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REVIEW

Tracking migration during human T cell development

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Abstract Specialized microenvironments within the thymus are comprised of unique cell types with distinct roles in directing the development of a diverse, functional, and self-tolerant T cell repertoire. As they differentiate, thymocytes transit through a number of developmental intermediates that are associated with unique localization and migration patterns. For example, during one particular developmental transition, immature thymocytes more than double in speed as they become mature T cells that are among the fastest cells in the body. This transition is associated with dramatic changes in the expression of chemokine receptors and their antagonists, cell adhesion molecules, and cytoskeletal components to direct the maturing thymocyte population from the cortex to medulla. Here we discuss the dynamic changes in behavior that occur throughout thymocyte development, and provide an overview of the cell-intrinsic and extrinsic mechanisms that regulate human thymocyte migration.

Keywords Human thymocytes · Migration · Chemokines · Integrins · Extracellular matrix · T cell receptor

Abbreviations

AIRE	Autoimmune regulator
BM	Bone marrow
CMJ	Cortical medullary junction
cTEC	Cortical thymic epithelial cell
DCs	Dendritic cells
DN	$CD4^{-}CD8^{-}$, double negative
DP	$CD4^{+}CD8^{+}$, double positive
ECM	Extracellular matrix
FTY720	Fingolimod
FTOC	Fetal thymic organ culture
HPCs	Hematopoietic progenitor cells
ICAM-1	Intercellular adhesion molecule 1
IL2Rgamma	IL2 receptor gamma chain
mTEC	Medullary thymic epithelial cell
MS	Multiple sclerosis
MST1	Mammalian sterile 20-like protein kinase 1
NOD	Non-obese diabetic
pMHC	Peptide-MHC complexes
PNAd	Peripheral node addressin
PSGL-1	P-selectin glycoprotein ligand 1
SCID	Severe combined immune deficiency
SP	$CD4^{+}CD8^{-}$ or $CD8^{+}CD4^{-}$, single positive
S1P	Sphingosine-1-phosphate
TCR	T cell receptor
TEC	Thymic epithelial cell
TSLP	Thymic stromal lymphopoietin
VCAM-1	Vascular cell adhesion molecule 1

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Introduction

The development of T cells capable of providing immunity against a diverse set of microbial and environmental antigens must be carefully balanced with deletion of potentially auto-reactive cells. The generation of these functionally mature, self-tolerant T cells occurs within anatomically and functionally distinct regions of the thymus, each containing specialized niches that regulate the sequential stages of thymocyte differentiation and maturation (Fig. 1). Thus, the complex and carefully coordinated journey of thymocytes through the thymus is essential for proper T cell development. Mechanistic insight into the events that direct thymocyte migration could guide the development of strategies to manipulate the T cell repertoire in the treatment of immune-mediated diseases or to increase thymic output and improve immune reconstitution following bone marrow (BM) transplantation.

Structurally, the thymus is divided into an outer cortex and an inner medulla, two functionally distinct regions with specific contributions to the differentiation of T cells. BM-derived thymus-seeding progenitor cells arrive predominantly via the vasculature and enter the cortex, where initial T cell commitment at the $CD4^{-}CD8^{-}$ (double negative, DN) progenitor stage occurs (Fig. 1a, b). Following the productive rearrangement of the TCR β chain and the developmental transition to the $CD4^{+}CD8^{+}$ (double positive, DP) stage, the process of positive selection ensures the development of T cells with functional αβ T cell receptors (TCRs) (Fig. 1c, d). Cortical thymic epithelial cells (cTECs) have distinct antigen-processing capabilities that result in the generation of a unique peptide repertoire optimized for positive selection [1–3]. During positive selection, DP thymocytes scan the unique repertoire of self-peptides presented by cTECs in the context of MHC, and those DP cells with TCRs that engage in low-affinity interactions with these self-peptide/MHC complexes go on to become $CD4^{+}$ or $CD8^{+}$ single positive (SP) thymocytes. The transition from the DP to SP stage is accompanied by the relocalization of thymocytes from the cortex to the thymic medulla (Fig. 1e). While there is evidence that negative selection of thymocytes bearing TCRs with high-avidity for ubiquitous self-antigens begins in the cortex [4], the thymic medulla is necessary for the deletion of additional self-reactive thymocytes. The specialized medullary microenvironment contains a higher concentration of dendritic cells (DCs) to present self-antigens for negative selection, and medullary thymic epithelial cells (mTECs) that express tissue-restricted antigens under the control of the transcription factor autoimmune regulator (AIRE) [5]. Thymocytes that successfully complete these stringent TCR selection events are exported from the thymus as mature $CD4^{+}$ and $CD8^{+}$ SP T cells to perform unique effector functions in host immune defense (Fig. 1f). Defective thymocyte migration at any of these stages of T cell development can result in lymphopenia or autoimmune disease.

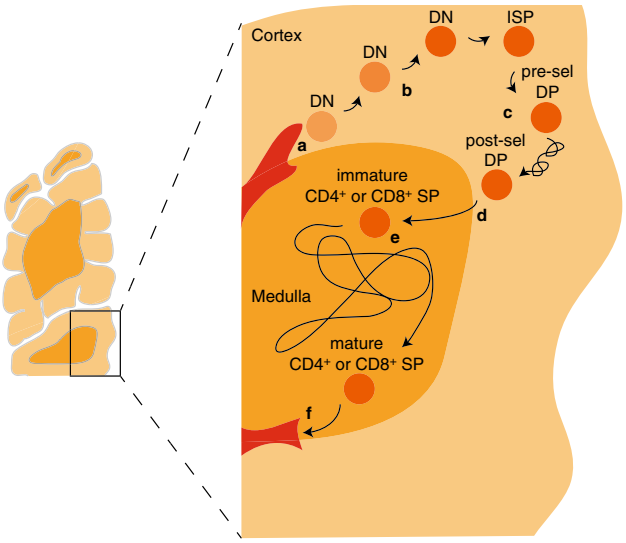


Fig. 1 Traffic of thymocytes in the human thymus. *Boxed area* represents an enlarged cortico-medullary region of the human multilobular thymic structure. **a** Circulating thymus-colonizing hematopoietic progenitor cells arrive via the vasculature and enter the postnatal thymus at the cortico-medullary junction. **b** Early progenitors commit to the T cell lineage and differentiate to DN thymocytes as they migrate through the cortex. **c** Those DN cells which productively rearrange the TCR β gene then proceed to up-regulate CD4 and CD8 and progress to a DP stage. DP thymocytes scan the cortex and interact with cTEC and cortical dendritic cells and proceed through positive and negative selection. **d** Positively selected DP thymocytes that have received a low-affinity TCR signal differentiate into SP thymocytes and migrate to the medulla. **e** The process of negative selection continues in the medulla, where thymocytes with high avidity for tissue-restricted antigens are deleted. **f** Mature SP thymocytes exit the thymus and enter the circulation

Much of what we know about thymocyte development and migration is based on studies in mice, but to what degree this is applicable to humans is not known. While there are many similarities between mouse and man, there are also salient differences, highlighting the need for the direct study of human T cell development. In this review, we will provide an overview of human thymocyte migration and how it impacts the organization of the human thymus, the differentiation of human T cells, and its role in human disease.

Models to study human thymocyte migration

Exploration of the molecular mechanisms underlying human primary immune deficiencies has led to the identification of many of the signaling cues involved in human T cell development and migration, and modeling these

Table 1 Differential expression of integrins implicated in thymocyte migration

Integrins	Expression of integrins by thymocyte subset				References
	DN	Pre-selection DP	Post-selection DP	SP	
α4β1	+++/adhesion	++/adhesion	++/migration	+/migration	[106, 107, 118, 119]
α5β1	+++/adhesion	±	++/migration	++/migration	[106, 107, 118, 119]

Table 2 Differential expression of chemokines and chemokine receptors implicated in human thymocyte migration

Chemokine receptors	Expression of chemokine receptors by thymocyte subset				References	Ligands	Location of ligand expression	References
	DN	Pre-selection DP	Post-selection DP	SP				
CXCR4	+++F, P	+++F, P	+F, P	++F, P	[16, 102, 103, 112, 113]	CXCL12/SDF-1	Cortex M	[9]
CCR7	+F, P	−F, P	+F, P	++/+++F, P	[16, 62]	CCL19/MIP-3 beta, ELC, exodus-3, CK beta 11	Medulla S	[62]
						CCL21/6Ckine, Exodus-2, SLC, TCA-4, CK beta 9	Medulla S	[20]
CCR9	+F	+++F	+++F	++F	[16, 104, 105]	CCL25/TECK, CK beta 15	Cortex S	[20]
CCR4		+F, H[a]	+F, H[a]	++F, H	[62]	CCL22/MDC	Medulla S	[62, 63]
CCR3	−F, P	+F, P[a]	+F, P[a]	++P, F	[65]	CCL11/Eotaxin	Medulla S	[65]
CXCR3	+F, P	−F, P[a]	−F, P[a]	++F, P	[64]	CXCL9/MIG	Medulla H, S	[64]
						CXCL10/IP-10	Subcapsular cortex S, medulla H, S	[64]
						CXCL11/I-TAC	Medulla H, S	[64]

The method of detection is indicated by: *F* functional chemotaxis assays, *P* protein expression by flow cytometry, *S* immunohistochemical or fluorescent antibody staining of sections, *H* in situ hybridization, *M* gene expression profiles by microarray analysis

CCR chemokine receptor, *CXCR* chemokine receptor, *CCL* chemokine ligand, *CXCL* chemokine ligand

[a] Results from DP population as a whole, no distinction between pre- and post-selection DP thymocytes

deficiencies in mice remains a powerful tool for the study of immune development. Thymic tissue is rarely available from immunodeficient patients. However, normal human thymic issue can be obtained from fetal products of conception or as a surgical byproduct during pediatric cardiac surgery. These tissues have provided invaluable information regarding the phenotype of human thymocytes and their compartmentalization during the different stages of development. Immunostaining of fixed human thymic tissue has enabled the characterization of human epithelial cells and DCs within the cortex and medulla and has also provided some information on the patterns of chemokine expression that direct human thymocyte migration within the thymus. However, technical challenges associated with immunostaining for secreted proteins such as chemokines have yielded conflicting results. For example, fluorescent and immunohistochemical staining suggests CXCL12 is expressed along the cortico-medullary junction (CMJ) or in the medulla of the human thymus [6, 7]. In contrast, gene expression profiles of human and murine purified thymic stromal cell populations clearly indicate higher CXCL12 expression in cortical versus medullary thymic epithelial cells [8, 9].

A number of in vitro assays have been developed to study human cell migration, each representing a simplified approximation of in vivo conditions [10]. In vitro transwell and two-dimensional migration studies have identified candidate chemokines and adhesion molecules that could potentially play a role in guiding human thymocyte migration at distinct stages of development (summarized in Tables 1, 2). Although it is well established that chemokine concentration gradients can direct cell migration in vitro, only recently has it been shown that the migration of DCs in vivo is dependent on a functional chemokine gradient

[11]. While in vivo evidence is lacking for human T cells, the development of 3D chemotaxis assays for live imaging of cell movement within a collagen matrix has allowed for the direct visualization of human T cell migration along a concentration gradient [12]. The migration of human thymocytes in vivo is likely governed by the integration of a multitude of different, and possibly opposing, signaling cues, whose additive effect determines the overall migration pattern of thymocytes at a given stage of development. Thus, these data should be viewed in context with data from complementary systems to study human thymocyte migration in situ.

To this end, several models to study human thymocyte migration in situ have been developed. Studies using chimeric fetal thymic organ cultures (FTOC), in which human progenitors are seeded onto murine fetal thymic lobes that can be maintained in culture for several weeks, have yielded fundamental information regarding the identity of human thymus-seeding progenitors and their differentiation potential, and have also helped characterize the stages of human thymocyte differentiation [13]. These interspecies organotypic culture systems allow for use of an expanded toolkit of the many genetic manipulations available in mice, but have the disadvantage that not all receptor:ligand interactions between species are conserved. Alternately, FTOCs composed of minced human thymic fragments provide a three-dimensional human thymic environment [14, 15]. However, these fragments do not necessarily recapitulate the subcapsular regions of the cortex nor do they reflect the cortico-medullary compartmentalization of a mature thymus. More recently, we have adapted a thymic slice model in which the cortical/medullary architecture is maintained, and in conjunction with two-photon microscopy, enables us to examine the dynamics of human thymocyte motility and behavior in their native tissue [16]. While human thymic organotypic culture systems provide excellent models in which to study human thymocyte migration, the lack of vascular or lymphatic connections makes them less suitable for examining thymic entry or egress.

Humanized mice, in which human hematopoietic progenitors give rise to a human immune system, could provide an important system to study certain aspects of human thymocyte migration that are best addressed in vivo. However, significant sequence divergence between mouse and human cytokines greatly contributes to the decreased cellularity and limited reconstitution of the murine thymus in humanized mice, and there are likely other inefficiencies in interspecies receptor:ligand interactions that further complicate this system [17–19]. Therefore, although humanized mice may represent an improvement over existing models, important caveats remain when considering the suitability of humanized mice as a model for the study of some aspects of human thymocyte development and migration.

In sum, varied and complementary systems ranging from single-cell suspension assays to organotypic cultures and humanized mice have been creatively designed to dissect the signaling cues guiding human thymocyte development and migration. Each of these models presents their own set of advantages and limitations, but if carefully applied, can provide a diverse set of tools to dissect the signaling cues directing the journey of developing human T cells through the thymus. As we review the literature, we will point out limitations that remain and challenges that lie ahead as we investigate the different aspects of human thymocyte migration.

Organization of the thymus

Thymic organogenesis

Several aspects of basic thymus anatomy differ in mice and humans. For example, the multi-lobular organization of the human thymus is in contrast to the bi-lobed thymus of mice, and there are well-developed Hassall's corpuscles in the human but not the murine thymus. Despite these differences, both the murine and human thymus develop exclusively from the third pair of pharyngeal pouches, and many of the genes involved in thymus organogenesis are conserved between the two species [20]. Following detachment from their site of origin in the pharynx, the epithelial primordia of mammals migrate ventrally to fuse at the pericardium. During murine embryogenesis, the migration of the thymus to the anterior chest occurs at approximately embryonic day 11.5 (e11.5), concurrent with the appearance of the first hematopoietic progenitors and prior to the formation of blood vessels [21]. Similarly, in humans, the thymic anlage reaches its final position in the body by the 8th week of gestation, along with the concomitant colonization of the tissue by lymphoid progenitors and prior to the establishment of the thymic vasculature [20, 22, 23]. Human TEC differentiation follows the onset of Foxn1 expression around week 8, resulting in the segregation of cTECs and mTECs and the establishment of a distinct cortico-medullary boundary by weeks 13–16 [20–24]. Further maturation of the medulla is evidenced by the onset of AIRE expression in mTECs by week 13–14, and the appearance of Hassall's corpuscles by 14–15 weeks of gestation [22]. Defects in Foxn1 expression lead to thymic aplasia and the development of severe combined immune deficiency (SCID) in both humans and mice [25–27], while

an absence of AIRE expression results in severe autoimmunity [5, 28, 29], underscoring the conservation of gene expression and function in thymic organogenesis between the two species.

Notwithstanding remarkable similarities in thymus organogenesis, the ontogeny of T cell development differs significantly between species. The appearance of TCR$\gamma\delta^+$ thymocytes precedes that of TCR$\alpha\beta^+$ cells in the murine fetal thymus, whereas $\alpha\beta$ and $\gamma\delta$ T cells emerge simultaneously during human fetal thymopoiesis [23, 30, 31]. Additionally, murine T cells are not evident in the periphery until e15, while the appearance of mature thymocytes occurs comparatively earlier in human development, with seeding of peripheral organs by mature naïve T cells as early as 12–14 weeks of gestation [22, 32, 33]. Perhaps one of the most striking differences between murine and human fetal T cell development is the appearance of human T_{reg} cells in the periphery concurrently with the appearance of naïve T cells, while murine T_{reg} cells are not detected in the periphery before day 3 of life [34–37].

There is ample evidence in mice that the normal differentiation of cortical and medullary epithelial cells, as well as formation of a mature medullary compartment during organogenesis, depends on thymocyte-derived signals [38–43]. Strong evidence for thymocyte:epithelial cell cross talk is also evident from the thymic biopsies of patients with severe combined immune deficiencies (SCID) caused by mutations in the genes encoding for the IL2 receptor gamma chain (IL2Rγ), JAK3, and adenosine deaminase, among others. These defects in the early stages of human T cell development result in profound abnormalities of TEC differentiation, loss of cortico-medullary demarcation, and an absence of AIRE$^+$ mTECs and Hassall's corpuscles [44]. For example, Omenn syndrome presents with the unique combination of immunodeficiency and autoimmunity. Although the primary defect is a hypomorphic mutation in recombination-activating genes resulting in impaired VDJ recombination and an oligoclonal T cell repertoire [45, 46], the expansion of these oligoclonal self-reactive T cells in the periphery has been suggested to result from defective negative selection [47]. Indeed, the thymi of patients with Omenn syndrome have markedly reduced AIRE$^+$ mTECS, and these findings are replicated in a mouse model of Omenn syndrome, suggesting that impaired negative selection due to absent AIRE expression may allow the escape of self-reactive T cells [47–49]. Treatment of SCID or Omenn syndrome patients with BM transplantation results in thymus-dependent T cell reconstitution and development of a polyclonal T cell repertoire [50, 51]. Similarly, reconstitution of the thymic rudiment in Rag$^{-/-}$IL-2R$\gamma^{-/-}$ mice with human CD34$^+$ progenitor cells results in the formation of a well-defined medulla containing differentiated mTECs [18].

The thymic microenvironment

Thymic epithelial cells are the most abundant stromal cells of the thymus and define anatomically and functionally distinct regions involved in directing the step-wise differentiation of thymocytes. Specialized epithelial cells of the thymic cortex provide positive selection signals, while the medulla contains a unique epithelial cell population with "promiscuous" expression of tissue-restricted antigens. The human thymic medulla also contains well-developed Hassall's corpuscles composed of keratinized epithelial swirls, which have been implicated in the development of human regulatory T (T_{reg}) cells [52]. In addition to epithelial cells, the human and murine thymus also contains BM-derived hematopoietic cells. These include antigen presenting cells such as DCs, involved in negative selection events [4, 53, 54], and macrophages, implicated in the clearance of dying thymocytes [55–57].

In addition to their role in thymocyte selection, thymic epithelial cells help produce another important part of the thymic microenvironment—the extracellular matrix (ECM). Generally, the ECM is composed of combinations of multiple collagens, and several isoforms of both laminin and fibronectin [58]. The ECM network is well preserved between humans and mice, and while extensive in the medulla, it is composed of only fine fibers in the cortex [59]. ECM components are important for the integrity of the tissue, the division of the thymus into lobules, and additionally provide developing T cells cues to proliferate, differentiate, and migrate. Accordingly, thymocytes demonstrate developmentally regulated expression of ECM specific receptors such as integrins, trans-membrane receptors that mediate cell:cell and cell:ECM adhesion (Table 1). Thymocyte motility depends on the regulation of repeated cycles of integrin-mediated adhesion and de-adhesion, along with cytoskeletal rearrangements and the generation of cell polarity. Essential to the high degree of flexibility required for cell adhesion during motility is the ability to alter the avidity of integrins for their ligands through an intra-cellular signaling process known as inside-out signaling [60].

The interactions of thymocytes with their microenvironment are also controlled by chemokines, a family of secreted proteins that induce chemotaxis. Traditionally, chemokines and their receptors are divided into four subfamilies (CC, CXC, C, or CX3C), identified according to the pattern of cysteine residues in the ligands. Each chemokine can bind one or more trans-membrane G-protein coupled chemokine receptors, and in turn, each chemokine receptor can bind many ligands, resulting in a functional redundancy that attenuates the phenotype of many mouse knockout models for single chemokines [61]. In the human thymus, chemokines are differentially expressed by epithelial cells of the cortex,

medulla, and CMJ in a pattern of expression similar to that in mouse [8, 20, 62–65]. Chemokines may be tethered to the cell surface or secreted into the environment and bound by the ECM to establish a gradient [61]. Thymocytes, in turn, respond to chemokines via developmentally regulated expression of chemokine receptors, which helps thymocytes tune their chemotactic response to the local expression of ligands, and aids in migration from one microenvironment to the next (Table 2). Additionally, chemokines are important regulators of integrin avidity through the activation of inside-out signaling, which is implicated in the modulation of integrin adhesion [60].

Several other factors are known to modify thymocyte interactions with their microenvironment and may provide additional levels of modulation of thymocyte migration. These include galectins, cytokines, matrix metalloproteases, and glycosylaminoglycans. Additionally, semaphorins have recently been shown to modulate thymocyte interactions with the thymic microenvironment. Semaphorins bind to neuropilins, transmembrane glycoproteins classically known for their role in neuronal guidance during development, and their co-receptors, plexins, expressed by thymocytes, TECs, and thymic DCs. Semaphorins have been shown to modulate the adhesive capacity of neuropilin-expressing thymocytes and can also dampen chemokine induced migration [66, 67].

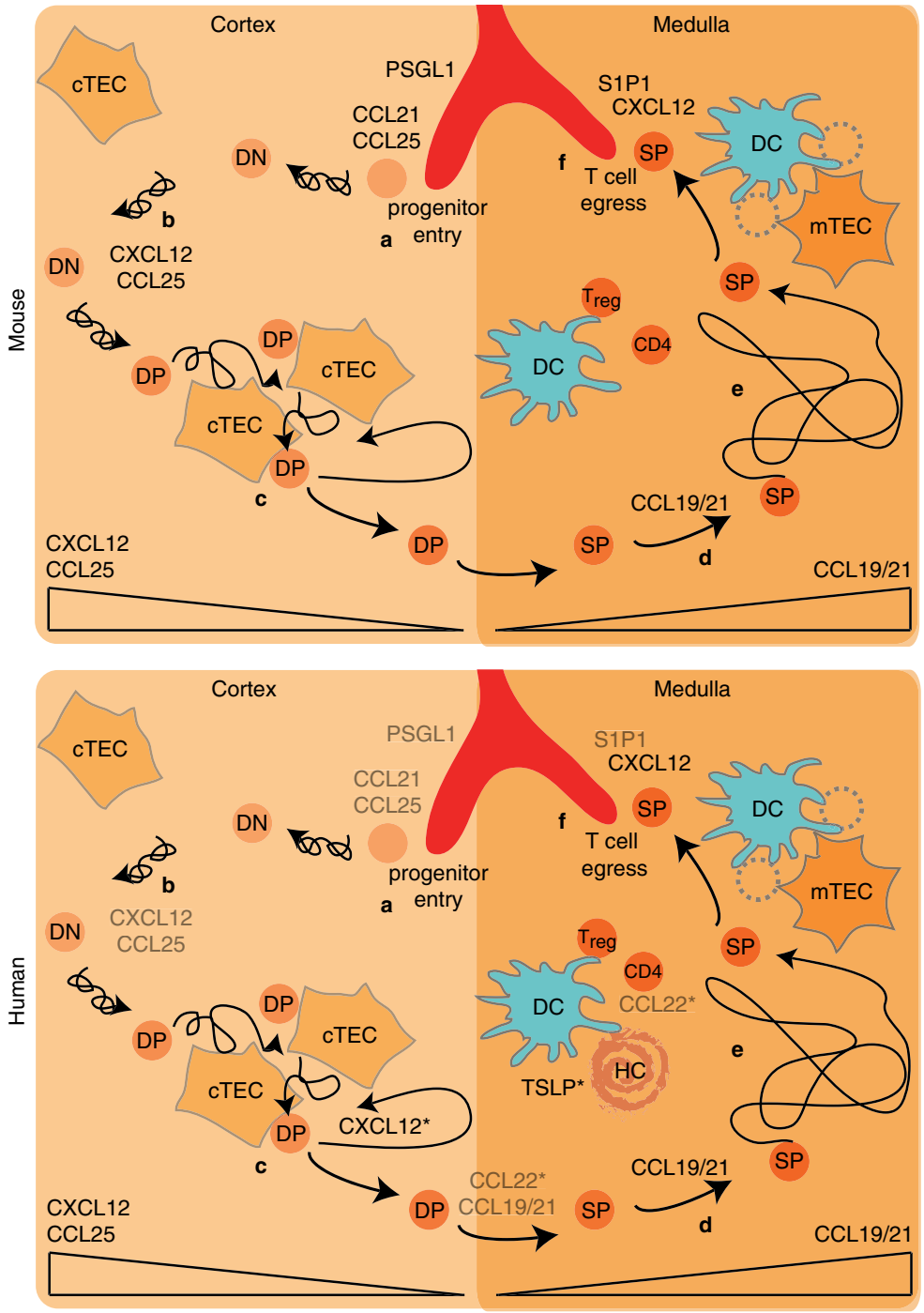
Fig. 2 Comparative overview of the factors that direct thymocyte migration in mice and men. **a** Entry of hematopoietic progenitors into the vascularized murine thymus at the CMJ requires binding of P-selectin on thymic endothelial cells through its ligand PSGL-1, on thymus-seeding progenitor cells, as well as expression of CCL21 and CCL25 by the thymic parenchyma. These factors likely also mediate progenitor entry into the human thymus. **b** In the mouse, the outward migration of DN thymocytes towards the sub-capsular region of the thymus is mediated by CCL25 and CXCL12. The preserved cortical predominance of these two chemokines in humans suggests they may guide the migration of DN thymocytes through the human cortex. **c** DP thymocytes scan the cortex in search of positive selecting signals, and CXCL12 is required to maintain the cortical localization of human DP cells, while the retention of murine DP thymocytes in the cortex may be a chemokine-independent process. **d** Positively selected DP thymocytes that have received a low-affinity TCR signal differentiate to a SP stage and migrate to the medulla in response to CCL19/21 in both human and mouse. **e** Negative selection of autoreactive T cells continues in the medulla, as well as maturation of SP thymocytes and differentiation of T_{reg} cells. In humans, TSLP-conditioned dendritic cells are essential for T_{reg} differentiation, and CCL22 may be involved in guiding T_{reg} progenitors to the specialized niche surrounding the Hassall's corpuscles. **f** In the mouse, mature SP thymocytes up-regulate $S1P_1$ and exit the thymus along an S1P gradient to enter the circulation, and CXCL12 has additionally been implicated in this process in both mice and humans. There is data to suggest that $S1P_1$ might also mediate the egress of human T cells from the thymus. *Black text* indicates factors that have been shown to influence thymocyte migration during development. *Grey text* indicates likely candidates to direct thymocyte migration that has not yet been proven. *Blue dotted lines* indicate dying cells. *Asterisk* indicates factors that appear to have unique, human-specific functions

Progenitor entry into the thymus

T cell progenitors arise from hematopoietic stem cells in the BM and journey through the blood to provide the thymus with a continuous supply of progenitors to maintain thymopoiesis. Several BM-derived human thymic progenitor cells have been identified. $CD34^{hi}CD45^{+}CD7^{+}$ hematopoietic progenitor cells (HPCs) are thought to be one of the earliest human thymus-seeding populations and retain the potential to differentiate into T, natural killer (NK), and B cells [68]. These HPCs emerge from the fetal BM at 8–9 weeks of gestation, and persist in significantly lower numbers into adult life [68]. Furthermore, $CD34^{+}CD7^{-}$ progenitors with erythroid, myeloid, and lymphoid differentiation potential have been found in postnatal human thymi, indicating that $CD7^{-}$ multipotent progenitors may also contribute to human thymopoiesis [69]. Evidence that these cells are thymus-settling progenitors derives from the detection of these populations in both the BM and thymus. Additionally, it has been shown that BM $CD34^{hi}CD45^{+}CD7^{+}$ hematopoietic cells are direct precursors of the earliest $CD34^{hi}CD1a^{-}$ fetal thymocytes and are attracted by, and have the ability to enter, the thymic parenchyma in ex vivo assays [68]. A separate group has further shown that in vitro-generated $CD34^{hi}CD45^{+}CD7^{+}$ HPCs have the ability to colonize the thymus in a humanized mouse model [70]. Whether additional BM-derived cells contribute to the thymic progenitor population and how these populations vary throughout ontogeny is not known.

While progenitor seeding of the postnatal murine thymus occurs in a focal manner via the vasculature at the CMJ, there is evidence of multifocal seeding of the human and murine thymus prior to establishment of the vasculature during fetal development [20, 68, 71]. First, there is scattered cortical distribution of the earliest $CD34^{+}$ immigrants in the human fetal thymus [20, 68]. In addition, it has been shown that human $CD34^{hi}CD45^{+}CD7^{+}$ HPCs displayed a heightened ability to enter the thymic parenchyma in ex vivo assays that closely mimic the earliest stages of thymus colonization prior to thymic vascularization. In these studies, fluorescently labeled human $CD34^{hi}CD45^{+}CD7^{+}$ HPCs seeded onto alymphoid thymic lobes from non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mouse embryos showed efficient and consistent infiltration into the thymic parenchyma across the basement membrane of the lobes and displayed a substantial capacity to generate $TCR\alpha\beta^{+}$ DP thymocytes [68].

Chemokines appear to play an important role in progenitor seeding of the human thymus. In the avascular murine fetal thymus, both CCL25 and CCL21 are required for



progenitor seeding of the tissue (Fig. 2a) [72–75]. Similarly, the expression of the chemokine CCL25 as early as the 8th week of gestation in the human thymic parenchyma, along with expression of its receptor, CCR9, on BM $CD34^{hi}CD45^{+}CD7^{+}$ cells, coincides with the appearance of the earliest HPCs within the human fetal thymus, suggesting a role for this chemokine in guiding human progenitor cells to the thymus (Fig. 2a) [20, 68]. Although expression of CCL21 is absent from the human fetal thymus until week 11, it is possible that, as in the mouse, CCL21 might instead be expressed by the adjacent parathyroid primordia, thus coordinating with thymic CCL25 expression to guide thymic progenitor entry into the avascular human fetal thymus [20, 75, 76]. Additionally, hematopoietic progenitors fail to enter the thymus of $Foxn1^{nu}$ (nude) mice, which may be accounted for by the absence of CCL25 expression by Foxn1-deficient thymic epithelial cells [72, 77, 78]. The common SCID phenotype in both mice and humans with

Foxn1 deficiencies suggests highly conserved Foxn1 gene expression and function, further implicating chemokine signaling in progenitor entry into the human thymus.

The role of other chemokines in human progenitor settling of the thymus is less clear. Human BM-derived $CD34^+$ progenitor cells with thymus-seeding potential also express CXCR4 and CCR7 and migrate in response to their respective ligands in vitro, suggesting a possible role for chemokine signaling in the recruitment and/or retention of human thymus colonizing cells during both fetal and adult stages of development [68, 79–81]. However, studies examining the effect of CXCL12:CXCR4 inhibition on the ability of human $CD34^+$ thymic precursors to repopulate murine fetal lobes have led to the suggestion that CXCR4 signaling is not required for progenitor entry into the thymus [7]. These studies used adult intrathymic progenitor cells that had presumably entered the thymus via the vasculature. Thus, it is important to also confirm these findings with early progenitor populations that are thought to seed the avascular fetal thymus. In addition, the ability of murine progenitor cells to settle the mature thymus is also contingent upon chemokine signaling through CCR9 and CCR7 [82, 83], but it is not yet known if these signals also influence human progenitor cell seeding of mature thymic tissue.

Entry of blood-borne lymphoid progenitors across the vascular bed of the mature human thymus likely mimics the requirements for entry into other tissues. Thus, it likely necessitates initiation of a rolling adhesion onto thymic microvasculature, followed by firm adhesion and cell arrest on vascular endothelium leading to extravasation of progenitors into the thymic parenchyma [60, 84]. In mice, it is well established that thymus-settling progenitors enter the adult thymic stroma at the well-perfused CMJ and that progenitor entry is dependent on the interaction between P-selectin on thymic endothelium and P-selectin glycoprotein ligand 1 (PSGL-1) on hematopoietic cells (Fig. 2a) [85, 86]. Data on the mechanism by which human progenitors enter the mature, vascularized thymus is scarce, likely due to the limited availability of models to address these questions. Assuming a certain degree of interspecies conservation, PSGL1 is expressed by early lineage marker negative ($CD2^-$, $CD3^-$, $CD14^-$, $CD16^-$, $CD19^-$, $CD24^-$, $CD56^-$, $CD66b^-$, and glycophorin-A^-) post-natal human thymocytes and to a lesser extent by in vitro generated $CD34^+CD7^+CD5^+$ progenitor cells with thymus-colonizing potential, and thus may also direct human progenitor seeding of the thymus (Fig. 2a) [70]. Another potential mediator of human progenitor thymic entry is via an L-selectin:peripheral node addressin (PNAd) interaction, as these molecules are expressed on BM-derived $CD34^+CD7^+$ cells and by the endothelium of the human thymus, respectively [87, 88]. It is interesting that BM endothelial cell-derived CXCL12 plays a role in the initiation of rolling adhesion and cell arrest on endothelial receptors of the human BM such as E-selectin, P-selectin, intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), and it is possible a similar process may mediate progenitor entry across the thymic vasculature [89, 90].

In sum, there is emerging evidence that chemokines and adhesion molecules direct and mediate hematopoietic progenitor cells to enter and seed the human thymus. Although direct confirmatory evidence of the signaling cues involved in progenitor settling of the thymus is lacking in humans, certain findings suggest interspecies similarities. In addition to further studies on these topics, it will also be interesting to note the interplay between chemokines and adhesion molecules in these processes.

Progression of cell surface marker expression during human thymocyte development

Human thymus-colonizing progenitor cells retain multilineage differentiation potential, although the capacity to develop along the B cell lineage is likely lost once exposed to the thymic environment [68, 91, 92]. Commitment to the T cell lineage requires Notch signaling and is marked by up-regulation of CD1a expression on human $CD34^+$ thymocytes [91–94]. The most immature cells in the human thymus are a heterogeneous population of DN cells that can be further subdivided on the basis of CD34 and CD1a expression into consecutive stages of differentiation similar to those in mouse. Human DN thymocytes then lose CD34 expression and progress to a $CD3^-CD4^+CD8^-$ intermediate single positive stage (CD4ISP), before developing into DP cells, and ultimately $CD4^+$ and $CD8^+$ mature SP T cells (Fig. 3) [95].

Human β-selection and initiation of TCRα rearrangement occurs at the $CD34^+CD38^+CD1a^+$ stage, similar to the timing of TCR gene rearrangement in mouse [94]. After completion of TCRα gene rearrangement and expression of an αβ TCR, DP cells that engage in productive low-affinity interactions with self-peptide:MHC complexes (pMHC) on thymic epithelial cells proceed through positive selection, while thymocytes that engage in high affinity interactions with pMHC are deleted by negative selection. As in mouse, CD69 expression marks the early stages of positive selection during human T cell development (Fig. 3) [96].

Positively selected thymocytes down-regulate either CD4 or CD8 to differentiate into SP thymocytes, which then undergo further negative selection and maturation in the medulla. Human SP thymocytes can be further classified according to expression of CD27, down-regulation of CD1a, acquisition of CD45RA, and loss of CD69 during terminal differentiation (Fig. 3) [96, 97].

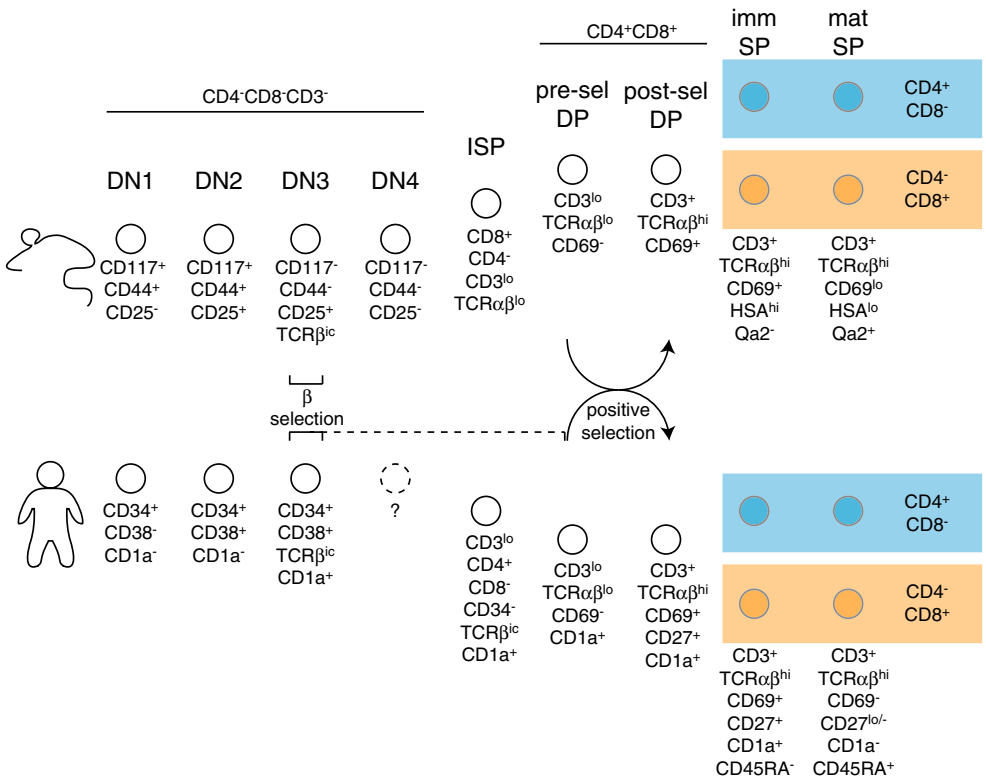


Fig. 3 Progression of cell surface marker expression during T cell development. Sequential view of the different phenotypes during mouse and human thymocyte development. Human DN thymocytes can be subdivided on the basis of CD34 and CD1a expression into maturational stages analogous to those in mouse. Transition from the DN to the DP stage proceeds through a $CD4^+$ ISP in humans, and CD69 up-regulation marks the early stages of positive selection. The final stages of human SP thymocyte maturation can be defined by the relative expression of CD69, CD1a, CD27, and CD45RA

Migration within the thymus

Cortical migration of thymocytes

In mice, the majority of chemokines expressed in the thymus are more abundant in the medulla relative to the cortex. The exceptions are CCL25 and CXCL12, and the outward migration of mouse DN thymocytes from the point of entry at the CMJ toward the sub-capsular zone is mediated by cortical gradients to these chemokines, as well as CCL21 (Fig. 2b) [8, 86, 98–101]. Conserved cortical-biased expression of CCL25 and CXCL12 point to a prominent role for CCR9 and CXCR4 signaling in guiding the migration of immature human cortical thymocytes (Fig. 2b) [9, 20]. Additionally, progressive differentiation of human DN thymocytes is accompanied by up-regulation of CCR7 and CCR9 surface expression, along with consistently high levels of CXCR4 expression [68, 102–104]. Further, it has been shown that human DN cells migrate in vitro in response to CXCL12, CCL25, and modestly to CCL21, the same three chemokines that have been suggested to play a role in the cortical migration of murine DN thymocytes [16, 105].

In addition to their ability to attract thymocytes, chemokines have also been shown to influence cell adhesion through modulation of integrin activation [89, 90]. In particular, CXCL12 has been shown to activate the integrins VLA-4/α4β1 and VLA-5/α5β1 on immature human $CD34^+$ hematopoietic cells. Interestingly, expression of CXCL12 is highest in the human cortex, and expression of its receptor, CXCR4, is highest among human DN thymocytes which also express high levels of both α4β1 and α5β1 and have been shown to adhere to fibronectin in vitro [9, 16, 106, 107]. Thus, it is likely that chemokines not only provide directional cues to developing thymocytes but also regulate thymocyte adhesion to the ECM, contributing to the distinct migration patterns of the different thymocyte populations [59].

Developmentally regulated expression of chemokine receptors continues at the DP stage of thymocyte differentiation, suggesting a role for chemokine signaling in the cortical localization of DP thymocytes. However, mice deficient for the receptors to CCL25 and CXCL12, the two exceptions to the predominantly medullary expression of chemokines, do not demonstrate overt defects at the DP stage of thymocyte development [73, 98, 99, 101,

108, 109]. Additionally, murine DP thymocytes overlaid on murine thymic slices were excluded from the medulla along a sharply defined boundary [110]. These observations have supported the notion that the cortical restriction of mouse DP thymocytes is a chemokine-independent process, and may instead be mediated by the inability of DP thymocytes to migrate on medullary substrate [110, 111]. In contrast, in vitro studies demonstrate that human pre-selection DP thymocytes ($CD3^{lo}CD69^{-}$) express high levels of CXCR4 and exhibit robust migration toward CXCL12 [16, 103, 112, 113]. Significantly, the transition through positive selection results in down-regulation of CXCR4 and loss of chemotaxis toward CXCL12, as well as improved migration to CCL21 coincident with CCR7 up-regulation in both humans and mice [16, 103, 112–117]. However, chemotaxis towards another predominantly cortical chemokine, CCL25, did not differ between pre- and post-selection human DP thymocytes [16]. To determine the role of chemokines in directing the migration of human thymocytes in situ, we adapted a thymic slice model in which purified human thymocytes are overlaid on either human or mouse thymic slices. Using this system, we demonstrated that inhibition of CXCR4 signaling resulted in the striking loss of cortical localization of pre-selection DP thymocytes, which instead accumulated in the medulla [16]. Importantly, this finding held true on both murine and human thymic stroma and was independent of whether CXCR4 signaling was inhibited before or after DP thymocytes were allowed to migrate into the cortex. These results indicate that CXCL12 is required to both direct and retain human pre-selection DP thymocytes to the cortex (Fig. 2c).

A prominent role for chemokine signaling in the accumulation of human DP thymocytes to the cortex does not preclude the involvement of integrin-mediated adhesion in mediating localization of human DP thymocytes. In fact, given the role of chemokines in the modulation of integrin avidity for their ligands, integrins are likely key players in the modulation of thymocyte motility [60, 90]. Additionally, expression of integrins is developmentally regulated in human thymocytes, suggesting distinct roles during T cell maturation [106, 118, 119]. Constitutive activation of α4β1 in $CD3^{lo}CD69^{-}$ pre-selection DP thymocytes mediates firm adhesion to fibronectin in vitro and results in a stationary state, whereas $CD3^{hi}CD69^{+}$ post-selection DP thymocytes do not adhere to fibronectin despite equivalent levels of α4β1 surface expression. In fact, the interaction of $CD3^{hi}CD69^{hi}$ DP thymocytes and mature SP thymocytes with fibronectin triggers migration rather than adhesion in a transwell assay, and involves ligation of both α4β1 and α5β1 [119].

Adding to the complexity of signaling interactions that are integrated by developing thymocytes to direct their migration in vivo, there is evidence for an additional level of control. TCR signaling has been demonstrated to induce expression of neuropilin-1 (NP-1) in human thymocytes, and ligation of its natural ligand, semaphorin-3A, on cortical and medullary TECs resulted in an inhibition of thymocyte adhesion [66]. More recent data demonstrates that semaphorin-3A additionally induces loss of chemotaxis towards CXCL12, and that this effect is mediated by down-regulation of CXCR4 expression among human DP and SP thymocytes [67]. These in vitro studies raise the interesting possibility that CXCR4 signaling, required for the cortical retention of human pre-selection DP thymocytes, may be modulated by TCR signaling during positive selection through the inhibitory effects of semaphorins. Chemokine signaling, in turn, affects integrin activation and thus modulates the ability of pre- and post-selection DP thymocytes to migrate on fibronectin. These findings suggest a tight integration of chemokine signaling, TCR activation, and adhesion to the ECM in the retention of human pre-selection DP thymocytes in the cortex.

Studies in the mouse have revealed a context-dependent effect of TCR signaling on thymocyte motility. We have noted that low-level TCR signals are required to sustain the motility of human polyclonal DP thymocytes consistent with indications of tonic TCR signaling in murine polyclonal DP thymocytes induced by MHC [16, 120]. This low-level tonic signaling is likely important for the generation of efficient positive selection signals, although the effect of altered motility on selection of the TCR repertoire and its influence on protective immunity has not been examined [53]. On the other hand, high-avidity TCR:MHC interactions associated with negative selection can lead to reduced motility and migratory arrest [53, 57, 121]. Using murine MHC class I- and II-deficient thymic slices in the chimeric human:mouse thymic slice model, we recently demonstrated that mouse MHC:human TCR signaling can support the activation and motility of polyclonal human DP thymocytes [16].

Both DN and DP murine thymocytes exhibit slow, confined migration patterns in the thymic cortex, and this behavior is conserved in immature human thymocytes. Two-photon imaging of human thymocyte migration within an intact thymic environment has revealed that DN and pre-selection DP thymocytes migrate with average speeds of 7 microns/min and ~4 microns/min, respectively [16]. These values are remarkably similar to those for mouse DN and DP thymocytes within the murine thymic cortex [120–122]. However, how cortical chemokines, adhesion molecules, and other factors regulate cortical thymocyte speed is not known. In mouse models, it is known that positive selection of DP thymocytes correlates with significant increases in motility [122]. Although similar data is not available at present to confirm this observation in human thymocytes, it is tempting to speculate that the significant

transition in chemokine receptor expression, chemokine sensitivity, integrin expression/activation, and TCR signaling contribute to these drastic changes in speed, and is consistent with the effects of some of these molecules on the in vitro migration of pre- and post-selection thymocytes. On the other hand, inhibition of chemokine signaling with pertussis toxin did not have a major impact on the overall motility of human pre-selection DP cells in situ [16]. However, the non-specific inhibition of all G-protein coupled receptors by pertussis toxin does not allow for a careful examination of the specific contribution of any one chemokine to motility, nor does it help discriminate between chemokines and other G-protein coupled receptors. Thus, the impact of chemokine signaling, integrin-mediated adhesion, and TCR signaling on the speed of immature human thymocytes requires further study.

Medullary migration of positively selected thymocytes

Prior to positive selection, high expression of CXCR4 allows the cortical predominance of CXCL12 to retain human DP thymocytes in the cortex. However, successful positive selection is accompanied by down-regulation of CXCR4 and up-regulation of CCR7, thereby promoting chemotaxis towards the medullary chemokines CCL19/21 [16, 62, 103, 112, 113]. The human medulla expresses a number of other chemokines, including MDC/CCL22, a ligand for CCR4 [63]. Interestingly, DP and SP human thymocytes also show enhanced chemotaxis towards CCL22, yet all $CCR4^+$ thymocytes localize to the medulla [62], suggesting that some post-selection DP thymocytes may migrate to the medulla prior to differentiation into mature SP cells.

Mutations in CCR7 signaling in mice result in impaired SP thymocyte migration to the medulla and the development of autoimmunity, highlighting the importance of appropriate migration in shaping the TCR repertoire (Fig. 2d, e) [123, 124]. As in the mouse, migration toward CCL21 is also developmentally regulated in human thymocytes, with the greatest response occurring in the most mature $CD1a^-$ SP cells [16, 62]. We recently examined the localization of human SP thymocytes on thymic slices from mice lacking CCR7 ligands (*plt/plt mice*), and found that CCR7 signaling is also necessary for the localization of mature human SP thymocytes to the medulla (Fig. 2d) [16]. A prominent role for CCR7 signaling in the medullary accumulation of human SP cells is not incompatible with a redundant role for CCL22, as the abnormal development of medullary epithelial cells in *plt/plt* mice may prevent the expression of CCL22, thus obscuring the contribution of CCL22 to the medullary localization of SP thymocytes.

In the human thymus, medullary epithelial cells of the Hassall's corpuscles are thought to play a role in the generation of human regulatory T cells (T_{reg} cells) through the production of thymic stromal lymphopoietin (TSLP). TSLP activates medullary DCs that mediate the selection of T_{reg} cells [52]. Interestingly, medullary epithelial cells of the Hassall's corpuscles also produce CCL22, and human T_{reg} cells in the periphery express CCR4 and migrate in response to CCL22 [125]. Therefore, it is conceivable that CCR4 signaling could direct CCL22-responsive post-selection DP and immature SP human thymocytes to specialized niches of the medulla, such as those surrounding the Hassall's corpuscles, and play a role in the generation of T_{reg} cells (Fig. 2e).

Other chemokines produced primarily in the human thymic medulla include the ligands for CXCR3: IP-10 (CXCL10), Mig (CXCL9), and I-TAC (CXCL11), the majority of which are produced by epithelial cells [64]. CXCR3 expression is confined to mature human $CD8^+TCR\alpha\beta^+$ SP thymocytes, and $CD3^+TCR\gamma\delta^+$ cells. Chemotactic activity towards CXCL9, 10, and 11 is highest among mature human $CD8^+$ SP thymocytes ($CD45RO^-CD45RA^+$), but has no influence on $CD4^+$ SP cells. $CD3^+TCR\gamma\delta^+$ T cells also migrate in response to these chemokines [64]. Human $CD8^+$ SP and $CD4^+$ SP thymocytes also migrate in response to CCL11/eotaxin, another medullary chemokine [65]. Whether these chemokines play a role in the medullary migration or localization of SP thymocytes in vivo is not yet known, but it is possible that thymocytes with distinct developmental potentials may be directed to defined medullary niches to finalize their maturation.

The migration of SP thymocytes in the medulla is associated with a striking increase in thymocyte motility, from the relatively slow migration of human DP thymocytes to average speeds of ~9 and 12 microns/min in $CD4^+$ and $CD8^+$ SP thymocytes respectively, similar to the speeds reported for mouse SP thymocytes [16, 110, 121, 126]. Remarkably, the speed of human and mouse SP thymocytes is maintained on both cortical and medullary substrates, suggesting that rapid migration is intrinsic to the developmental stage of the cell [16, 110]. Studies in mice have revealed that CCR7 signaling is additionally involved in determining the rapid motility of SP medullary thymocytes [110]. The contribution of G-protein coupled receptors, such as chemokines, to SP thymocyte motility was confirmed in humans, as treatment with pertussis toxin significantly reduced the average speed of SP thymocytes [16]. Thus, chemokine signaling, in combination with the developmentally regulated modulation of adhesion through integrin receptors, is likely responsible for the characteristic patterns of migration at different stages of thymocyte differentiation. Dissecting the contribution of individual integrins and the factors that control their activation merits further investigation and should yield important insight into

the regulation of thymocyte migration both in the cortex and the medulla.

Exit from the thymus

Emigration of mature thymocytes into the circulation is required to maintain the peripheral naïve T cell pool and requires careful timing of thymic egress to ensure that thymocytes are effectively screened for reactivity to self-antigens in the medulla before they enter the circulation. In mice, emigration from the thymus is mediated by the regulated expression of $S1P_1$, the receptor for the sphingolipid metabolite sphingosine-1-phosphate (S1P), on the most mature thymocytes (Fig. 2f) [127]. Lymphocyte egress from the thymus and from peripheral lymphoid organs is thought to occur in response to an S1P gradient, with low S1P levels in lymphoid tissue and high S1P levels in the circulation [128, 129], while the timing of thymocyte egress was shown to be tightly regulated by the effects of TCR signaling and CD69 expression on surface expression of $S1P_1$ [129, 130]. Furthermore, transgenic expression of $S1P_1$ on immature thymocytes led to their premature exit from the thymus, leading to insufficient negative selection and autoimmunity [129].

The role of S1P in T cell trafficking was initially revealed by the effect of FTY720 (fingolimod), an agonist/functional antagonist of $S1P_1$ [131, 132]. FTY720 was consistently shown to prevent lymphocyte egress from secondary lymphoid tissue in mice, and this effect is thought to be mediated by the initial activation and eventual down-regulation and degradation of $S1P_1$ [133]. Since then, FTY720 has been found to be effective in multiple animal models of autoimmune disease, particularly in experimental autoimmune encephalomyelitis, a model of human multiple sclerosis (MS) [133, 134]. Following promising clinical trials, FTY720 was approved for the treatment of relapsing MS. Similar to its effect on mouse lymphocytes, FTY720 treatment of MS patients was found to cause significant lymphopenia, specifically reducing the number of circulating naïve T cells and central memory T cells but not effector memory T cells [135]. Despite the absence of data regarding the effect of S1P modulation on human thymocyte egress, the conserved interspecies effect of FTY720 in peripheral T cell trafficking would suggest a prominent role for S1P in the exit of human lymphocytes from the thymus (Fig. 2f).

The blood vessels of the CMJ have been identified as the primary site of egress for the majority of murine thymocytes, while a role for lymphatic-mediated egress has been excluded [129, 136]. The absence of lymphatics in the human thymic medulla, as well as the presence of thymocytes within the perivascular spaces of human thymi, suggests that human thymocytes likely egress via blood vessels as well, although direct experimental evidence for this is lacking [129, 137, 138].

A role for chemokines in mediating thymic egress has also been proposed. Specifically, data from both FTOC and in vivo studies in mice demonstrated a role for CXCR4 signaling in the emigration of $CD4^+$, but not $CD8^+$, SP thymocytes (Fig. 2f) [139]. This suggests that murine $CD4^+$ and $CD8^+$ SP thymocytes may exit the thymus by two different mechanisms. In human thymocytes, however, the opposite appears to be true; both expression of CXCR4 and migration towards CXCL12 are greater in human $CD8^+$ compared to $CD4^+$ SP thymocytes [16]. Additionally, it has been previously demonstrated that both $CD4^+$ and $CD8^+$ human fetal SP thymocytes migrate away from thymic fragments and from a novel artificial thymic organoid (generated on a 3-D inert matrix) in a CXCL12-dependent manner, suggesting that human SP thymocyte egress is mediated by chemorepulsion to the chemokine CXCL12 produced by the thymic stroma (Fig. 2f) [140]. It will be interesting to determine the contribution of CXCL12 to thymocyte egress in the vascularized thymus of humanized mice that might more closely approximate conditions for thymic egress in vivo.

Chemokine-induced migration requires cell polarization that results from cytoskeleton remodeling. Coronin 1A, involved in actin cytoskeletal rearrangement, was found to be deficient in some patients with SCID, and coronin 1A-deficient mice have impaired thymic egress [141, 142]. Mammalian STE20-like protein kinase 1 (MST1), another molecule involved in the regulation of cytoskeletal remodeling, has been shown to be essential for murine thymic egress and lymphocyte migration [143]. It has therefore been suggested that the T cell lymphopenia observed in MST1-deficient patients might also be a consequence of impaired egress of mature T cells from the thymus [144].

Concluding remarks

Thymic T cell development is essential for the generation of protective immunity against foreign antigens, and the carefully orchestrated journey of thymocytes through the thymus is crucial to the correct development and maturation of T cells. Simultaneously, thymocyte signaling supports the differentiation of the thymic stroma, further underscoring the importance of thymocyte migration. Signaling interactions that guide the journey of thymocytes are complex and involve integration of a multitude of cues, some of which have been identified, and many others that are inferred from mouse studies. While a variety of in vitro systems and organ culture systems are available, the humanized mice model remains the only “in vivo” model

for the dissection of human immune development. Despite significant advances, many aspects of human thymocyte migration, and their relation to T cell development, remain unknown.

Although candidate chemokines and adhesion molecules have been identified, studies of human thymic progenitor entry into and lymphocyte exit from the thymus are particularly hampered by the lack of models to efficiently recapitulate the trafficking of human cells between the intravascular space and the thymus. These studies are complicated by the fact that multiple different hematopoietic populations, some of which may not yet have been identified, may enter the thymus via unique mechanisms. Humanized mice are a promising experimental platform to study the signaling cues guiding the migration of human cells to and from the thymus, but certain limitations, such as inefficient interspecies receptor:ligand interactions and differences in the vascularization of the murine and human thymus, may complicate the effectiveness of these models. Despite the challenges, these questions remain an area of paramount importance, as the slow recovery of T cells following BM transplantation affects the morbidity and mortality of a large number of patients. Additionally, the current clinical use of FTY720, as well as the development of next generation S1P receptor modulators for the treatment of a wide range of inflammatory diseases, stresses the need for a closer characterization of the role of the S1P:$S1P_1$ axis in the regulation of human thymocyte egress.

The thymic cortex harbors immature thymocytes as they progress through the early stages of T cell development, yet the signals directing the localization of human cortical thymocytes remains elusive. Assuming that the vessels of the CMJ are the site of hematopoietic cell entry into the vascularized human thymus as they are in mouse, it is still not known whether an outward migration to the sub-capsular region of the thymus is essential for progression through β-selection. Moreover, while CXCL12 signaling has been implicated in the retention of pre-selection DP thymocytes in the cortex, the identity of the signaling cues that guide the migration of human DN thymocytes in situ have yet to be identified. Additionally, integrins and adhesion molecules are likely implicated in the unique migration signatures of thymocytes and may influence the outcome at the various selection checkpoints in the thymic cortex. Thus, the contribution of integrins to thymocyte migration and development warrants further investigation.

The requirement for CCR7 signaling in the relocalization of post-selection thymocytes from the cortex to the medulla appears to be preserved between human and mouse. Whether some post-selection DP thymocytes migrate to the medulla prior to maturation to the SP stage, and whether other chemokine signals or alterations in thymocyte adhesion additionally contribute to the localization of immature SP thymocytes to the medulla is not known. It is likely that the human medulla contains specialized niches with distinct functional capacities, such as the contribution of Hassall's corpuscles to the induction of human T_{reg} cells. The medulla is rich in chemokine expression, and specific human thymocyte subsets have been shown to respond differentially to many of these in vitro. The physiologic relevance of directed thymocyte migration within specific niches of the medulla and its possible contribution to the selection of the TCR repertoire remains to be determined.

The ability to modulate thymocyte seeding of, migration within, and exit from the thymus is of great clinical interest in the treatment of disease. In addition to their role in thymocyte adhesion and migration, chemokines and integrins have been implicated in a significant number of pathologies, including autoimmune disorders, transplant rejection, cancer, and human immunodeficiency virus (HIV) infection. Thus, many small molecule antagonists targeting these receptor/ligand interactions are already in use, many others are in clinical trials, and the repertoire of targeted molecules is likely to continue to expand. Therefore, it is of paramount importance to understand the contribution of these molecules to thymocyte migration and development for the safe design of chemokine- and integrin- antagonists for clinical use.

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