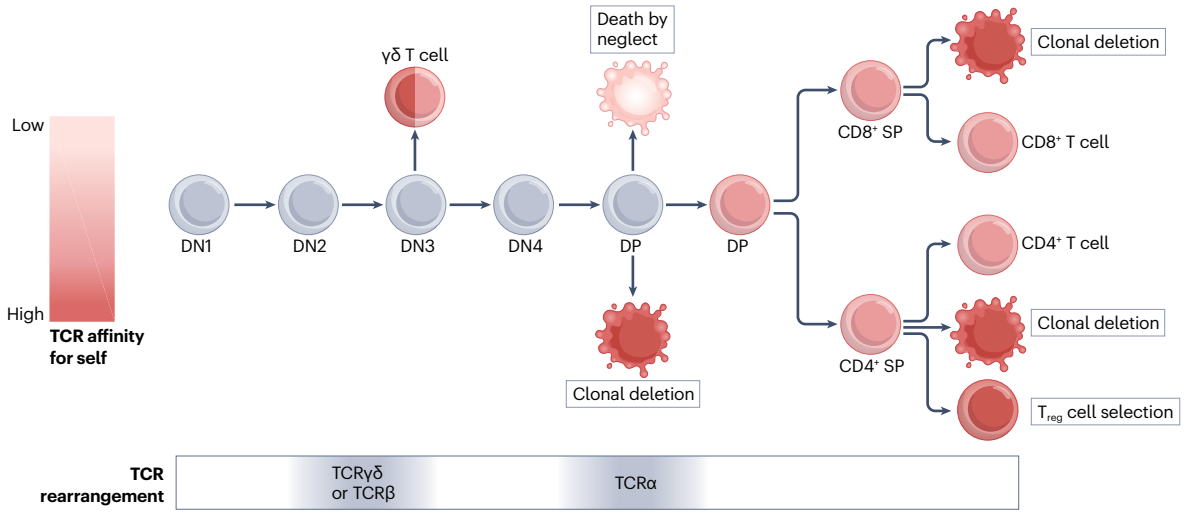


Fig. 3 | The thymus selects for self-reactive T cells. The development of double-negative (DN) thymocytes can be divided into four stages (DN1–DN4), during which cells undergo commitment to the T cell lineage and successive rearrangement of T cell receptor (TCR) genes. Cells that rearrange TCRγ and TCRδ genes to generate a γδ TCR diverge from the major T cell lineage (which expresses αβ TCR) at the DN3 stage. Cells that rearrange TCRβ genes are subject to a developmental checkpoint known as β-selection that ensures that thymocytes have rearranged a functional TCRβ gene before continuing with rearrangement of the TCRα locus. Completion of β-selection and initiation of TCRα rearrangement stimulate thymocytes to upregulate expression of the co-receptors CD4 and CD8, becoming double-positive (DP) thymocytes. DP thymocytes must interact with peptide–MHC (pMHC) molecules on cortical thymic epithelial cells to survive and continue in their development. DP thymocytes that rearrange non-functional αβ TCRs that cannot recognize the pMHC molecules expressed by that individual undergo apoptosis, termed 'death by neglect'. However, DP thymocytes that recognize pMHC with high affinity can also undergo programmed cell death at this stage, termed 'clonal deletion'. In this way, cells with moderate affinity for pMHC molecules expressed on cortical thymic epithelial cells are selected. Positive selection initiates lineage commitment, during which MHC reactivity informs whether a DP thymocyte develops into a $CD4^+$ or $CD8^+$ single-positive (SP) thymocyte, by downregulating one of the co-receptors. SP thymocytes, which accumulate in the medulla following positive selection, are subject to an additional round of negative selection. In the medulla, high-affinity interactions with self-pMHC molecules on medullary thymic epithelial cells, B cells, dendritic cells or other medullary antigen-presenting cells can stimulate programmed cell death (clonal deletion) or diversion of $CD4^+$ SP thymocytes into the regulatory T (T_{reg}) cell lineage.

were reconstituted with wild-type (MHC-sufficient) bone marrow and wild-type mice were reconstituted with MHC-deficient bone marrow — thus restricting MHC expression to either radioresistant APCs (in wild-type recipient mice) or radio-sensitive bone-marrow-derived APCs (in MHC-deficient recipients). T cells matured fully when MHC was expressed by the recipient wild-type mice but failed to mature in MHC-deficient mice that were reconstituted with wild-type bone marrow[34], confirming that the APC required for positive selection was radio-resistant and likely part of the thymic stroma. Further work showed that the relevant non-bone-marrow-derived cell type required for MHC expression and positive selection was the relatively rare, but quite remarkable, cTEC.

MHC molecules must be present on cTECs to effectively select T cells in the thymus[34,35]. However, growing evidence suggests that the nature of the peptides displayed by cTECs also affects the outcome of positive selection. The generation of surface pMHC requires the coordination of several pathways that regulate MHC expression and protein processing (which is necessary to break down proteins into short peptides that can bind MHC molecules)[36]. The peptides displayed by cTECs differ from those displayed by other APCs because cTECs express unique molecules (such as the proteasome subunit PSMB11 (also known as β5t), cathepsin L and thymus-specific serine protease) that modify how proteins are broken down into peptides within the cell[15,37–39]. This unique 'peptidome' of cTECs positively selects T cells that are unlikely, therefore, to encounter the same self-peptide elsewhere in the body (Fig. 5b), which may allow more positively selected thymocytes to survive and differentiate. Furthermore, several studies have shown that mice with deficiencies in these cTEC-specific protein-processing molecules have defects in positive selection, highlighting a requirement for this unique peptidome in selecting functional T cells[37–39].

In addition to their capacity to process and present unique self-antigens, cTECs form a 3D sponge-like network that provides a special environment for positive selection[15]. Disruption of this environment impairs T cell selection, as evidenced by the observation that simple cultures of thymocytes and epithelial cells do not support positive selection; rather, the cells need to be 'reaggregated'[40] under special conditions for positive selection to occur (Box 1). This is currently a major obstacle to the development of in vitro approaches to generate functional T cells[41].

Lineage commitment

Positive selection functions not only to select thymocytes that recognize host MHC molecules but also to coordinate MHC reactivity with T cell lineage and function. Thus, the positive selection response is different when DP thymocytes recognize MHC-I molecules versus MHC class II (MHC-II) molecules in the cortex (Figs. 3 and 6a). Those DP thymocytes that recognize MHC-I develop into $CD8^+$ SP thymocytes that lose CD4 expression. Those DP thymocytes that recognize MHC-II develop into $CD4^+$ SP thymocytes that lose CD8 expression. The CD4 and CD8 co-receptors, because they bind MHC independently of the TCR[42], are crucial in this process[43]. But this step involves much more

than simply downregulation of the inappropriate co-receptor gene. Rather, an MHC-II-restricted thymocyte upregulates the transcription factor ZBTB7B (also known as ThPok) and substantially remodels its chromatin architecture, such that when a CD4$^+$ T cell becomes activated in the periphery it will have helper cell effector functions[44] (Fig. 5a). Likewise, an MHC-I-restricted thymocyte upregulates the transcription factor RUNX3 and remodels its chromatin such that when a CD8$^+$ T cell becomes activated in the periphery it will have killer cell effector functions. Therefore, this step is commonly referred to as lineage commitment.

A mouse model, coined the 'flip flop' mouse, was recently created that demonstrates the importance of coordinating MHC recognition with lineage commitment[45]. These mice have the *Cd4* gene engineered into the *Cd8* locus and vice versa. In such mice, thymocytes with MHC-II-restricted TCRs are misdirected to the killer cell lineage, and those with MHC-I-restricted TCRs are misdirected to the helper cell lineage. Flip flop mice were not able to mount effective immune responses to infection, which underscores the importance of appropriate helper versus killer lineage commitment being established in the thymus[45].

Positive selection of specialized subsets

Naive CD4$^+$ and CD8$^+$ T cells are the major cellular products of thymic selection, but the thymus also produces numerous specialized T cell populations that are less abundant but nonetheless have crucial roles in the immune system. Naive CD4$^+$ and CD8$^+$ T cells, which are positively selected on self-pMHC ligands, populate secondary lymphoid tissues upon export from the thymus and take part in inflammatory responses when activated by recognition of foreign antigens in the periphery — the classic 'soldiers' of the immune system (Fig. 6b). The less abundant, specialized T cell populations are positively selected on distinct ligands (for the most part) and seem to have unique roles in maintaining lymphoid, metabolic and tissue homeostasis — the 'peacekeepers' of the immune system. Such cells include γδ T cells, which develop from earlier progenitors at the DN stage (Fig. 3) and have distinct developmental requirements and functions (reviewed elsewhere[19]). The development of iNKT cells from DP thymocytes requires TCR recognition of self-lipids — as opposed to peptide antigens — presented by the non-polymorphic MHC-like molecule CD1d and signalling from the SLAM family of costimulatory molecules[46]. iNKT cells are highly abundant in many tissues of mice (whereas naive CD4$^+$ and CD8$^+$ T cells are restricted to secondary lymphoid organs), in which they regulate immune responses and adipose tissue homeostasis[47]. Likewise, MAIT cells develop upon recognition of small microbial metabolites presented by the non-polymorphic MHC-like molecule MR1 (ref. 48). MAIT cells are highly abundant in humans and are thought to regulate responses to infection in mucosal tissues. IELs, which reside between epithelial cells of the small and large intestine, develop in the thymus upon recognition of self-peptide ligands presented by MHC-I molecules in the absence of costimulation, and they are thought to function in the maintenance of barrier surface homeostasis[49]. Finally, T_{reg} cells are a subset of CD4$^+$ T cells that recognize self-peptide ligands presented by MHC-II molecules. They have crucial roles in maintaining lymphoid homeostasis and preventing autoimmunity, as discussed in detail later. Interestingly, all of these specialized T cells recognize steady-state ligands (peptide, lipid or metabolite) with high affinity[50]. Nonetheless, because of the involvement of unique costimulatory factors and cytokines in their development, they do not undergo programmed cell death following TCR signalling. For this reason, their development has been referred to as agonist selection.

Selecting T cells that are safe

Several synergistic layers of immune regulation exist to prevent the activation and propagation of self-specific immune responses (known as autoimmunity). The first two of these layers occur in the thymus,

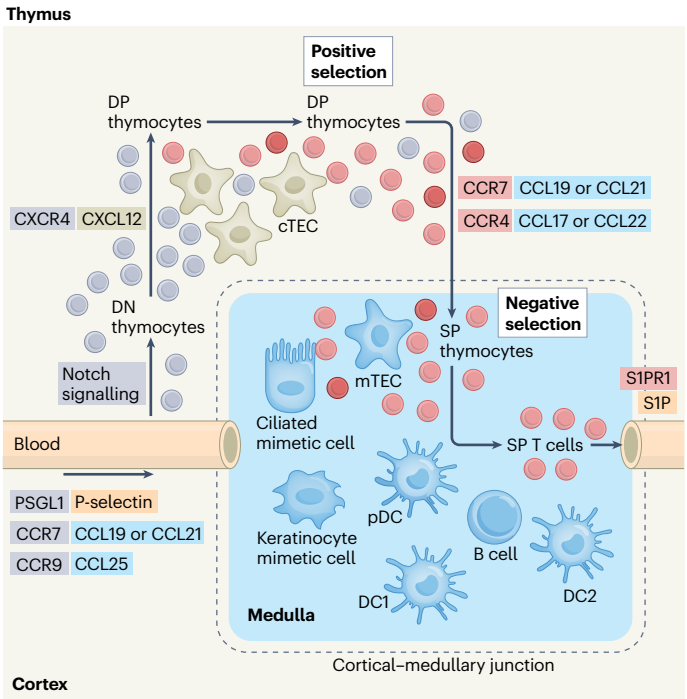


Fig. 4 | Thymocytes traffic through various regions of the thymus as they undergo positive and negative selection. The earliest haematopoietic progenitors enter the thymus at the cortical–medullary junction. Thymic entry is facilitated by P-selectin glycoprotein ligand 1 (PSGL1) expression by progenitor cells, which recognizes P-selectin on endothelial cells, and by the chemokine receptors CCR9 and CCR7, whose ligands (CCL25 and CCL19 or CCL21, respectively) are expressed on thymic epithelial cells. Notch signalling drives commitment to the T cell lineage early after thymic entry, when progenitor cells do not express CD4 or CD8 co-receptors and are known as double-negative (DN) cells. DN thymocytes migrate to the distal cortex while committing to the T cell lineage and undergo T cell receptor gene rearrangements. Retention in the cortex is facilitated by expression of the chemokine receptor CXCR4 by DN thymocytes, which recognizes the ligand CXCL12 on cortical thymic epithelial cells (cTECs.) DN cells give rise to CD4 and CD8 double-positive (DP) cells, which express rearranged T cell receptor proteins at the cell surface. If these cells interact with MHC class I or MHC class II molecules on cTECs, they can undergo positive selection, which initiates migration towards the medulla. Concurrently, cells lose expression of the inappropriate co-receptor — such that MHC class I-interacting cells become CD8$^+$ and MHC class II-interacting cells become CD4$^+$ single-positive (SP) cells. CCR4 and CCR7 expression on thymocytes after positive selection supports entry into the medulla, where the ligands for these receptors (CCL17 or CCL22 and CCL19 or CCL21, respectively) are expressed on medullary thymic epithelial cells (mTECs). SP thymocytes migrate through the medulla and interact with multiple unique antigen-presenting cells, such as dendritic cells (DCs), plasmacytoid DCs (pDCs), B cells, mTECs and mimetic cells. These interactions often lead to negative selection. Mature SP thymocytes that have survived positive and negative selection processes upregulate expression of sphingosine-1-phosphate receptor 1 (S1PR1) and exit the thymus at the cortical–medullary junction, supported by recognition of sphingosine-1-phosphate (S1P), which is present at high concentrations in the circulation. Further details about the thymocyte-intrinsic changes that occur during the course of T cell development are described in Fig. 3.

IEL - intraepithelial lymphocytes

in which T cell progenitors that recognize self-antigens with high affinity are directed to undergo programmed cell death (clonal deletion) or develop into the T_{reg} cell lineage.

Clonal deletion

Clonal deletion is initiated by high-affinity interactions between TCRs and self-pMHC and results in the induction of a form of programmed cell death known as apoptosis[51]. This removes thymocytes expressing self-reactive TCRs from the repertoire (Fig. 7). A key intracellular mediator of clonal deletion is the pro-apoptotic protein BIM[52]. DP thymocytes, which reside in the cortex, are highly sensitive to clonal deletion, and the same is true for young SP thymocytes ($CD4^+$ or $CD8^+$), which traffic through the medulla and are exposed to distinct APCs from those in the cortex. Clonal deletion requires costimulation by CD28 together with a strong TCR signal, and many (but not all) thymic APCs express CD80 and CD86, the ligands for CD28 (refs. 23,27). In the absence of costimulation, self-reactive thymocytes can be diverted from the conventional ($CD4^+$ or $CD8^+$) T cell lineages and become IELs that traffic to the gut[53], where their precise role is unclear[54].

T_{reg} cell selection

Alternatively to clonal deletion, when $CD4^+$ SP thymocytes experience strong TCR interactions with self-pMHC-II, they can develop into T_{reg} cells[55] (known as T_{reg} cell selection) (Fig. 7). T_{reg} cells express the master transcription factor FOXP3, which is required for their development and function. Mature T_{reg} cells have a crucial role in immune regulation outside the thymus by inhibiting immune activation against self-antigens[56–58]. In fact, the discovery of this crucial population was made possible in part by studying mice and humans with mutations in FOXP3, who lack T_{reg} cells and experience a fatal lymphoproliferative disease[56] (Box 2). Thus, T_{reg} cell selection functions not only to remove self-reactive $CD4^+$ SP thymocytes from the conventional $CD4^+$ T cell repertoire but also to select for a self-reactive T_{reg} cell repertoire[58–60]. T_{reg} cell development requires TCR ligation, CD28 costimulation[30] and IL-2 signalling[61] and results in stable expression of FOXP3 and the high-affinity IL-2 receptor (CD25)[55].

Distinguishing deletion and T_{reg} cell selection

Similar to the puzzle of TCR signalling in positive selection, an important question in the field of T_{reg} cell selection has been why some TCR signals received by $CD4^+$ SP thymocytes lead to T_{reg} cell selection, whereas others lead to death[62]. Data show that as their self-reactivity increases, thymocyte clones are more likely to develop into T_{reg} cells[63]. For example, in *Nur77*^{GFP} reporter mice (Box 1), T_{reg} cells have slightly greater expression of green fluorescent protein (GFP) than conventional T cells, which is consistent with them having received a stronger TCR signal. However, a relatively broad range of TCR affinities supports T_{reg} cell selection, and this range overlaps with that of clonal deletion[63], which suggests that differences in TCR affinity alone cannot explain differences in selection outcome. Instead, the distinction between

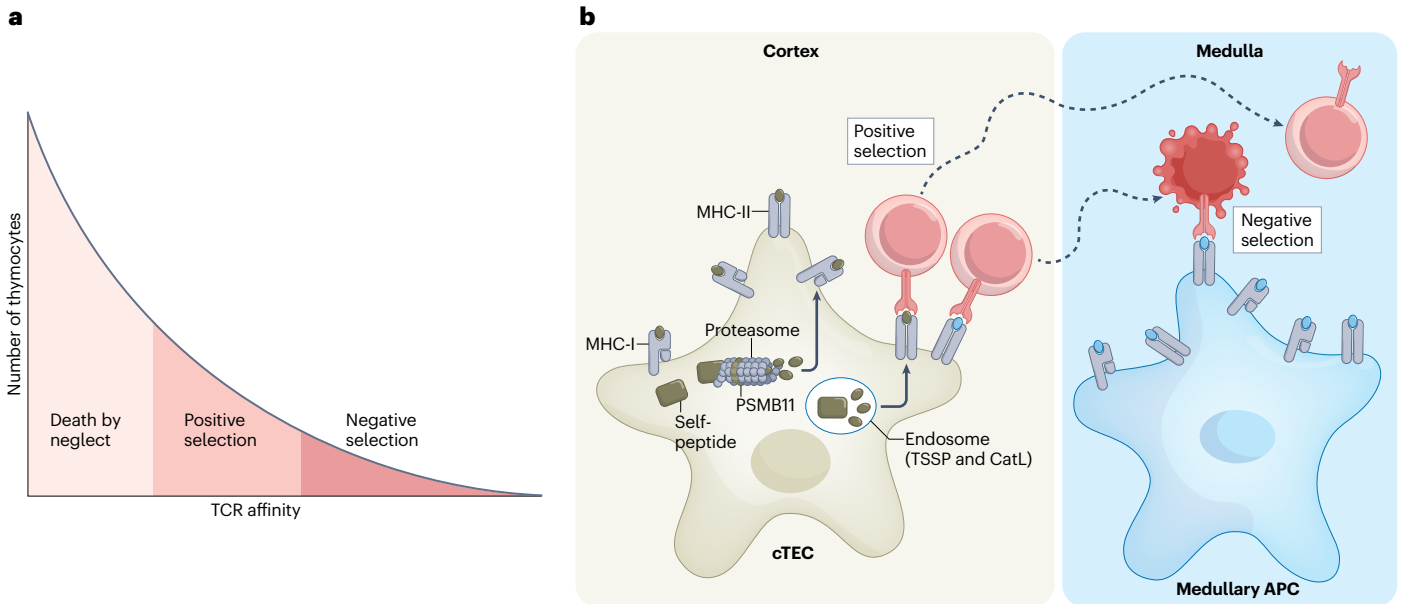


Fig. 5 | The balance between positive and negative selection. The number of thymocyte clones that undergoes positive selection must be greater than the number that undergoes negative selection to select a net positive number of T cells. Two major factors contribute to this balance. **a**, The strength of interaction between T cell receptors (TCRs) and MHC molecules is a crucial factor in selection outcome. Most thymocyte clones fail to interact with MHC and die by neglect. Thymocyte clones that make weak interactions with MHC are triggered to survive and mature (positive selection). Thymocyte clones that make strong interactions with MHC can trigger programmed cell death (clonal deletion; one outcome of negative selection). Because the TCR is randomly generated, thymocyte clones are more likely to have weak interactions with MHC than strong interactions. **b**, Positive selection is triggered by TCR interactions with peptide–MHC on cortical thymic epithelial cells (cTECs), whereas negative selection is most often triggered by TCR interactions with peptide–MHC on medullary antigen-presenting cells (APCs). cTECs express several genes that modify antigen processing and presentation, such that cTECs present unique self-peptides that are not presented by medullary APCs. These genes include those encoding the proteasome subunit PSMB11 (also known as β5t), which determines how self-peptides are degraded for presentation by MHC class I (MHC-I), and thymus-specific serine protease (TSSP) and cathepsin L (CatL), which degrade peptides in endosomes for presentation by MHC class II (MHC-II). The unique 'peptidome' of cTECs increases the number of clones that are positively selected in the cortex but not deleted (negatively selected) in the medulla.

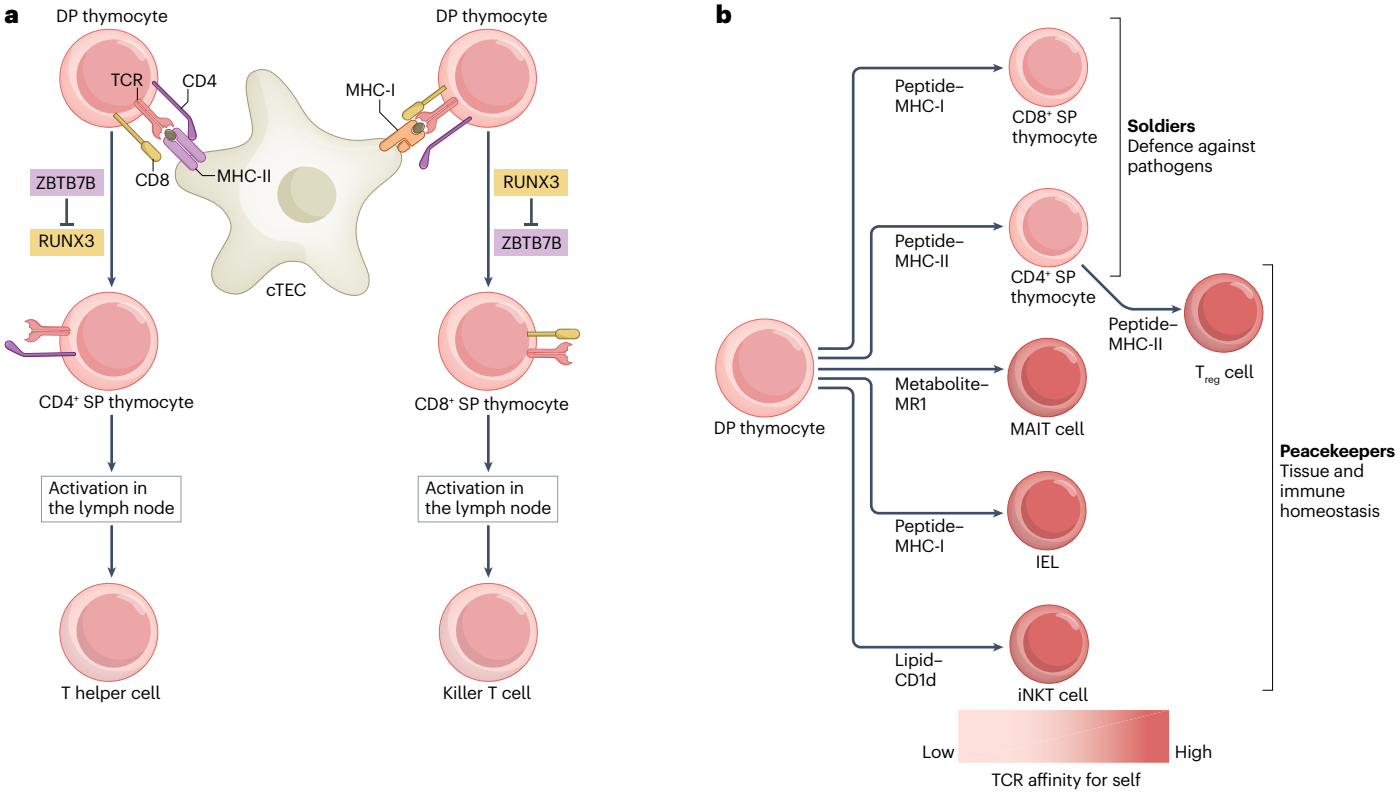


Fig. 6 | MHC recognition determines T cell lineage and function. a, When a double-positive (DP) thymocyte expresses a T cell receptor (TCR) that interacts with MHC class II (MHC-II) on a cortical thymic epithelial cell (cTEC), the CD4 co-receptor assists this interaction. This leads to loss of expression of the uninvolved co-receptor (CD8 in this case) and upregulation of expression of the transcription factor ZBTB7B (also known as ThPok). The actions of ZBTB7B poise the thymocyte to have helper cell activity when activated in a lymph node during an infection. By contrast, when a DP thymocyte expresses a TCR that interacts with MHC class I (MHC-I) on a cTEC, the CD8 co-receptor assists the interaction, leading to loss of CD4 expression and upregulation of the transcription factor RUNX3. The actions of RUNX3 poise the thymocyte to have killer cell activity during an infection. ZBTB7B and RUNX3 negatively regulate each other, so that the helper cell and killer cell lineages are quite stable. **b**, In addition to the specification of the major αβ T cell lineages ($CD4^+$ helper cells and $CD8^+$ killer cells), the thymus generates several minor subsets of T cells with their own unique developmental requirements and functions. γδ T cells diverge from the major αβ T cell lineage at the double-negative DN3 stage (Fig. 3). Invariant natural killer T (iNKT) cells and mucosal associated invariant T (MAIT) cells diverge from the $CD4^+$ and $CD8^+$ lineages at the DP stage, initiated by recognition of non-classical MHC molecules (CD1d and MR1, respectively) in the thymus. CD8αα intraepithelial lymphocytes (IELs) are selected from DP thymocytes on self-peptide–MHC-I molecules in the absence of costimulatory molecules. Finally, $CD4^+$ thymocytes that recognize self-antigens in the medulla can be diverted into the regulatory T (T_{reg}) cell lineage. Importantly, almost all of these minor lymphocyte subsets are thought to undergo agonist selection, during which they receive strong TCR signals but do not undergo apoptosis. Thus, these lineages are generally thought to have a higher affinity for self-antigens than $CD4^+$ helper cells and $CD8^+$ killer cells, which are positively selected by interactions of low-to-intermediate affinity with peptide–MHC molecules. Once mature, cells of each lineage have unique effector functions. Generally, they can be divided into 'solider' lineages that are essential for defence against pathogens and 'peacekeeper' lineages that have important roles in tissue and immune homeostasis. However, it is important to acknowledge that there are exceptions to both of these classifications. SP, single-positive.

clonal deletion and T_{reg} cell selection is in part attributable to the availability of IL-2, which is present at limiting concentrations in the thymus (Fig. 7). $CD4^+$ SP thymocytes that receive TCR and costimulatory signals but not IL-2 die by apoptosis, suggesting that T_{reg} cell development might be a two-step process that requires IL-2-mediated 'rescue' of TCR-signalled cells to prevent apoptosis and drive completion of the T_{reg} cell differentiation programme[64–66]. The availability of self-antigen is another crucial determinant of negative selection outcomes in the medulla. Some self-antigens, such as those derived from ubiquitously expressed genes, are present at high densities in the thymus medulla and are likely to be encountered by a thymocyte multiple times during the negative selection window. Other self-antigens are rare in the medulla, owing to low levels of gene expression or presentation by a rare APC population. Ubiquitous self-antigens are more likely to result in clonal deletion of $CD4^+$ SP thymocytes, whereas low-density or rare self-antigens favour T_{reg} cell selection (Fig. 7). This was shown to be the case in model antigen systems, in which the same antigen was expressed at different densities in the thymus[67].

Specialized APCs in the medulla

Both clonal deletion and T_{reg} cell selection require interactions with self-pMHC in the thymus, and medullary APCs are uniquely equipped to present a broad range of self-pMHC to T cell progenitors (Fig. 8). Key among these APCs are mTECs, which can express otherwise tissue-specific genes (such as myelin basic protein or insulin) through several mechanisms. A subset of mTECs expresses the transcriptional

regulator AIRE. *AIRE* was initially discovered as the gene involved in a rare, inherited, multi-organ autoimmune disease[68] (Box 2); a similar disease was also seen in *Aire*-knockout mice[12]. These studies and others showed that AIRE binds to repressive elements in the genome and de-represses the transcription of tissue-specific genes that would otherwise be silenced in mTECs[12] (Fig. 8). This results in a population of AIRE$^+$ mTECs that collectively expresses a large number of proteins that are normally only expressed in a single (or select few) tissues in the body[69,70]. In this way, developing T cells expressing TCRs that recognize tissue-specific proteins can be deleted or diverted into the T_{reg} cell lineage as they develop in the thymus. In humans and mice with deficiencies of AIRE, these tissue-specific antigens are not expressed in the thymus, and thymocytes with specificity for tissue-specific antigens escape negative selection, developing instead into conventional T cells. These 'escaped' self-reactive T cells become activated when they encounter tissue-specific self-antigens outside the thymus, causing substantial pathology[12,60]. However, not all tissue-specific antigens expressed in the thymus are AIRE dependent. Some rely on another transcription factor, FEZF2, for their expression[71]. Furthermore, recent work has uncovered small populations of 'mimetic cells' among mTECs, which may have a key role in tissue-specific antigen expression in the thymus. These mimetic cells are generated from mTECs by co-opting lineage-defining transcription factors to take on the characteristics of peripheral cell types, such as muscle cells, keratinocytes or microfold cells[72] (Fig. 8). There is still much to learn about the exact roles of FEZF2 and mimetic mTECs in thymic selection, as these mechanisms were only recently described. However, it is important to note that AIRE, FEZF2 and mimetic cells all result in the rare, low-density expression of otherwise tissue-restricted self-antigens in the thymus, which is ideal for T_{reg} cell selection.

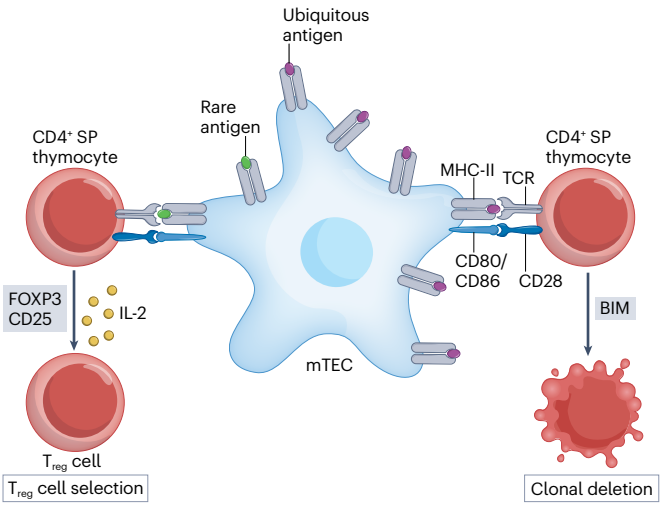


Fig. 7 | Clonal deletion and regulatory T cell selection are the major thymic tolerance mechanisms. Thymocytes that interact strongly with MHC class I or MHC class II (MHC-II) on medullary antigen-presenting cells, such as medullary thymic epithelial cells (mTECs), often trigger apoptosis (clonal deletion). This involves the upregulation of a pro-apoptotic protein BIM and requires costimulation through CD28. However, some CD4$^+$ single-positive (SP) thymocytes that interact strongly with MHC-II on medullary antigen-presenting cells upregulate the transcription factor forkhead box protein 3 (FOXP3) and the IL-2 receptor CD25 and differentiate into regulatory T (T_{reg}) cells. This outcome (known as T_{reg} cell selection) also requires costimulation through CD28, but additionally requires IL-2 signalling, and is favoured when the self-antigen recognized by the cell is rare. TCR, T cell receptor.

The medulla also supports a complex, uniquely activated haematopoietic APC compartment that is involved in negative selection[73]. Thymic B cells undergo class-switching in the thymus and have been shown to participate in clonal deletion[74] and T_{reg} cell selection[75–77]. The thymus also contains three major types of dendritic cell (DC) — DC1, DC2 and plasmacytoid DC[78,79]. Evidence suggests that thymic DCs can promote clonal deletion and T_{reg} cell selection[80,81], and these cells have even been shown to acquire self-antigens from other cells in the thymus (such as mTECs)[82]. The unique roles of the different thymic DC populations in negative selection are still being worked out. For example, it was recently found that CX3CR1$^+$ DCs bring bacterial DNA from the intestine to the thymus, suggesting a possible mechanism for selecting or deleting T cell progenitors dependent on reactivity to commensal-derived antigens[83]. Meanwhile, DC2s seem to be specialized for promoting clonal deletion[84].

Central tolerance

Central tolerance is a term used to describe the collective outcomes of clonal deletion and T_{reg} cell selection in the thymus. 'Central' refers to the thymus as a primary lymphoid organ, and 'tolerance' refers to the idea that these processes are essential for maintaining broader immune tolerance to self-antigens, which is supported by observations in humans and mice with deficiencies in thymic selection (Box 2). However, determining the relative effects of clonal deletion and T_{reg} cell selection on maintaining tolerance is not straightforward and it is difficult to deconvolute the effects of thymic selection from the additional layers of immune regulation that occur outside the thymus (such as peripheral T_{reg} cell induction and immune checkpoint molecules). Although clonal deletion is efficient at removing high-affinity self-reactive clones, this process is imperfect, and many self-reactive cells do develop into conventional CD4$^+$ and CD8$^+$ T cells. Mice lacking the pro-apoptotic protein BIM (which is required for clonal deletion) produce large numbers of self-reactive T cells, but they do not have lethal autoimmune disease[52]. However, mice and humans with deficiencies in thymic T_{reg} cell selection present with multi-organ autoimmune disease[56,85] (Box 2). This has led to the suggestion that clonal deletion debulks the T cell repertoire, but that other tolerance processes, such as the thymic induction of T_{reg} cells and/or peripheral tolerance mechanisms, can regulate T cell self-reactivity under most circumstances. In this context, it is interesting to note that mice lacking AIRE (presumed to result mainly in a central tolerance defect) or the immune checkpoint molecule PD1 (presumed to mainly affect peripheral tolerance) individually have relatively mild autoimmune disease. Yet when combined, these deficiencies cause a spontaneous lethal autoimmunity before adulthood[86]. Therefore, most human autoimmune diseases likely reflect a breakdown in more than one arm of tolerance.

Final maturation and thymic emigration

Thymocytes that survive both positive and negative selection processes undergo final maturation steps in the medulla before exiting the thymus. Crucial among these are the switch in metabolic machinery that allows for mature T cells to proliferate and become activated following strong TCR stimulation (provided that the right costimulatory molecules and cytokine environment are present), instead of undergoing apoptosis as happens in immature thymocytes[87]. This 'proliferation competence' allows for mature naive T cells to participate in immune responses upon antigen recognition in secondary lymphoid organs. The final maturation

Box 2

Human diseases that result from failures of thymic selection

Most diseases that affect thymic function and selection of T cells are inborn errors of immunity, such as primary immunodeficiencies (PIDs), caused by inherited or somatic gene mutations[106]. Among these, there are mutations intrinsic to the developing thymocyte progenitors and mutations in genes expressed by thymic stromal cells. Some autoimmune diseases, such as myasthenia gravis, result from failures of thymic selection also.

Mutations intrinsic to thymocyte progenitors

PIDs were classically defined as mutations causing increased susceptibility to infection. However, as our understanding of PIDs has grown exponentially in the past decade, it has become apparent that autoimmunity co-occurs in a surprisingly large fraction of patients with PIDs[107]. This is likely due, at least in part, to the fact that developing thymocytes provide signals to the stroma during thymic development, such that when thymocyte development is impaired, the ability of the stroma to support immune tolerance is also compromised[108]. Thus, many patients with primary T cell immunodeficiency also experience autoimmunity — particularly endocrinopathies, enteropathy and arthritis.

Severe combined immunodeficiency. Severe combined immunodeficiency affects the development of T cells and B cells and is frequently caused by mutations in genes that are involved in cytokine signalling in developing lymphocytes (such as *IL2RG*, *IL7R* and *JAK3*)[109]. This can also be caused by mutations in genes that affect nucleotide metabolism (such as *ADA*, *AK2* and *PNP*) and have toxic effects on developing lymphocytes.

Omenn syndrome. This T cell and B cell combined immunodeficiency is caused by mutations in *RAG1*, *RAG2* or other genes involved in the somatic gene rearrangements that are required to generate a T cell receptor or B cell receptor[110]. Thymic output can be partially to severely reduced in these individuals.

ZAP70 deficiency. In humans, this causes a profound loss of $CD8^+$ T cells and dysfunction in $CD4^+$ T cells. ZAP70 is a kinase that has a crucial role in the positive selection of T cells. Individuals with ZAP70 deficiency are susceptible to life-threatening infections and require a bone marrow transplant to survive[111].

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is caused by mutations in the *FOXP3* gene, which is required for the development of $CD4^+$ regulatory T (T_{reg}) cells[56,112]. The syndrome is inherited in males in an X chromosome-linked recessive manner[56]. Without T_{reg} cells, severe enteropathy symptoms present in the first few days of life and bone marrow transplantation has been shown to be effective in treating these patients.

Mutations in genes expressed by thymic stroma

DiGeorge syndrome. This is caused by a microdeletion on the long arm of chromosome 22 (22q11.2), which contains the *TBX1* gene that has a key role in early thymic organogenesis[113]. Symptoms vary widely and can involve mild-to-severe thymic hypoplasia, with resulting T cell deficiency and increased susceptibility to infections and autoimmune manifestations.

CHARGE syndrome. This is caused by mutations in the *CHD7* gene, which is involved in chromatin remodelling in thymic epithelial cells early in thymic development[114]. Patients with CHARGE syndrome can benefit from thymic transplantation.

Autoimmune polyendocrine syndrome type 1. APS1 (also known as APECED) is an autoimmune syndrome involving the destruction of multiple and varied endocrine tissues, including the thyroid and parathyroid glands and the pancreas[85]. The genetic basis is mutations in the autoimmune regulator (*AIRE*) gene, which regulates the expression of tissue-specific antigens in the thymus and is essential for developing tolerance to those antigens.

Bare lymphocyte syndrome. This results from mutations in genes that are essential for the expression of MHC molecules at the cell surface (such as *CIITA* and *TAP*). Individuals with this syndrome have very low levels of MHC class II or MHC class I, and thus impaired selection of $CD4^+$ or $CD8^+$ T cells, respectively, and have reduced ability to fight infections[115].

Myasthenia gravis

Myasthenia gravis is an autoimmune disease caused by the generation of autoantibodies to the acetyl choline receptor. These autoantibodies target muscle tissue and have deleterious effects on vision, speech, mobility and swallowing. Somewhat surprisingly, the thymus is involved in the generation of these autoantibodies, although the precise mechanisms are unclear, and may have to do with thymic 'muscle-like' mimetic cells[116]. Thymectomy (surgical removal of the thymus) has proven effective in treating some forms of myasthenia gravis[117].

of thymocytes is dependent on nuclear factor-κB signalling[88]. It also induces the cellular changes that enable mature naive T cells to home to the circulation and enter secondary lymphoid organs (spleen and lymph nodes), namely, 'migration competence'. In particular, upregulation of the transcription factor KLF2 in late-stage thymocytes drives expression of sphingosine-1-phosphate receptor 1, which recognizes the soluble ligand sphingosine-1-phosphate that is present at high concentrations in blood. KLF2 also induces expression of L-selectin (also known as CD62L) on mature naive T cells, which facilitates their homing to lymph nodes through interaction with ligands on high endothelial venules.

Glossary

αβ T cells
T cells that express a T cell receptor (TCR) composed of rearranged TCRα and TCRβ chains. Most αβ T cells are 'conventional' $CD8^+$ or $CD4^+$ T cells that recognize peptide antigens presented by highly polymorphic MHC class I or MHC class II molecules, respectively. However, several less abundant subsets of T cells also express αβ TCRs, including regulatory T cells, invariant natural killer T cells, mucosal associated invariant T cells and intraepithelial lymphocytes.

γδ T cells
T cells that express a T cell receptor (TCR) composed of rearranged TCRγ and TCRδ chains. They are less abundant than αβ T cells in mice and humans. They recognize diverse ligands and have physiological roles in homeostasis and tissue protection.

Agonist selection
The process through which T cell receptor signalling in the thymus directs differentiation into a specific T cell lineage. Agonist selection is required for thymocyte differentiation into regulatory T cells, invariant natural killer T cells, mucosal associated invariant T cells and intraepithelial lymphocytes. In addition to T cell receptor signalling, agonist selection often also requires additional cues for completion of lineage specification, such as costimulatory molecules and cytokines.

$CD4^+$ T helper cell
Upon activation in a lymph node, $CD4^+$ T helper cells acquire effector functions associated with providing activating or 'helper' signals to other immune cells, such as B cells and macrophages.

$CD8^+$ killer T cell
Upon activation in a lymph node, $CD8^+$ killer T cells acquire the ability to directly kill target cells through T cell receptor-directed release of cytotoxic molecules.

Clonal deletion
One possible outcome of negative selection. Clonal deletion is initiated by T cell receptor interaction with peptide–MHC and results in programmed cell death (apoptosis), thus eliminating or 'deleting' a T cell clone expressing that specific T cell receptor from the developing repertoire.

Immune tolerance
The inability to mount immune responses against self-proteins. Importantly, immune tolerance is distinct from immunodeficiency in that the ability to mount immune responses against foreign antigens is preserved.

Intraepithelial lymphocytes
(IELs). T cells that reside in the epithelial layer of mucosal linings, such as the gastrointestinal tract. IELs are composed of both γδ T cells and αβ T cells. A prominent subpopulation of IELs expresses CD8αα homodimers, unlike conventional $CD8^+$ T cells. It is the thymic precursors of CD8αα IELs that we refer to in this article.

Invariant natural killer T cells
(iNKT cells). A small subset of αβ T cells that recognizes lipid antigens presented by the non-polymorphic MHC-like molecule CD1d.

Lineage commitment
The process by which a double-positive thymocyte acquires the characteristics of either the helper ($CD4^+$) lineage or killer ($CD8^+$) lineage and loses the potential to differentiate into the alternative lineage.

MHC restriction
Refers to the fact that a T cell, through its T cell receptor, recognizes a combinatorial ligand of an MHC molecule presenting a foreign peptide and does not directly recognize the foreign peptide alone.

Mucosal associated invariant T cells
(MAIT cells). A small subset of αβ T cells that recognizes bacterial metabolites presented by the non-polymorphic MHC-like molecule MR1.

Negative selection
The process by which the interaction between T cell receptor and peptide–MHC in the thymus triggers the apoptosis of a thymocyte expressing that receptor or its diversion away from the conventional T cell fate.

Peptide–MHC
(pMHC). During infection, MHC molecules can be loaded with 'foreign' or microbial peptides derived from the invading pathogen and presented to T cell receptors on T cells.

Positive selection
The process by which the interaction between T cell receptor and peptide–MHC in the thymus promotes the survival and differentiation of a double-positive thymocyte expressing that receptor.

Regulatory T cell
(T_{reg} cell). A $CD4^+$ MHC class II-restricted T cell that is self-reactive and suppresses the activation of other immune cells by interfering with antigen presentation, producing immunosuppressive cytokines and/or sequestering pro-inflammatory cytokines.

Self-pMHC
Peptide–MHC complexes in which the peptide is derived from a self-protein. MHC molecules expressed at the surface of the cell are typically bound to self-peptides in the absence of infection, as empty MHC molecules are not stable.

Thymic selection
The T cell receptor-dependent cell fate events that shape the repertoire of T cells present in an individual.

T_{reg} cell selection
The process by which T cell receptor signalling in the thymus directs differentiation into the regulatory T cell lineage. T_{reg} cell selection requires recognition of self-peptide–MHC class II molecules and IL-2 signalling.

In this way, thymocytes complete their development into mature T cells by acquiring the ability to patrol secondary lymphoid organs, where they can become activated during infections.

SP thymocytes undergo this final transition over a period of about 4–5 days[89], as indicated by data from *Rag2*^{GFP} mice[90] (Box 1), in which the stability of GFP acts as a sort of molecular timer. Use of these mice and other studies[91] has helped to elucidate the timing of thymocyte egress. In these studies, it was important to distinguish maturing thymocytes from those that recirculate back to the thymus from peripheral tissues[89,92,93]. It is now appreciated that maturing thymocytes leave in a 'conveyor belt' manner, with a continual egress of the oldest thymocytes. *Rag2*^{GFP} mice have also been useful for studying those thymocytes that have recently egressed from the thymus — known as recent thymic emigrants — as GFP expression is retained in these T cells for about 2 weeks. Recent thymic emigrants are subject to some additional maturation events in their first few weeks outside the thymus, which have been reviewed previously[94].

Future directions

This Review summarizes the myriad ways in which selection processes in the thymus shape both the TCR repertoire and the mature effector functions of T cell subsets. Attempts to replicate T cell development in vitro, for therapeutic purposes, must ensure that all of the complex self-ligand-dependent selection events function with high fidelity.

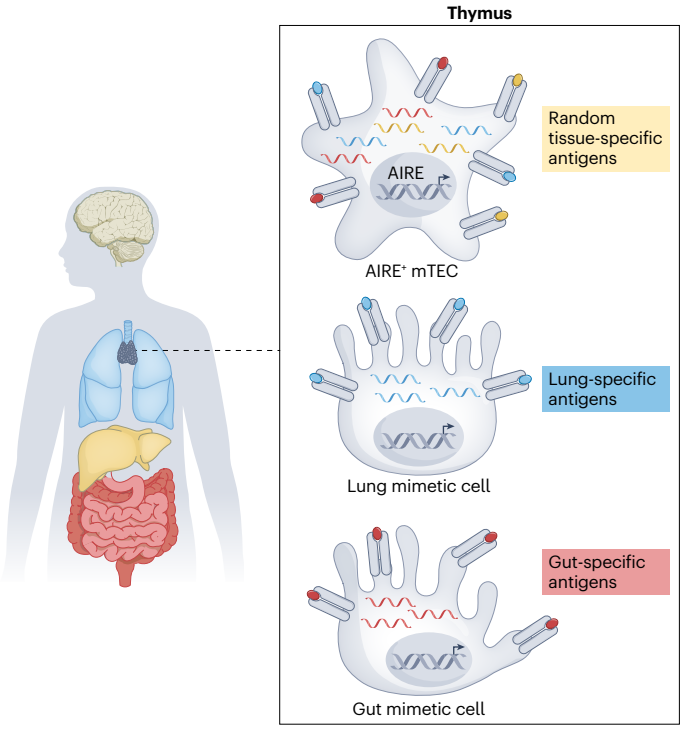


Fig. 8 | Tissue-specific antigens are produced in the thymus in an autoimmune regulator-dependent manner. Tissue-specific expression of genes results in distinct composition of self-antigens between different tissues and organs (denoted here by different colours.) This poses a crucial problem for tolerizing T cell progenitors to self-antigens before thymic egress. The expression of tissue-specific antigens in the thymus is achieved in part through the actions of autoimmune regulator (AIRE), which drives the expression of tissue-restricted genes in medullary thymic epithelial cells (mTECs). In addition, we now know that some mTECs co-opt lineage-defining transcription factors to 'mimic' the characteristics of peripheral cell types. Mimetic mTECs express tissue-specific genes in a biologically logical manner — co-expressing genes that are also co-expressed in peripheral tissues, such as genes that define ciliated cells in the lung. Together, these mechanisms allow for the production of tissue-specific antigens in the thymus, which is crucial for the negative selection of self-reactive T cell progenitors. Humans (and mice) with deleterious AIRE mutations have multi-organ autoimmune disease, which is driven by self-reactive T cells that escape thymic selection when the thymus is depleted of tissue-specific self-antigens.

As the field moves forward, it will be crucial to fully understand how various types of thymic APC are specialized to support the different selection processes — positive selection, negative selection, T_{reg} cell selection or other agonist selection events. Once that is clear, we will need to know how the thymic microenvironment directs these APCs. It is already clear that haematopoietic APCs, such as B cells and DCs, are uniquely programmed in the thymus compared with other tissues[73,95,96]. What signals do they receive in the thymus to generate these programmes? And how does that serve the thymic selection of a functional and safe T cell repertoire?

Much more work is needed to understand the unique requirements for agonist selection of T_{reg} cells, γδ T cells, iNKT cells, IELs and MAIT cells. We still do not understand the exact nature of the self-ligands that select these populations, particularly in the case of γδ T cells and iNKT cells. We understand some of the factors that are involved in specifying lineage commitment, such as FOXP3 for T_{reg} cells, ZBTB16 (also known as PLZF) for iNKT cells and MAIT cells, and IRF2 for IELs, but more work needs to be done in understanding how the synergy of signals from the TCR, costimulatory molecules and cytokine receptors induces these key factors. The field also needs to develop methods and best practices to guide the assessment of all of the diverse cellular products of the thymus when evaluating therapies and strategies to enhance thymic reconstitution, particularly in human studies.

Another area of key interest is how age affects thymic selection[97]. There are early-life events that are crucial in shaping the T cell repertoire, including a not well-understood requirement for T_{reg} cells generated early in life to protect from lymphoproliferative disease[98] and a recent discovery that commensal bacteria shape thymic selection early in life[83]. Also, a recent report showed that the ability of the thymus to support the key tolerance functions of clonal deletion and T_{reg} cell selection is decreased in aged animals[99]. So, the drive to enhance thymic reconstitution in the elderly may be not simply to increase the output of naive T cells but also to enhance the fidelity of selection mechanisms that shape these T cells. Furthermore, the creation of a mouse model that allows for tamoxifen-induced labelling of developing αβ thymocytes has provided a new and exciting tool for the field to track fate and function of T cells that develop at different times throughout ontogeny[100].

Despite an accepted role for thymic dysfunction in the generation of some human autoimmune diseases, there are no clinically approved therapies that target the thymus to prevent or treat autoimmune diseases. Thus, we perceive great opportunities in this area in the future. Although thymic selection is indeed complex, principles have been established now that should allow for the future development of therapeutic strategies to enhance thymic output, recapitulate T cell development in vitro and treat autoimmunity by targeting the thymus.

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References

1. Burnet, F. M. A modification of Jerne's theory of antibody production using the concept of clonal selection. *CA Cancer J. Clin.* **26**, 119–121 (1976).
2. Boehm, T. & Swann, J. B. Origin and evolution of adaptive immunity. *Annu. Rev. Anim. Biosci.* **2**, 259–283 (2014).
3. Laios, K. The thymus gland in ancient Greek medicine. *Horm. Athens Greece* **17**, 285–286 (2018).
4. Miller, J. F. Immunological function of the thymus. *Lancet* **2**, 748–749 (1961).
5. Martinez, C., Kersey, J., Papermaster, B. W. & Good, R. A. Skin homograft survival in thymectomized mice. *Proc. Soc. Exp. Biol. Med.* **109**, 193–196 (1962).
6. Zinkernagel, R. M. & Doherty, P. C. Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. *Nature* **248**, 701–702 (1974).
7. Fink, P. J. & Bevan, M. J. H-2 antigens of the thymus determine lymphocyte specificity. *J. Exp. Med.* **148**, 766–775 (1978).
8. Kisielow, P., Teh, H. S., Blüthmann, H. & von Boehmer, H. Positive selection of antigen-specific T cells in thymus by restricting MHC molecules. *Nature* **335**, 730–733 (1988).
9. Kappler, J. W., Staerz, U., White, J. & Marrack, P. C. Self-tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. *Nature* **332**, 35–40 (1988).
10. MacDonald, H. R. et al. T-cell receptor V beta use predicts reactivity and tolerance to Mlsa-encoded antigens. *Nature* **332**, 40–45 (1988).
11. Kisielow, P., Blüthmann, H., Staerz, U. D., Steinmetz, M. & von Boehmer, H. Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD4⁺8⁺ thymocytes. *Nature* **333**, 742–746 (1988).
12. Anderson, M. S. et al. Projection of an immunological self shadow within the thymus by the aire protein. *Science* **298**, 1395–1401 (2002).
13. Hori, S., Nomura, T. & Sakaguchi, S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* **299**, 1057–1061 (2003).
14. Lancaster, J. N., Li, Y. & Ehrlich, L. I. R. Chemokine-mediated choreography of thymocyte development and selection. *Trends Immunol.* **39**, 86–98 (2018).
15. Abramson, J. & Anderson, G. Thymic epithelial cells. *Annu. Rev. Immunol.* **35**, 85–118 (2017). **This paper reviews the functions of thymic epithelial cells in positive and negative selection.**

16. Hosokawa, H. & Rothenberg, E. V. How transcription factors drive choice of the T cell fate. *Nat. Rev. Immunol.* **21**, 162–176 (2021).
An in-depth review of the transcriptional networks that establish T cell identity early during thymic development.
17. Zhang, S. L. & Bhandoola, A. Trafficking to the thymus. *Curr. Top. Microbiol. Immunol.* **373**, 87–111 (2014).
18. Krangel, M. S. Mechanics of T cell receptor gene rearrangement. *Curr. Opin. Immunol.* **21**, 133–139 (2009).
19. Schmolka, N., Wencker, M., Hayday, A. C. & Silva-Santos, B. Epigenetic and transcriptional regulation of γδ T cell differentiation: programming cells for responses in time and space. *Semin. Immunol.* **27**, 19–25 (2015).
This paper summarizes how γδ T cells develop in the thymus and how their functional properties are established. It also describes key differences between fetal and adult development.
20. Nitta, T., Murata, S., Ueno, T., Tanaka, K. & Takahama, Y. Thymic microenvironments for T-cell repertoire formation. *Adv. Immunol.* **99**, 59–94 (2008).
21. Hu, Z., Lancaster, J. N., Sasiponganan, C. & Ehrlich, L. I. R. CCR4 promotes medullary entry and thymocyte–dendritic cell interactions required for central tolerance. *J. Exp. Med.* **212**, 1947–1965 (2015).
22. James, K. D., Jenkinson, W. E. & Anderson, G. T-cell egress from the thymus: should I stay or should I go? *J. Leukoc. Biol.* **104**, 275–284 (2018).
23. Starr, T. K., Jameson, S. C. & Hogquist, K. A. Positive and negative selection of T cells. *Annu. Rev. Immunol.* **21**, 139–176 (2003).
24. Zerrahn, J., Held, W. & Raulet, D. H. The MHC reactivity of the T cell repertoire prior to positive and negative selection. *Cell* **88**, 627–636 (1997).
25. Hogquist, K. A. et al. T cell receptor antagonist peptides induce positive selection. *Cell* **76**, 17–27 (1994).
26. Alam, S. M. et al. T-cell-receptor affinity and thymocyte positive selection. *Nature* **381**, 616–620 (1996).
27. Punt, J. A., Osborne, B. A., Takahama, Y., Sharrow, S. O. & Singer, A. Negative selection of $CD4^+CD8^+$ thymocytes by T cell receptor-induced apoptosis requires a costimulatory signal that can be provided by CD28. *J. Exp. Med.* **179**, 709–713 (1994).
28. Breed, E. R., Watanabe, M. & Hogquist, K. A. Measuring thymic clonal deletion at the population level. *J. Immunol. Baltim. Md. 1950* **202**, 3226–3233 (2019).
29. Buhlmann, J. E., Elkin, S. K. & Sharpe, A. H. A role for the B7-1/B7-2:CD28/CTLA-4 pathway during negative selection. *J. Immunol.* **170**, 5421–5428 (2003).
30. Salomon, B. et al. B7/CD28 costimulation is essential for the homeostasis of the $CD4^+CD25^+$ immunoregulatory T cells that control autoimmune diabetes. *Immunity* **12**, 431–440 (2000).
31. Tai, X., Cowan, M., Feigenbaum, L. & Singer, A. CD28 costimulation of developing thymocytes induces Foxp3 expression and regulatory T cell differentiation independently of interleukin 2. *Nat. Immunol.* **6**, 152–162 (2005).
32. Williams, J. A. et al. Regulation of thymic NKT cell development by the B7-CD28 costimulatory pathway. *J. Immunol.* **181**, 907–917 (2008).
33. Watanabe, M., Lu, Y., Breen, M. & Hodes, R. J. B7-CD28 co-stimulation modulates central tolerance via thymic clonal deletion and Treg generation through distinct mechanisms. *Nat. Commun.* **11**, 6264 (2020).
This paper explores how CD28 costimulation contributes to both life (T_{reg} cell generation) and death (clonal deletion) during thymic selection.
34. Markowitz, J. S., Auchincloss, H., Grusby, M. J. & Glimcher, L. H. Class II-positive hematopoietic cells cannot mediate positive selection of $CD4^+$T lymphocytes in class II-deficient mice. *Proc. Natl Acad. Sci. USA* **90**, 2779–2783 (1993).
35. Lo, D. & Sprent, J. Identity of cells that imprint H–2-restricted T-cell specificity in the thymus. *Nature* **319**, 672–675 (1986).
36. Pishesha, N., Harmand, T. J. & Ploegh, H. L. A guide to antigen processing and presentation. *Nat. Rev. Immunol.* **22**, 751–764 (2022).
37. Murata, S. et al. Regulation of $CD8^+$T cell development by thymus-specific proteasomes. *Science* **316**, 1349–1353 (2007).
38. Gommeaux, J. et al. Thymus-specific serine protease regulates positive selection of a subset of $CD4^+$thymocytes. *Eur. J. Immunol.* **39**, 956–964 (2009).
39. Honey, K., Nakagawa, T., Peters, C. & Rudensky, A. Cathepsin L regulates $CD4^+$ T cell selection independently of its effect on invariant chain. *J. Exp. Med.* **195**, 1349–1358 (2002).
40. Anderson, K. L., Moore, N. C., McLoughlin, D. E., Jenkinson, E. J. & Owen, J. J. Studies on thymic epithelial cells in vitro. *Dev. Comp. Immunol.* **22**, 367–377 (1998).
41. Sharma, H. & Moroni, L. Recent advancements in regenerative approaches for thymus rejuvenation. *Adv. Sci.* **8**, 2100543 (2021).
42. Janeway, C. A. The T cell receptor as a multicomponent signalling machine: CD4/CD8 coreceptors and CD45 in T cell activation. *Annu. Rev. Immunol.* **10**, 645–674 (1992).
43. Robey, E. A. et al. Thymic selection in CD8 transgenic mice supports an instructive model for commitment to a CD4 or CD8 lineage. *Cell* **64**, 99–107 (1991).
44. Egawa, T. Regulation of CD4 and CD8 coreceptor expression and CD4 versus CD8 lineage decisions. in *Advances in Immunology* Vol. 125, 1–40 (Elsevier, 2015).
45. Shinzawa, M. et al. Reversal of the T cell immune system reveals the molecular basis for T cell lineage fate determination in the thymus. *Nat. Immunol.* **23**, 731–742 (2022).
This study used genetic engineering of mice to switch the co-receptor proteins encoded by *Cd4* and *Cd8* gene loci. These mice produce $CD4^+$ cytotoxic T cells and $CD8^+$ T helper cells, and the report explores why such an immune system fails to provide pathogen protection.
46. Gapin, L. Development of invariant natural killer T cells. *Curr. Opin. Immunol.* **39**, 68–74 (2016).
This paper reviews the development of lipid-specific T cells.
47. Crosby, C. M. & Kronenberg, M. Tissue-specific functions of invariant natural killer T cells. *Nat. Rev. Immunol.* **18**, 559–574 (2018).
This paper reviews the functions of lipid-specific T cells.
48. Salou, M., Legoux, F. & Lantz, O. MAIT cell development in mice and humans. *Mol. Immunol.* **130**, 31–36 (2021).
This paper reviews the development and functions of MAIT cells.
49. Ruscher, R. & Hogquist, K. A. Development, ontogeny and maintenance of TCRαβ+CD8αα IEL. *Curr. Opin. Immunol.* **58**, 83–88 (2019).
This paper reviews the development and functions of intraepithelial lymphocytes.
50. Stritesky, G. L., Jameson, S. C. & Hogquist, K. A. Selection of self-reactive T cells in the thymus. *Annu. Rev. Immunol.* **30**, 95–114 (2012).
51. Irla, M. Instructive cues of thymic T cell selection. *Annu. Rev. Immunol.* **40**, 95–119 (2022).
52. Bouillet, P. et al. BH3-only Bcl-2 family member Bim is required for apoptosis of autoreactive thymocytes. *Nature* **415**, 922–926 (2002).
53. Pobezinsky, L. A. et al. Clonal deletion and the fate of autoreactive thymocytes that survive negative selection. *Nat. Immunol.* **13**, 569–578 (2012).
54. McDonald, B. D., Jabri, B. & Bendelac, A. Diverse developmental pathways of intestinal intraepithelial lymphocytes. *Nat. Rev. Immunol.* **18**, 514–525 (2018).
55. Savage, P. A., Klawon, D. E. J. & Miller, C. H. Regulatory T cell development. *Annu. Rev. Immunol.* **38**, 421–453 (2020).
An in-depth consideration of how T_{reg} cells are selected in the thymus.
56. Bennett, C. L. et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat. Genet.* **27**, 20–21 (2001).
57. Fontenot, J. D., Gavin, M. A. & Rudensky, A. Y. Foxp3 programs the development and function of $CD4^+CD25^+$ regulatory T cells. *Nat. Immunol.* **4**, 330–336 (2003).
58. Kim, J. M., Rasmussen, J. P. & Rudensky, A. Y. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. *Nat. Immunol.* **8**, 191–197 (2007).
59. Hsieh, C.-S., Zheng, Y., Liang, Y., Fontenot, J. D. & Rudensky, A. Y. An intersection between the self-reactive regulatory and nonregulatory T cell receptor repertoires. *Nat. Immunol.* **7**, 401–410 (2006).
60. Malchow, S. et al. AIRE enforces immune tolerance by directing autoreactive T cells into the regulatory T cell lineage. *Immunity* **44**, 1102–1113 (2016).
61. Toomer, K. H. & Malek, T. R. Cytokine signaling in the development and homeostasis of regulatory T cells. *Cold Spring Harb. Perspect. Biol.* **10**, a028597 (2018).
62. Klein, L., Robey, E. A. & Hsieh, C.-S. Central $CD4^+$ T cell tolerance: deletion versus regulatory T cell differentiation. *Nat. Rev. Immunol.* **19**, 7–18 (2019).
A comprehensive review of $CD4^+$ T cell tolerance.
63. Lee, H.-M., Bautista, J. L., Scott-Browne, J., Mohan, J. F. & Hsieh, C.-S. A broad range of self-reactivity drives thymic regulatory T cell selection to limit responses to self. *Immunity* **37**, 475–486 (2012).
64. Tai, X. et al. Foxp3 transcription factor is proapoptotic and lethal to developing regulatory T cells unless counterbalanced by cytokine survival signals. *Immunity* **38**, 1116–1128 (2013).
65. Burchill, M. A. et al. Linked T cell receptor and cytokine signaling govern the development of the regulatory T cell repertoire. *Immunity* **28**, 112–121 (2008).
66. Lio, C.-W. J. & Hsieh, C.-S. A two-step process for thymic regulatory T cell development. *Immunity* **28**, 100–111 (2008).
67. Malhotra, D. et al. Polyclonal $CD4^+$ T cell tolerance is established by distinct mechanisms, according to self-peptide expression patterns. *Nat. Immunol.* **17**, 187–195 (2016).
This study tracked T cells reactive to a model self-antigen, GFP, in various mouse strains that express GFP in different tissues. It revealed that T cells specific for abundant self-antigens tend to be deleted, whereas those that recognize rare self-antigens tend to develop into T_{reg} cells.
68. Nagamine, K. et al. Positional cloning of the APECED gene. *Nat. Genet.* **17**, 393–398 (1997).
69. Mathis, D. & Benoist, C. Aire. *Annu. Rev. Immunol.* **27**, 287–312 (2009).
70. Su, M. A. & Anderson, M. S. Aire expands: new roles in immune tolerance and beyond. *Nat. Rev. Immunol.* **16**, 247–258 (2016).
71. Takaba, H. et al. Fezf2 orchestrates a thymic program of self-antigen expression for immune tolerance. *Cell* **163**, 975–987 (2015).
72. Michelson, D. A., Hase, K., Kaisho, T., Benoist, C. & Mathis, D. Thymic epithelial cells co-opt lineage-defining transcription factors to eliminate autoreactive T cells. *Cell* **185**, 2542–2558.e18 (2022).
This study describes the development of mTECs into multiple subtypes that mimic epithelial subtypes in peripheral tissues (such as muscle, skin and neuronal tissue). It shows that lineage-defining transcription factors are expressed in mTECs and facilitate the development of such 'mimetic' cells, which promote tolerance to the antigens expressed in different tissues.
73. Breed, E. R., Lee, S. T. & Hogquist, K. A. Directing T cell fate: how thymic antigen presenting cells coordinate thymocyte selection. *Semin. Cell Dev. Biol.* **84**, 2–10 (2018).
An overview of the various types of antigen-presenting cell in the thymus and their roles in selection.
74. Perera, J., Meng, L., Meng, F. & Huang, H. Autoreactive thymic B cells are efficient antigen-presenting cells of cognate self-antigens for T cell negative selection. *Proc. Natl Acad. Sci. USA* **110**, 17011–17016 (2013).

75. Lu, F.-T. et al. Thymic B cells promote thymus-derived regulatory T cell development and proliferation. *J. Autoimmun.* **61**, 62–72 (2015).
76. Walters, S. N., Webster, K. E., Daley, S. & Grey, S. T. A role for intrathymic B cells in the generation of natural regulatory T cells. *J. Immunol.* **193**, 170–176 (2014).
77. Martinez, R. J. et al. Type III interferon drives thymic B cell activation and regulatory T cell generation. *Proc. Natl Acad. Sci. USA* **120**, e2220120120 (2023).
78. 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).
79. Guilliams, M. et al. Unsupervised high-dimensional analysis aligns dendritic cells across tissues and species. *Immunity* **45**, 669–684 (2016).
80. Lei, Y. et al. Aire-dependent production of XCL1 mediates medullary accumulation of thymic dendritic cells and contributes to regulatory T cell development. *J. Exp. Med.* **208**, 383–394 (2011).
81. Bonasio, R. et al. Clonal deletion of thymocytes by circulating dendritic cells homing to the thymus. *Nat. Immunol.* **7**, 1092–1100 (2006).
82. Vobořil, M. et al. Toll-like receptor signaling in thymic epithelium controls monocyte-derived dendritic cell recruitment and Treg generation. *Nat. Commun.* **11**, 2361 (2020).
83. Zegarra-Ruiz, D. F. et al. Thymic development of gut-microbiota-specific T cells. *Nature* **594**, 413–417 (2021).
This paper reports that bacterial antigens from the gut are presented in the thymus under some conditions. These surprising findings present a new paradigm for T cell development.
84. Breed, E. R. et al. Type 2 cytokines in the thymus activate Sirpα+dendritic cells to promote clonal deletion. *Nat. Immunol.* **23**, 1042–1051 (2022).
85. Ferré, E. M. N., Schmitt, M. M. & Lionakis, M. S. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *Front. Pediatr.* **9**, 723532 (2021).
86. Policheni, A. N. et al. PD-1 cooperates with AIRE-mediated tolerance to prevent lethal autoimmune disease. *Proc. Natl Acad. Sci. USA* **119**, e2120149119 (2022).
87. Kishimoto, H. & Sprent, J. Negative selection in the thymus includes semimature T cells. *J. Exp. Med.* **185**, 263–271 (1997).
88. Xing, Y., Wang, X., Jameson, S. C. & Hogquist, K. A. Late stages of T cell maturation in the thymus involve NF-κB and tonic type I interferon signaling. *Nat. Immunol.* **17**, 565–573 (2016).
89. McCaughtry, T. M., Wilken, M. S. & Hogquist, K. A. Thymic emigration revisited. *J. Exp. Med.* **204**, 2513–2520 (2007).
90. Boursalian, T. E., Golob, J., Soper, D. M., Cooper, C. J. & Fink, P. J. Continued maturation of thymic emigrants in the periphery. *Nat. Immunol.* **5**, 418–425 (2004).
91. James, K. D. et al. Endothelial cells act as gatekeepers for LTβR-dependent thymocyte emigration. *J. Exp. Med.* **215**, 2984–2993 (2018).
92. Agus, D. B., Surh, C. D. & Sprent, J. Reentry of T cells to the adult thymus is restricted to activated T cells. *J. Exp. Med.* **173**, 1039–1046 (1991).
93. Santamaria, J., Darrigues, J., van Meerwijk, J. P. M. & Romagnoli, P. Antigen-presenting cells and T-lymphocytes homing to the thymus shape T cell development. *Immunol. Lett.* **204**, 9–15 (2018).
94. Fink, P. J. The biology of recent thymic emigrants. *Annu. Rev. Immunol.* **31**, 31–50 (2013).
95. Perera, J. & Huang, H. The development and function of thymic B cells. *Cell. Mol. Life Sci.* **72**, 2657–2663 (2015).
96. Ardouin, L. et al. Broad and largely concordant molecular changes characterize tolerogenic and immunogenic dendritic cell maturation in thymus and periphery. *Immunity* **45**, 305–318 (2016).
97. Srinivasan, J. et al. Age-related changes in thymic central tolerance. *Front. Immunol.* **12**, 676236 (2021).
98. Yang, S., Fujikado, N., Kolodin, D., Benoist, C. & Mathis, D. Immune tolerance. Regulatory T cells generated early in life play a distinct role in maintaining self-tolerance. *Science* **348**, 589–594 (2015).
99. Lancaster, J. N. et al. Central tolerance is impaired in the middle-aged thymic environment. *Aging Cell* **21**, e13624 (2022).
100. Zhang, B. et al. Glimpse of natural selection of long-lived T-cell clones in healthy life. *Proc. Natl Acad. Sci. USA* **113**, 9858–9863 (2016).
101. Kong, Y., Jing, Y. & Bettini, M. Generation of T cell receptor retrogenic mice. *Curr. Protoc. Immunol.* **125**, e76 (2019).
102. Perry, J. S. A. et al. Transfer of cell-surface antigens by scavenger receptor CD36 promotes thymic regulatory T cell receptor repertoire development and allo-tolerance. *Immunity* **48**, 923–936.e4 (2018).
103. Perry, J. S. A. et al. Distinct contributions of Aire and antigen-presenting-cell subsets to the generation of self-tolerance in the thymus. *Immunity* **41**, 414–426 (2014).
104. Jenkinson, E. J., Franchi, L. L., Kingston, R. & Owen, J. J. Effect of deoxyguanosine on lymphopoiesis in the developing thymus rudiment in vitro: application in the production of chimeric thymus rudiments. *Eur. J. Immunol.* **12**, 583–587 (1982).
105. Kurd, N. & Robey, E. A. T-cell selection in the thymus: a spatial and temporal perspective. *Immunol. Rev.* **271**, 114–126 (2016).
106. Pala, F., Notarangelo, L. D. & Bosticardo, M. Inborn errors of immunity associated with defects of thymic development. *Pediatr. Allergy Immunol.* **33**, e13832 (2022).
107. Padron, G. T. & Hernandez-Trujillo, V. P. Autoimmunity in primary immunodeficiencies (PID). *Clin. Rev. Allergy Immunol.* https://doi.org/10.1007/s12016-022-08942-0 (2022).
108. Akiyama, T. et al. The tumor necrosis factor family receptors RANK and CD40 cooperatively establish the thymic medullary microenvironment and self-tolerance. *Immunity* **29**, 423–437 (2008).
109. Fischer, A. Severe combined immunodeficiencies (SCID). *Clin. Exp. Immunol.* **122**, 143–149 (2000).
110. Villa, A., Santagata, S., Bozzi, F., Imberti, L. & Notarangelo, L. D. Omenn syndrome: a disorder of Rag1 and Rag2 genes. *J. Clin. Immunol.* **19**, 87–97 (1999).
111. Walkovich, K. & Vander Lugt, M. ZAP70-related combined immunodeficiency. in *GeneReviews®* (eds Adam, M. P. et al.) (University of Washington, 1993).
112. Huang, Q. et al. Molecular feature and therapeutic perspectives of immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. *J. Genet. Genom.* **47**, 17–26 (2020).
113. Sullivan, K. E. Chromosome 22q11.2 deletion syndrome and DiGeorge syndrome. *Immunol. Rev.* **287**, 186–201 (2019).
114. Hsu, P. et al. CHARGE syndrome: a review. *J. Paediatr. Child Health* **50**, 504–511 (2014).
115. Reith, W. & Mach, B. The bare lymphocyte syndrome and the regulation of MHC expression. *Annu. Rev. Immunol.* **19**, 331–373 (2001).
116. Yasumizu, Y. et al. Myasthenia gravis-specific aberrant neuromuscular gene expression by medullary thymic epithelial cells in thymoma. *Nat. Commun.* **13**, 4230 (2022).
117. Cooper, J. D. History of thymectomy for myasthenia gravis. *Thorac. Surg. Clin.* **29**, 151–158 (2019).

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