Journey through the thymus: stromal guides for T-cell development and selection

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Abstract | Lympho–stromal interactions in multiple microenvironments within the thymus have a crucial role in the regulation of T-cell development and selection. Recent studies have implicated that chemokines that are produced by thymic stromal cells have a pivotal role in positioning developing T cells within the thymus. In this Review, I discuss the importance of stroma-derived chemokines in guiding the traffic of developing thymocytes, with an emphasis on the processes of cortex-to-medulla migration and T-cell-repertoire selection, including central tolerance.

"…and yet men are so foolishly venturous as to set out lightly on pilgrimage, and to come without a guide."
John Bunyan

The thymus is an organ that supports the differentiation and selection of T cells[1–3]. The thymic development of T cells consists of several processes that require the dynamic relocation of developing lymphocytes into, within and out of the multiple environments of the thymus (FIG. 1). These processes include: first, the entry of lymphoid progenitor cells into the thymus; second, the generation of $CD4^+CD8^+$ double-positive (DP) thymocytes at the outer cortex of the thymus; third, the positive and negative selection of DP thymocytes in the cortex; fourth, the interaction of positively selected thymocytes with medullary thymic epithelial cells (mTECs) to complete thymocyte development and ensure central tolerance; and last, the export of mature T cells from the thymus[4–9] (FIG. 2).

For developing thymocytes to experience these key events at the right places and in the right order, the developing thymocytes and thymic stromal cells have to communicate with each other both in close proximity and remotely. However, this lympho–stromal communication does not readily fit a typical textbook view of thymic education, in which the stromal 'teacher' unilaterally instructs the lymphocyte 'apprentices' in the thymus 'classroom'. In fact, thymocyte development involves a stringent repertoire selection in which only 1–3% of thymocytes succeed in survival and export from the thymus[10–12]. In addition, thymic stromal cells need to be closely coached by developing thymocytes to provide the appropriate microenvironments for promoting and regulating further thymocyte development[13–16]. Therefore, the lympho–stromal communication is a bilateral coordination, or crosstalk, between architectural stromal cells and travelling thymocytes[14]. The travelling thymocytes have to create their own path by letting their presence be known to the stromal cells and by changing the stromal environment for further development. Despite this ability, however, most of the thymocytes are unable to complete their journey through the thymus, so that they are seldom 'happy tourists' travelling through various thymic scenes. Consequently, the crosstalk between thymocytes and thymic stromal cells offers the view that developing thymocytes resemble 'path-making pilgrims' who make their way through the thymus, struggling for their life beyond the thymus.

Among such crosstalk signals, chemokines that are produced by thymic stromal cells in individual microenvironments seem to have a pivotal role in guiding the direction of migrating thymocytes, and developing thymocytes seem to find their way by sequentially expressing different chemokine receptors. Recent studies of mice that are deficient for certain chemokines or chemokine receptors have shown the important role of chemotactic guidance in controlling T-cell development in the thymus (TABLE 1). This Review summarizes our current understanding of the trafficking of developing thymocytes and the crosstalk in the thymus, and provides a discussion of the role of chemotactic signals in regulating thymocyte trafficking and development.

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Thymic primordium
The primordium refers to an organ or tissue in its earliest recognizable stage of development. The primordium of the thymus is generated at the ventral aspect of the third pharyngeal pouch as early as embryonic day 10.5 in mice.

Thymic parenchyma
The parenchyma refers to the functional part of an organ. The parenchyma of the thymus is surrounded by the capsule, the trabeculae and the perivascular spaces.

Entry of T-progenitor cells into the thymus

The seeding of the thymus with lymphoid progenitor cells (BOX 1) occurs as early as embryonic day 11.5 (E11.5) in mice and the eighth week of gestation in humans[17,18], and is mediated by at least two different pathways: the vasculature-independent pathway, which probably occurs during the early stage of embryonic development before vascularization of the thymus, and the vasculature-dependent pathway, which probably occurs in the late stage of embryogenesis and postnatally after vascularization. It is thought that vasculature-independent colonization of the fetal thymus is regulated by the chemotactic attraction of lymphoid progenitor cells to the thymic primordium (FIG. 3a). The partial but significant roles of two chemokines, CC-chemokine ligand 21 (CCL21) and CCL25, in this early stage of fetal thymus colonization have been reported[19–21]. CCL21 and CCL25 are expressed by the fetal thymus primordium, along with several other chemokines[19,20]. In a naturally occurring mutant mouse strain *plt*/*plt*, which is deficient for CCL21, or in mice that are deficient for CC-chemokine receptor 7 (CCR7), which is the receptor for CCL21, the number of thymocytes was partially but significantly lower than that in normal mice until E14.5 (REF. 20). Mice deficient for CCR9, the receptor for CCL25, showed a three-fold decrease in total thymocyte cellularity until E17.5 (REF. 21). These results indicate that CCL21 and CCL25 are partially involved in fetal thymus colonization. Whether the combination of CCL21 and CCL25 is sufficient for fetal thymus colonization or whether other chemokines also have a role in this process is unclear. It has also been shown that CXC-chemokine ligand 12 (CXCL12) and its receptor CXC-chemokine receptor 4 (CXCR4) are not involved in early fetal thymus colonization[22].

In the postnatal thymus, lymphoid progenitor cells that have just entered the thymic parenchyma are found mainly in the area close to the cortico–medullary junction, where the vasculature is well developed, indicating that the lymphoid progenitor cells enter the postnatal thymus by transmigrating from the blood to the thymic parenchyma, mainly through the area around the cortico–medullary junction[23] (FIG. 2a). Whereas the role of chemokines in postnatal thymus seeding is unclear, it has been reported that thymus seeding of the adult thymus is regulated by the adhesive interaction between platelet (P)-selectin glycoprotein ligand 1 (PSGL1), which is expressed by circulating lymphoid progenitor cells, and P-selectin, which is expressed by the thymic endothelium[24]. Interestingly, the entry of lymphoid progenitor cells into the thymus is not a continuous event but an intermittent and gated event that occurs in waves during embryogenesis and in adulthood[25–27]. During embryogenesis, distinct waves of thymus-colonized cells give rise to distinct progenies of γδ T cells with different usages of T-cell receptor (TCR)-Vγ and Vδ chains, indicating that T-lymphoid progenitor cells that colonize the fetal thymus differ in developmental potential from T-lymphoid progenitor cells that enter the postnatal thymus[27–31]. In the adult thymus, the receptivity to chimerism of total thymocytes (largely of the αβ T-cell lineage) varies cyclically with a periodicity of 3–5 weeks in non-irradiated mice, as shown by quantitative intravenous bone-marrow adoptive-transfer experiments and the timed separation of parabiotic adult mice[25].

Cortex formation and outward traffic

Following entry into the thymus, lymphoid progenitor cells begin their development into T cells through the developmental pathway that is commonly identified by the expression profiles of CD25 and CD44,

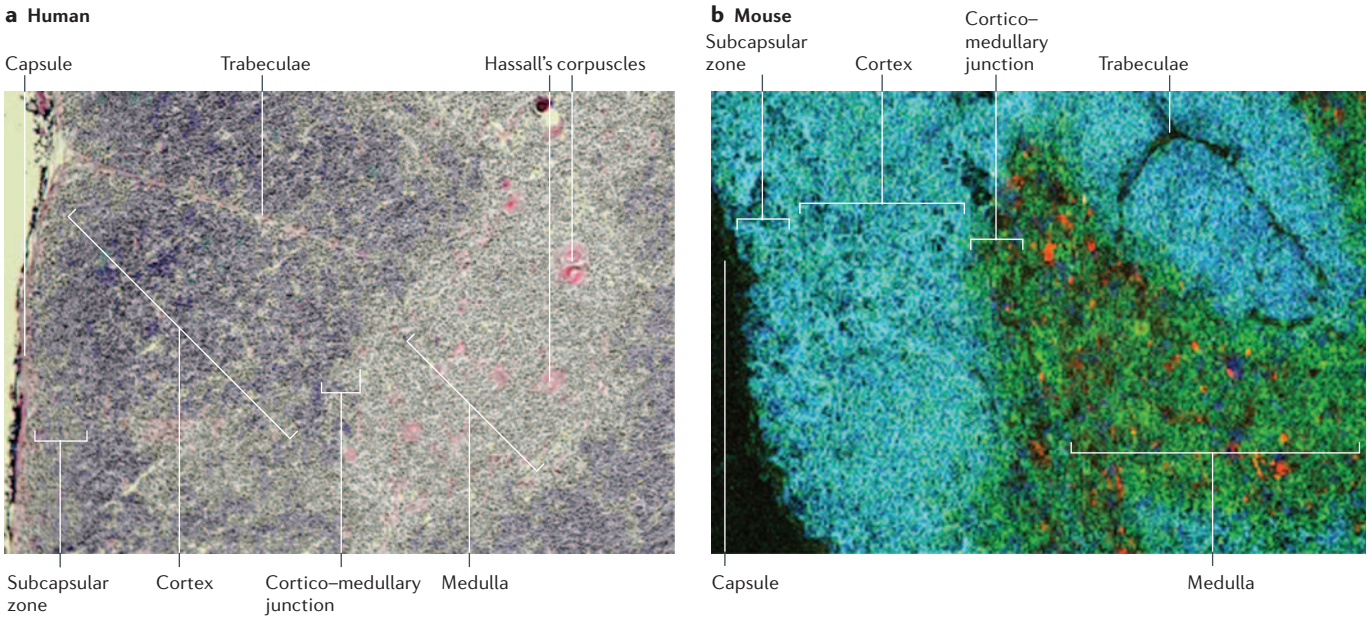

Figure 1 | **The thymus architecture. a** | The human thymus section from a newborn child was stained with haematoxylin and eosin. **b** | The thymus section from an adult C57BL/6 mouse was stained for CD4 (green), CD8 (blue), and medullary thymic epithelial cells defined using *Ulex europaeus* agglutinin 1 (red). Cells in cyan indicate the co-expression of CD4 and CD8, therefore being $CD4^+CD8^+$ double-positive cells.

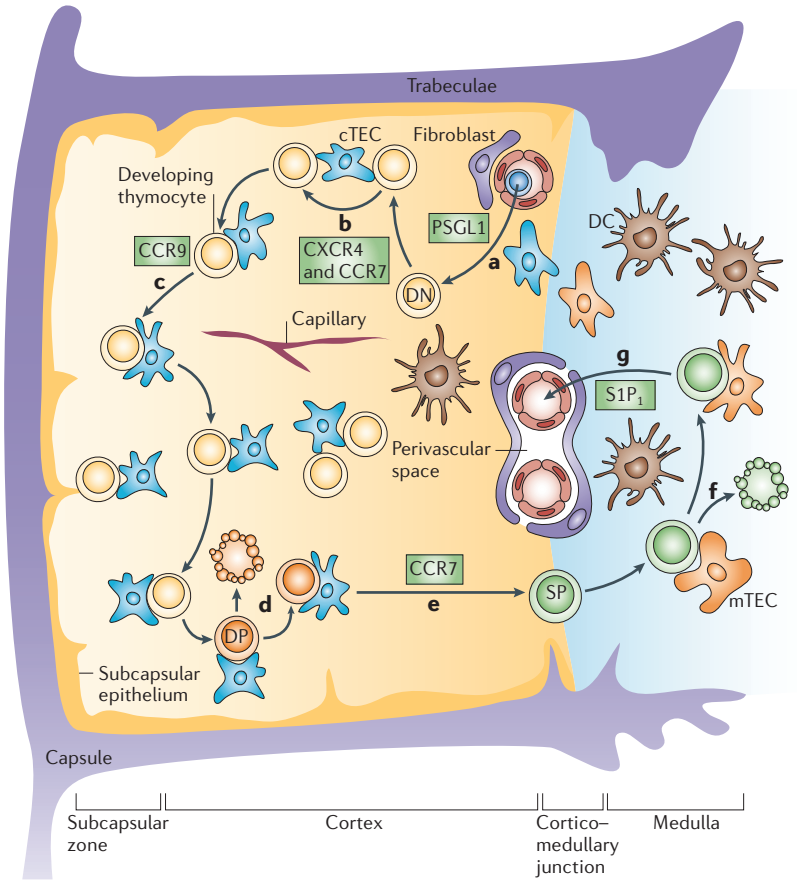

Figure 2 | **Traffic of thymocytes for T-cell development and selection. a** | In the postnatal thymus, circulating T-lymphoid progenitor cells migrate into the thymic parenchyma through the vasculatures that are enriched around the cortico–medullary junction. **b** | The outward migration of $CD4^-CD8^-$ double-negative (DN) thymocytes to the capsule is regulated by chemokine signals through CXC-chemokine receptor 4 (CXCR4) and CC-chemokine receptor 7 (CCR7). **c** | Further outward migration of the DN thymocytes to the subcapsular region is mediated by CCR9 signals. **d** | $CD4^+CD8^+$ double-positive (DP) thymocytes generated in the outer cortex are motile, interacting with stromal cells that are localized in the cortex for positive and negative selection. **e** | Positively selected DP thymocytes that gain the capability to survive and differentiate into CD4 or CD8 single-positive (SP) thymocytes show an increase in the surface expression of CCR7, through which the cells are attracted to the medulla, which expresses CCR7 ligands. **f** | In the medulla, further selection of SP thymocytes includes the deletion of tissue-specific-antigen-reactive T cells and the generation of regulatory T cells. **g** | Mature SP thymocytes express sphingosine-1-phosphate receptor 1 ($S1P_1$), through which the cells are attracted back to the circulation that contains a high concentration of sphingosine-1-phosphate. cTEC, cortical thymic epithelial cell; DC, dendritic cell; mTEC, medullary thymic epithelial cell; PSGL1, platelet-selectin glycoprotein ligand 1.

until the developmental checkpoint at the $CD4^-CD8^-CD25^+CD44^-$ (double-negative 3, DN3) stage[32,33]. Only the cells that succeed in in-frame rearrangement of the gene encoding the TCR β-chain are selected for further differentiation beyond this DN3 stage. The initial thymocyte development until the DN3 stage is promoted by Notch-mediated signals delivered by binding of Delta ligands[34,35] and is supported by signals delivered by interleukin-7 (IL-7)[36,37]: the signals derived from cortical thymic epithelial cells (cTECs) (FIG. 3c).

Along this developmental pathway, immature DN thymocytes promote the differentiation of thymic stromal cells and trigger the formation of the cortical-epithelial environment in the thymus[15,16,38,39]. In mice that are deficient for thymocyte development beyond the DN1 stage, such as a mouse strain carrying a transgene encoding human CD3ε, TECs are arrested at the immature stage, at which they express both keratin 5 and keratin 8, and they are unable to differentiate into cTECs that express keratin 8 but not keratin 5 ($keratin\ 5^-keratin\ 8^+$)[38]. Accordingly, the thymus in these mice does not form a histologically normal cortex and contains large cysts[15,16]. However, in mice that are deficient for thymocyte development beyond the DN3 stage, such as recombination-activating gene 1 (RAG1)-deficient mice, $keratin\ 5^-keratin\ 8^+$ cTECs and a histologically normal cortex are generated in the thymus[38]. Therefore, the differentiation of thymocytes from the DN1 stage to the DN3 stage regulates the differentiation of TEC precursor cells into cTECs that form the cortical environment in the thymus (FIG. 3b).

Concomitantly, DN thymocytes relocate outwards from the cortico–medullary junction to the subcapsular region of the thymic cortex[23] (FIG. 2b). Several chemokine receptors, including CXCR4, CCR7 and CCR9, have been suggested to be involved in this movement of immature thymocytes[40–42]. Using a proximal *Lck*-promoter-driven immature-thymocyte-specific deletion of *Cxcr4*, it was found that the CXCR4-deficient DN thymocytes fail to move efficiently outwards from the cortico–medullary junction to the cortex and fail to differentiate beyond the DN stages[40]. An independent report also showed that the development of thymocytes that are derived from CXCR4-deficient progenitor cells is severely affected at stages before the generation of $CD4^+CD8^+$ DP thymocytes[22]. It was also shown that DN2 thymocytes that are deficient for CCR7 (the receptor for CCL19 and CCL21) are partially arrested at the cortico–medullary junction[41]. By contrast, another group showed that DN2 and DN3 thymocytes in CCR9-deficient mice are distributed normally throughout the cortex but are inefficient at accumulating in the subcapsular region[42]. The development of DN thymocytes was partially arrested in the absence of CCR7 but was not disturbed in CCR9-deficient mice[41,42]. Therefore, the chemokine-dependent outward localization of the thymus-seeded progenitor cells from the cortico–medullary junction to the outer cortex seems to be essential for optimal initiation of thymocyte development, although the accumulation of DN3 thymocytes in the subcapsular region might not be required for further thymocyte development.

In the thymic cortex, on their way to the subcapsular region, DN thymocytes begin to rearrange their *Tcrb* locus and the cells that succeed in generating the in-frame *Tcrb* rearrangement begin assembling TCRβ and pre-TCRα (pTα) chains to form the cell-surface pre-TCR complex[43,44]. The successful expression of the pre-TCR complex on the cell surface[44,45], along with the Delta–Notch interaction[46], initiates the signals for further development to DP thymocytes that express TCRαβ antigen receptors. This is the first checkpoint of

Two-photon laser fluorescence microscopy
A fluorescence-imaging technique that takes advantage of the fact that fluorescent molecules can absorb two photons simultaneously during excitation before they emit light. This technique greatly reduces photodamage of living specimens, improves tissue penetration depth, allows the distinct separation between excitation and emission wavelengths, and confines the excitation to a discrete focal point.

T-cell development at the DN3 stage that was mentioned earlier, which censors the cells that have succeeded in in-frame TCRβ rearrangement and allows further development of thymocytes beyond the DN3 stage. It has been shown that the subcapsular region is rich in transforming growth factor-β (TGFβ), which retards the cell-cycle progression of pre-DP thymocytes and negatively regulates the generation of DP thymocytes[47], indicating that the migration of pre-DP thymocytes to the subcapsular region might have a role in regulating the rate of production of DP thymocytes (FIG. 2c).

Positive selection guides to the medulla

DP thymocytes that are newly generated in the cortex express low levels of the TCRαβ antigen–receptor complex. The cortical DP-thymocyte population contains the unselected repertoire of T cells, making this population the main target of the most rigorous positive and negative selection[48,49]. Visualization of green-fluorescent-protein-expressing thymocytes within an isolated intact thymus using two-photon laser fluorescence microscopy showed that most thymocytes in the cortex of the adult thymus are highly motile (with a velocity of ~3–8 m/min)[50,51]. The motile thymocytes pause to interact through their TCR with peptide–MHC complexes that are expressed by stromal cells, such as cTECs, and dendritic cells in the cortex[51]. Following TCR recognition of peptide–MHC ligands at low-avidity interactions, DP thymocytes are induced to receive signals for survival and further differentiation into single-positive (SP) thymocytes. This process, referred to as positive selection, enriches 'useful' T cells that are potentially reactive to foreign antigens, but not to self antigens, presented by self-MHC molecules. By contrast, high-avidity interactions elicit signals that lead to the deletion of thymocytes. Apoptosis is the process that has been best characterized as the mechanism of negative selection. The process of negative selection contributes to the deletion of self-reactive T cells, thereby avoiding autoimmunity. In addition to the negatively selected thymocytes, most cortical DP thymocytes fail to receive TCR signals and are also destined to die at this stage. Therefore, only 3–5% of developing thymocytes survive this checkpoint of T-cell development at the cortical DP-thymocyte stage[11,12] (FIG. 2d; FIG. 3d; FIG. 4a).

Table 1 | **Chemokines that are implicated in guiding thymocytes**

Receptor	Receptor-expressing cells in the thymus‡	Ligand§	Ligand-expressing cells in the thymus‡	Role in the thymus as identified in gene-knockout mice
CCR4	$CD62L^-CD69^+$ SP thymocytes (R, F)[53] $CD3^+CD4^+CD8^{low}$ thymocytes (F)[82] Medullary thymocytes (R)[83]* $CD4^+CD8^{low}$ and DP thymocytes (F)[83]*	CCL17 (TARC)	Dendritic cells (R)[80,81] Mostly in the medulla (R)[81]	Not determined, although possibly associated with negative selection
		CCL22 (MDC)	Outer walls of Hassall's corpuscles (H)[82,83]* A fraction of mTECs (H)[82]*	
CCR7	SP thymocytes (R, F)[52,53,82] (P)[41,54] TCR-stimulated DP thymocytes (P)[54] DN1 and DN2 thymocytes (P)[41] SP and a fraction of DP thymocytes (F)[83,108]*	CCL19 (ELC, MIP3β)	A fraction of mTECs (H)[54] Endothelial venules in the medulla (H)[88] Medulla, CMJ, blood vessels (H)[41] mTECs (high levels), cTECs (low levels), dendritic cells (R)[88] mTECs (H)[83]*	Attraction of positively selected thymocytes to the medulla[54] Guidance of postnatal thymus exit[88] Outward relocation of DN thymocytes towards the outer cortex[41] Attraction of fetal progenitor cells to pre-vascular fetal thymus[20]
		CCL21 (SLC, 6Ckine)	Most mTECs (H)[54] Medulla, CMJ, cortical dendritic cells and macrophages (H)[41] mTECs (high levels), cTECs (low levels)[88]	
CCR9	DP thymocytes and $CD62L^-CD69^+$ SP thymocytes (F)[53,109–111] DN3–DN4, DP and a fraction of SP thymocytes (P)[42] DP thymocytes and γδ T cells (P)[112] DP thymocytes (high levels), SP thymocytes (low levels) (R)[110] DP and SP thymocytes (F)[113]*	CCL25 (TECK)	cTECs and mTECs (H)[41] cTECs and a fraction of mTECs (H)[109] mTECs (high levels), cTECs (low levels) (R)[109]	Outward relocation of DN2 or DN3 thymocytes to the subcapsular region[42] Fetal thymus accumulation[21]
CXCR4	DN1–DN4 thymocytes (R, F)[22,40,52] DN, DP and SP thymocytes (R, F)[22,52] Dendritic cells (H)[114]* DP and SP thymocytes (P, F)[108]*	CXCL12 (SDF1, SDF1α)	Medulla, CMJ (H)[41] A subset of cTECs, not in the medulla (R)[40] Hassall's corpuscles, epithelial cells (H)[114]*	Outward relocation of DN thymocytes towards the outer cortex[40] Development of DN thymocytes[22]

*These data were obtained from human tissues and cells; all other data are derived from mouse studies. ‡The method of detection is indicated in parentheses by: R, RNA analysis including *in situ* hybridization; F, functional analysis of chemotaxis; P, protein detection by flow cytometry; H, histological analysis of sections using antibodies. §The popular synonym(s) for the ligand are shown in parentheses. CCL, CC-chemokine ligand; CCR, CC-chemokine receptor; CMJ, cortico–medullary junction; cTECs, cortical thymic epithelial cells; CXCL, CXC-chemokine ligand; CXCR, CXC-chemokine receptor; DN, double negative; DP, double positive; ELC, Epstein–Barr virus-induced molecule 1 ligand chemokine; MDC, macrophage-derived chemokine; MIP3β, macrophage inflammatory protein 3β; mTECs, medullary thymic epithelial cells; SDF, stromal-cell-derived factor; SLC, secondary lymphoid-tissue chemokine; SP, single positive; TARC, thymus and activation-regulated chemokine; TCR, T-cell receptor; TECK, thymus-expressed chemokine.

Box 1 | Entry into the thymus and commitment to T cells

Whether or not the thymus-colonizing lymphoid progenitor cells are committed to the T-cell lineage and have lost the potential to become other cell lineages, such as the B-cell lineage, remains unclear and is discussed by other authors in this issue[101]. Using a single-cell assay for progenitor potential, it was shown that the lymphoid progenitor cells that colonize the fetal thymus are not multi-lineage lymphoid progenitor cells but are a mixture of T-lymphoid progenitor cells, B-lymphoid progenitor cells and myeloid progenitor cells, and that the cells that are committed to the T-lymphoid lineage (which includes αβ T cells and γδ T cells, as well as natural killer cells) no longer retain the capacity to become B cells or myeloid cells[102]. Accordingly, the choice between B cells and T cells during embryogenesis occurs before contact with the fetal thymic epithelium is made[103,104]. Also, in the adult thymus, the canonical T-lymphoid progenitor cells detected in the thymus (that is, the double-negative 1 a-b fraction[105] or the early T-cell lineage progenitor (ETP) fraction[106]) seem to retain limited or no B-cell potential[105,106], indicating that similar to the fetal thymus, the adult thymus might be seeded by lymphoid progenitor cells that are already committed to the T-cell lineage and have lost the potential to become B cells. However, recent single-cell assays for immature thymocytes isolated from the adult thymus (of the CC-chemokine-receptor-9-enhanced green-fluorescent-protein knock-in allele) have shown that the adult thymus is seeded by multi-lineage progenitor cells that give rise to both B cells and T cells[107].

Positively selected DP thymocytes then begin relocating from the cortex to the medulla[50]. Early studies using a chemotaxis assay and mRNA measurement showed that the expression of the chemokine receptor CCR7 by developing thymocytes is associated with the phenotypic stage of cortex-to-medulla migration during the development of immature DP thymocytes to mature SP thymocytes[52,53]. It has been recently shown that following TCR ligation, cortical DP thymocytes show an increase in cell-surface expression of CCR7 (REF. 54). By contrast, CCR7 ligands (CCL19 and CCL21) in the postnatal thymus are predominantly produced by mTECs[54]. Therefore, cortical DP thymocytes that have received TCR-mediated signals are attracted to the medulla through CCR7-mediated chemotaxis (FIGS 2e,4b). Because negatively selected DP thymocytes are destined to die, irrespective of CCR7 expression, the chemotactic attraction to the medulla through CCR7 and its ligands is applied to positively selected thymocytes. Indeed, in mice that are deficient for CCR7 or CCR7 ligands, positively selected mature thymocytes are found in the cortex because they fail to be attracted to the medulla[54], whereas the forced expression of CCR7 in premature thymocytes causes the relocation of DP thymocytes to the medulla[55]. How the CCR7-mediated signals in the thymus regulate both the outward movement of DN thymocytes[41] and the inward movement of TCR-engaged DP thymocytes is still unclear.

In addition to the chemotactic attraction for the migration of positively selected thymocytes, there is an inward flow of interstitial fluid within the thymic parenchyma. Intraperitoneal administration of labelled proteins or small particles has indicated that liquid flow is present in the postnatal thymus from the capsular and subcapsular areas to the medullary area[56,57]. Although the regulation of thymocyte movement by this inward flow was not detected in isolated thymus lobes[50], the *in vivo* migration of developing thymocytes from the cortex to the medulla might be regulated by the combination of active chemotaxis and passive inward flow. However, the migration of developing thymocytes to the medulla cannot be solely mediated by the passive inward flow, because the migration of thymocytes to the medulla is defective in mice that are deficient for positive selection[13,58,59] and in mice that are deficient for CCR7 or CCR7 ligands[54].

In addition to the migration of thymocytes to the medulla, the formation of the medullary architecture is severely defective in mice that are deficient for positive selection, such as TCRα- or ZAP70 (ζ-chain-associated protein kinase of 70 kDa)-deficient mice[13,58,59], and is mildly defective in mice that are deficient for CCR7 or CCR7 ligands[54]. Therefore, the positive selection of thymocytes is a prerequisite for both the formation of the medulla and the migration of positively selected thymocytes to the medulla (FIG. 3e). How positive selection of thymocytes leads to the formation of the medulla is unclear, although it is known that the signals of the nuclear factor-κB (NF-κB)-mediated signal pathway, occuring through lymphotoxin-β receptor, tumour-necrosis-factor-receptor-associated factor 6 (TRAF6), NF-κB-inducing kinase (NIK) and the NF-κB subunit RelB are crucial for the development of the thymic medulla[60–63].

Tolerance in the medulla and thymic export

Positively selected DP thymocytes are induced to differentiate into SP (that is, $CD4^+CD8^-$ or $CD4^-CD8^+$) thymocytes and relocate to the medulla. The SP thymocytes are assumed to spend approximately 12 days in the medulla before being exported from the thymus[11]. During this

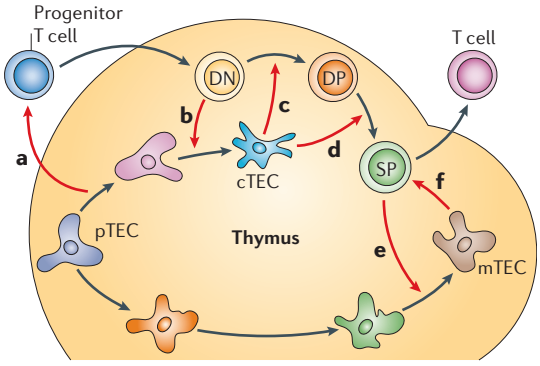

Figure 3 | **Crosstalk between thymocytes and thymic stromal cells.** Thymic stromal cells, including the common progenitor thymic epithelial cells (pTECs), attract the entry of T-lymphoid progenitor cells to the thymus (signal **a**). The developing double-negative (DN) thymocytes are required for pTECs to generate cortical thymic epithelial cells (cTECs) (signal **b**) that form the cortical environment that is needed to promote the generation of double-positive (DP) thymocytes (signal **c**) and the positive selection of DP thymocytes (signal **d**). The generation of single-positive (SP) thymocytes by the positive selection of DP thymocytes is required for the development of mature medullary thymic epithelial cells (mTECs) (signal **e**) that form the medullary environment to support the maturation, further selection and export of mature SP thymocytes (signal **f**) to supply the peripheral T-cell pool. Red arrows indicate crosstalk signals.

Interstitial fluid
The fluid in the spaces between cells and tissues, outside the lymphatic or cardiovascular systems. Its composition is similar to plasma and lymph.

period, the SP thymocytes go through a maturation process that is commonly identified by the expression profiles of CD62 ligand (CD62L; also known as lymphocyte (L)-selectin) and CD69, and with the acquisition of functional capability of these cells as mature but naive T cells[64–66]. The newly generated SP thymocytes are CD62L^{low}CD69^{hi} semi-mature cells that are functionally incompetent and susceptible to various apoptotic signals, including dexamethasone, and undergo further maturation to become mature SP thymocytes that are functional, dexamethasone-resistant and CD62L^{hi}CD69^{low}.

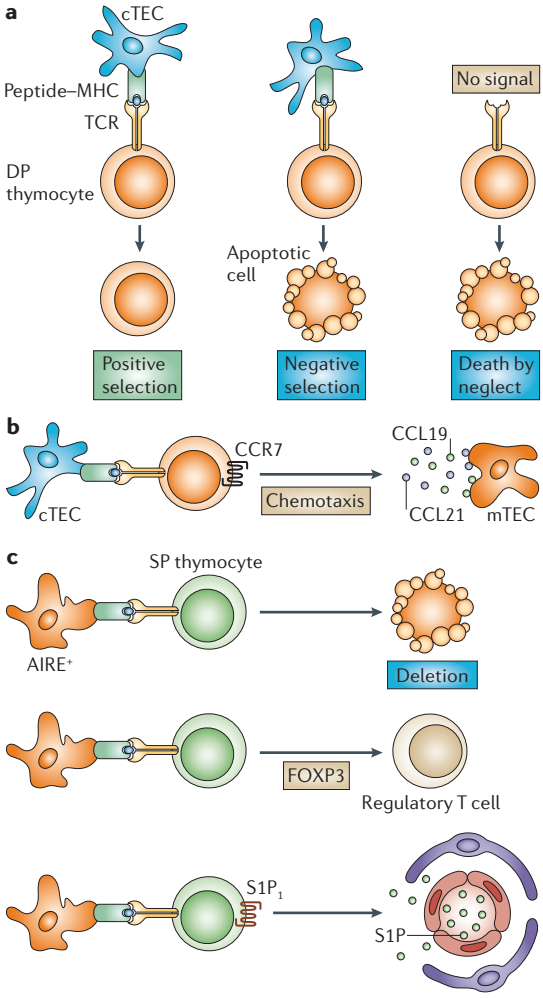

Figure 4 | **Positive selection and migration to the medulla. a** | Double-positive (DP) thymocytes that are generated in the thymic cortex are selected for their T-cell receptor (TCR) recognition specificity by interacting with peptide–MHC complexes that are presented in the cortex by cortical thymic epithelial cells (cTECs) and dendritic cells. **b** | Positively selected thymocytes are induced to express CC-chemokine receptor 7 (CCR7) as well as to undergo the programme of differentiation into single-positive (SP) thymocytes, and CCR7-expressing thymocytes are attracted to the CCR7 ligands, CC-chemokine ligand 19 (CCL19) and CCL21, which are produced by medullary TECs (mTECs) and mainly localized in the medulla. **c** | In the medulla, newly generated SP thymocytes are further selected by the medullary stromal cells, including autoimmune regulator (AIRE)-expressing mTECs, so that the cells that are reactive to tissue-specific antigens can be deleted. The maturation of SP thymocytes in the medulla includes the production of regulatory T cells and the expression of sphingosine-1-phosphate receptor 1 ($S1P_1$). $S1P_1$-expressing mature T cells seem to be attracted to the circulation, where the concentration of S1P is high. FOXP3, forkhead box P3.

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy
(APECED or autoimmune polyendocrine syndrome type 1). APECED is characterized by the presence of two of three clinical symptoms: Addison's disease and/or hypoparathyroidism and/or chronic mucocutaneous candidiasis. It is caused by a mutation in the gene autoimmune regulator (*AIRE*).

Hassall's corpuscles
Small clusters or concentric whorls of stratified keratinizing epithelium in the thymic medulla, possibly involved in the negative selection of thymocytes, the generation of regulatory T cells and/or undergoing apoptosis themselves. They are found clearly in the human thymus, but are unclear in the mouse thymus.

G-protein-coupled receptor
(GPCR). A receptor that is composed of seven membrane-spanning helical segments. These receptors associate with G-proteins, which are a family of trimeric intracellular-signalling proteins with common β- and γ-chains, and one of several α-chains. The α-chain determines the nature of the signal that is transmitted from a ligand-occupied GPCR to downstream effector systems.

The process of SP-thymocyte maturation presumably occurs in the medulla and is accompanied by further deletion of self-reactive thymocytes that have escaped negative selection in the cortex (FIGS 2f,4c). Such additional deletion in the medulla seems to be particularly important in establishing central tolerance to tissue-specific antigens, as mTECs express tissue-specific antigens promiscuously[67]. The expression of tissue-specific antigens by mTECs is at least partially dependent on the transcriptional factor autoimmune regulator (AIRE)[68,69]. Indeed, AIRE deficiency causes failure in establishing central tolerance to tissue-specific antigens, resulting in autoimmune polyendocrinopathy-candidiasis-ectodermal-dystrophy (APECED) in humans[70,71] and similar autoimmune diseases in mice[72–74]. This process of medullary tolerance is shown to occur, at least in part, through the deletion of self-reactive T cells[73,75,76]. In addition to the deletional mechanism that ensures self-tolerance, it is also important to note that the medulla is thought to be the place for the production of regulatory T cells[77–79]. It has been shown that most forkhead box P3 (FOXP3)-expressing regulatory T cells within the thymus are found in the medulla[78]. Therefore, the period of maturation in the medulla seems to be essential for SP thymocytes to establish central tolerance by ensuring the deletion of T cells that are reactive to tissue-specific antigens and by producing regulatory T cells (FIGS 3f,4c).

A recent report has highlighted the role of Hassall's corpuscles in the generation of regulatory T cells through production of the cytokine thymic stromal lymphopoietin (TSLP)[79]. It was shown that Hassall's corpuscles express TSLP, which activates thymic dendritic cells that in turn induce the generation of regulatory T cells[79]. Independent studies have indicated that CCL17 is expressed by dendritic cells that are mostly localized in the medulla[80,81], and CCL22 is expressed by the outer walls of Hassall's corpuscles[82,83], whereas CCR4 (a receptor for CCL22 and CCL17) is expressed by semi-mature SP thymocytes[53,82,83]. These chemokine signals might direct medullary semi-mature SP thymocytes to Hassall's corpuscles and have a role in establishing central tolerance by enhancing the generation of regulatory T cells and/or optimizing negative selection.

It has been long appreciated that thymocyte emigration is an active process controlled by signals mediated by G-protein-coupled receptors[84]. It was recently shown that sphingosine-1-phosphate receptor 1 ($S1P_1$), one of the S1P receptors, is expressed by mature SP thymocytes and that $S1P_1$ is required for the egress of T cells from the adult thymus[85]. During maturation from DP thymocytes, SP thymocytes acquire the expression of $S1P_1$ (REFS 85,86). However, the concentration of S1P seems to be higher in serum than in most tissues[87]. These results indicate that the chemoattraction of

$S1P_1$-expressing mature thymocytes to circulating S1P regulates the egress of mature T cells from the postnatal thymus (FIG. 2g). In addition to S1P-mediated chemotaxis, the role of some chemokines in thymocyte emigration has been reported. It was shown that CCL19-mediated CCR7 signals contribute to thymocyte emigration in newborn mice but not in adult mice[88], indicating that there might be a developmental switch between CCR7-mediated emigration and $S1P_1$-mediated emigration during ontogeny. It was also shown that the repulsive movement of SP thymocytes repelled from a high concentration of CXCL12 has a role in the emigration of T cells from artificial thymus organoids[89].

The export of mature thymocytes from the thymic parenchyma to the circulation is thought to occur through the perivascular space, which is channelled to post-capillary venules, arterioles and lymphatics[90,91]. However, whether emigrating T cells in the perivascular space are subsequently released into the blood, into the lymphatics or both is still unclear. The recirculation of mature T cells back to the thymic medulla has also been described[92], although both its rationale and mechanism are unknown.

Concluding remarks

It has become evident that the chemotactic signals that are delivered by several chemokines have an essential role in positioning developing thymocytes among multiple microenvironments within the thymus. Certainly, chemokines are not the only molecules that guide the migration of thymocytes. Other molecules, such as adhesion molecules (for example, selectins and integrins) and cell-guiding molecules other than chemokines (for example, ephrins and metalloproteinases), might be involved in navigating thymocyte migration[93–95], probably in cooperation with the chemokine signals[96,97]. It is also apparent that the molecular basis for the other aspect of crosstalk — that is, the signals from lymphoid cells to stromal cells — remains largely unclear and should be explored in the future.

The contribution of other facets of chemokine signals, such as the promotion of cell survival in thymocyte development, is still unclear. How developing thymocytes are released from a chemokine signal after reaching the corresponding chemokine-expressing stromal cells is another mystery. Nonetheless, it is important to note that the chemokine-mediated positioning of developing thymocytes greatly affects the developmental efficiency and central tolerance of developing T cells. In this context, we now realize that the control of chemotactic signals might serve as an attractive tool in designing and manipulating the therapeutic regulation of T-cell development[98]. For example, controlling the outward chemokine signals should aid in promoting and regulating the development of pre-selected thymocytes, which might be useful for resetting the T-cell repertoire in autoimmunity or transplantation. Conversely, the selective control of the inward chemokine signals might selectively support or regulate the development of positively selected thymocytes, which might be useful for enhancing T-cell generation and T-cell-mediated immunity. By contrast, the medullary migration of developing thymocytes and their intimate contact with mTECs should be carefully considered in ensuring central tolerance to tissue-specific antigens and in pursuing the therapeutic reconstruction of the functional thymus for the regeneration of the T-cell system in various diseases[99,100]. Further understanding of the molecular and cellular mechanisms that regulate thymocyte pilgrimage are warranted.

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Competing interests statement
The author declares no competing financial interests.

DATABASES
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