Review

Thymic Output: Influence Factors and Molecular Mechanism

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Thymus is a primary lymphoid organ, able to generate mature T cells that eventually colonize secondary lymphoid organs, and is therefore essential for peripheral T cell renewal. Recent data showed that normal thymocyte export can be altered by several influence factors including several chemokines, sphingosine1-phosphate (S1P), transcription factors such as Foxj1, Kruppel-like transcription factor 2 (KLF2) and antigen stimulation, etc. In this review, we summarized the recent reports about study strategies, influence factors and possible molecular mechanisms in thymic output. *Cellular & Molecular Immunology*. 2006;3(5):341-350.

Key Words: RTE, GPCR, S1P, homeostasis

Introduction

The completion of T cell-lineage differentiation depends on active movement of hemopoietic progenitors from the blood to the thymus, and then on the intricate migratory pathway that developing thymocytes follow from the corticomedullary junction to the outer cortex, and finally to the thymic medulla (1). Systemic immune surveillance requires the emigration of newly derived T cells from the thymus into the peripheral circulation to maintain the T cell specificity, as well as the peripheral homeostasis to shape the T cell repertoire.

Although many aspects of intrathymic thymocyte migration have been described, the mechanism by which mature thymocytes exit the thymus is less well understood. There is evidence to support the viewpoint that chemokinetic agents, including chemokines that signal *via* G protein-coupled receptors (GPCR), are involved in thymic emigration by virtue of the fact that this process is pertussis toxin (PTX)3 sensitive (2, 3). However, there is also evidence that the action of chemoattractants alone does not fully explain the process of thymic emigration. The homeostatic regulation of thymic export in the thymus and the periphery involved is also very important in this process. With age, thymus atrophy dramatically reduces the export of new T cells and predisposes an individual to impaired T-cell function, reduced T-cell immunity, and increased autoimmunity. Thymus atrophy is also the primary obstacle to restoration of the T-cell pool in the aftermath of HIV treatment or lymphoablative therapies. Therefore, further research on the molecular mechanism of thymic emigration would be therapeutically beneficial.

Overview of the study methods of recent thymic emigrants

Since the 1970s, when it was first proposed that T cells exited the thymus in an immature state and completed their development in lymphoid periphery (4), several techniques have been used to identify and study murine recent thymic emigrants (RTEs). One method, direct intrathymic injection of fluorescein isothiocyanate (FITC), efficiently labels murine thymocytes, enabling their subsequent identification in the lymphoid periphery. Using this technique, it is discovered that RTEs are phenotypically mature in their CD4 and CD8 profiles and are functionally competent within 16 hours of exiting the thymus (5, 6). With intrathymic FITC injection, the maximal time elapsed after cellular exit from the thymus can be known precisely. Disadvantages of this technique include the surgical stress to which the animals are subjected, often within the day of analysis (4-8). In addition, FITC fades quickly, preventing investigation of phenotypic changes accrued over time.

The second method, giving the animals the thymidine analog bromodeoxyuridine (BrdU), RTEs have been

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Abbreviations: RTE, recent thymic emigrant; S1P, sphingosine1-phosphate; KLF2, Kruppel-like transcription factor 2; Foxj1, forkhead box j1; GPCR, G protein-coupled receptor; PTX, pertussis toxin; Egr1, early growth response gene 1; TREC, T cell receptor rearrangement excision circle; LTβR, lympho-toxin receptor; NIK, nuclear factor-κB-inducing kinase; SDF, stromal-derived factor.

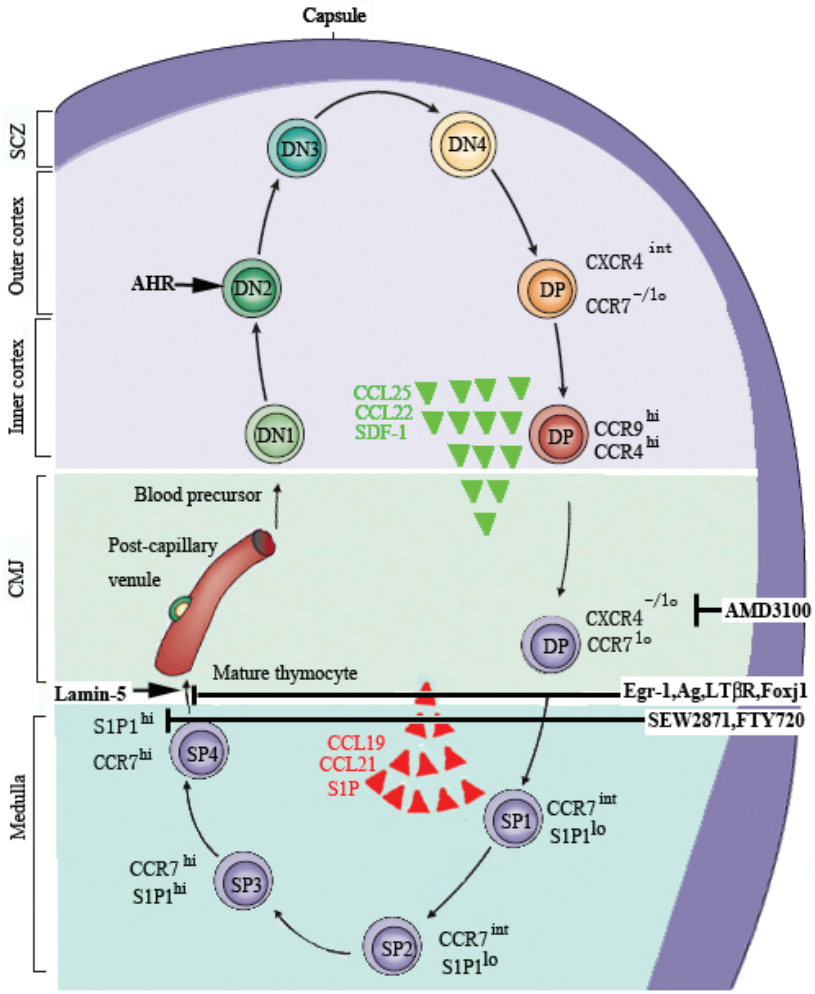

Figure 1. Influence factors of cell migration during T-cell differentiation in the thymus. Chemokines CCL25, CCL22 and SDF-1 are involved in the migration of DP thymocytes from cortex to CMJ. CCL19, CCL21 and S1P recruit DP thymocytes to the medulla. FTY720, SEW2871, Foxj1, KLF2, Lamin-5, etc., regulate the thymic output.

identified as $BrdU^{lo}$ (9). With this method, tough DF demonstrated that $BrdU^{lo}$ cells are not the direct descendants of positively selected cortical cells, but rather are the progeny of mature "single-positive" (SP) cells dividing in the medulla (10). Analyzing cell surface markers and BrdU labeling by four-color cytofluorometry, Penit C and colleagues found these DNA-synthesizing mature thymocytes had a complete mature phenotype ($CD4^-CD8^+$ or $CD4^+CD8^-TCR^{hi}$, HSA^-, $Qa\text{-}2^{hi}$) and expanded only weakly after BrdU incorporation. These data demonstrated the existence of a late intrathymic expansion phase, and involving phenotypically mature cells renewed each day. Double FITC/BrdU detection showed that a high proportion (10-20%) of recent thymic emigrants (labeled by FITC) were $BrdU^+$ just post cycling cells and that around 50% of cycling mature thymocytes were just ready to emigrate to the periphery in the few hours after DNA synthesis. The late intrathymic expansion phase demonstrated here increased the daily thymic cell export by at least 30%. It could play a role in the adjustment of the T cell repertoire before emigration and in the regulation of the thymic cell output into the peripheral T cell pool (9).

Although RTEs were heterogeneous for HSA and Qa-2 expression, they were quite uniform with regard to the expression of other molecules. In contrast to medullary SP thymocytes, most RTEs were $L\text{-selectin}^{hi}CD69^-$. In addition, $CD4^+CD8^-$ and $CD4^-CD8^+$ RTEs were phenotypically distinct from each other in that the former were $\beta7\ integrin^{-/lo}$, $CD45RB^{int}$ and $CD45RC^-$, while the latter were $\beta7\ integrin^{hi}$, $CD45RB^{hi}$ and $CD45RC^{lo}$. These phenotypes were comparable to only a minor (as little as 6%) subpopulation of medullary SP thymocytes. Overall, export of cells from the medullary pool of SP thymocytes is not random, but that a series of maturational events within the SP stage are necessary before export can occur (8). Although BrdU labeling does allow quantification of RTEs in unmanipulated mice, the $BrdU^{lo}$ population is contaminated with post-division $BrdU^{hi}$ T cells, blurring the distinction between RTEs and older peripheral T cells. Furthermore, detection of BrdU incorporation precludes

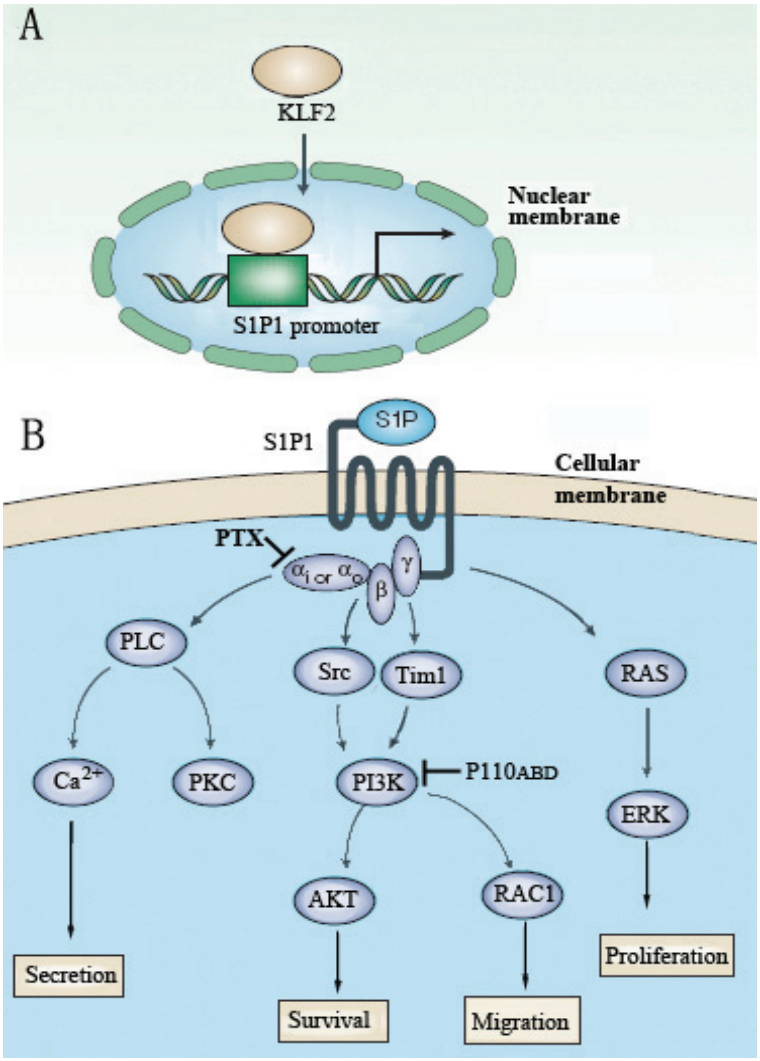

Figure 2. S1P signal pathway. (A) Transcription factor KLF2 regulating S1P1 expression. KLF2 both binds and transactivates the promoter for S1P1-a receptor that is critical for thymocyte egress and recirculation. (B) S1P1 pathways of intracellular trafficking and signaling. $G\alpha_i$- and $G\alpha_o$-coupled signaling mechanisms are depicted for S1P1 expressed by a lymphocyte. G proteins that contain $G\alpha_i$ or $G\alpha_o$ can link to, and activate, numerous effector pathways, each of which elicits specific cellular responses, such as secretion, proliferation, survival and migration. PLC, phospholipase C; ERK, extracellular-signal-regulated kinase; PI3K, phosphatidylinositol 3-kinase; PTX, pertussis toxin; p110ABD, p110 adaptor binding domain.

analysis of functional competence.

The third method, studies of human RTEs rely on T cell receptor (TCR) rearrangement excision circles (TRECs) (10-15). Analysis of TRECs is problematic because long-lived naïve T cell populations can still carry these DNA circles (16, 17). In addition, because TRECs are not replicated, half of cells have undergone a division after rearrangement can no longer be identified as RTEs (18). Thus, analysis of TRECs is an imperfect strategy for RTEs, with the added disadvantage that further phenotypic and functional characterization of cell populations enriched for TRECs is not possible.

Recently, a new method was developed to detect RTEs. Mice transgenic for green fluorescent protein (GFP) driven by the recombination activating gene 2 (*Rag2*) promoter (called RAG2p-GFP transgenic mice) were generated. In RAG2p-GFP transgenic mice, the multicopy transgene generated a bright GFP signal whose induction occurred at the predicted late $CD4^-CD8^-$ double-negative stage. The signal remained bright untill 1-2 weeks after egress. GFP^+ peripheral T cells act as RTEs. So both the changes of phenotype and function of RTEs can be traced with this method. Boursalian and colleagues showed that T cell differentiation continues post-thymically, with progressive maturation of both surface phenotype and immune function. In addition, the relative contribution of CD4 and CD8 recent thymic emigrants was modulated as they entered the peripheral T cell pool. Thus, T cell maturation and subset

contribution are both finalized in the lymphoid periphery (19). Our recent data indicated that phenotypic mature thymocytes still underwent further functional maturation during their retention in the medulla. It implied that the functional maturation of T cells was a continuous process from the thymus to the periphery.

However, attempts to distinguish phenotypically RTEs from longer-lived naïve T cells, without thymic labeling, have been relatively unsuccessful. This is not entirely surprising, given that RTEs are generally regarded as members of the naïve T-cell pool; they express low levels of CD44 and high levels of CD62L, characteristic of naïve T cells, although many have divided immediately before export, like other naïve cells they are usually non-dividing except in circumstances of lymphopenia (20). Some broad differences exist between RTEs and longer-lived naïve T cells, at the population level, in the expression of Qa-2, CD24 (21), CTLA-4 and TSA-2 (mouse) (22), CD45RA (sheep), CD45RC and RT6 (rat) and CD103, CD31 and CCR9 (human), but with the exception of the chT1 thymocyte antigen in chickens, no single marker has been identified that is uniquely expressed by either population.

Influence factors of RTE egress

G protein coupled receptors

During T cell development, the organism generates a T cell population with an extended repertoire of Ag specificities, but lacking autoreactivity. Thymocytes undergo a phenotypic and functional maturation in the medulla before exiting to the periphery. Based on gross phenotypic alterations, such as the down-regulation of 6C10, CD69 and HAS, and the up-regulation of CD62L and Qa-2 (23), we report the first practical, ontogenetically and functionally relevant CD4 SP thymocyte maturation stages, including SP1 ($6C10^{+}CD69^{+}$ $Qa\text{-}2^{-}$), SP2 ($6C10^{-}CD69^{+}Qa\text{-}2^{-}$), SP3 ($6C10^{-}CD69^{-}Qa\text{-}2^{-}$) and SP4 ($6C10^{-}CD69^{-}Qa\text{-}2^{+}$) (Figure 1). However the signals that regulate emigration of mature T cells to peripheral lymphoid tissues remain unknown. Chemokines were reported to have important roles in the process. Mechanisms to explain the chemotaxis during thymocyte exit have mostly fallen into three models: chemorepulsion from thymic stromal-derived elements, chemoattraction to peripheral signals and the loss of responsiveness to thymic retention signals. At least five kinds of receptor-ligand interactions, between sphingosine-1-phosphate (S1P) and its receptor S1PR (24), the chemokine stromal-derived factor (SDF)-1, and its cognate receptor CXCR4 (25), CCL19 and its receptor CCR7 (27), CCL25 and CCR9, CCL22 and CCR4, are involved in this process.

S1P is a platelet-derived sphingolipid that activates G protein-coupled S1P receptors and initiates a broad range of responses in vascular endothelial cells (24, 28, 29). Two S1P receptors, S1P1 and S1P4, are highly expressed in T and B lymphocytes. T cells in S1P1 transgenic mice showed higher chemotactic responses to S1P. The total number of $CD3^{+}$ T cells in the blood was markedly increased but in the spleen and lymph nodes was the same as wild-type mice (30).

Because deletion of S1P1 results in embryonic lethality, the basic genetic approaches to study its role in lymphocyte migration include reconstitution of lethally irradiated wild-type mice with hepatic precursors of lymphocytes from S1P1-deficient mice (29) and the selective deletion of $S1P1^{+}$ early thymocytes and all subsequent T-cell stages using LCK-CRE conditional knockout technology (28). Both of these models show that S1P1 has an important role in T-cell development: S1P1-deficient thymocytes do not emigrate from the thymus, which results in increased numbers of mature thymocytes in the thymus and in medullary hyperplasia, and few S1P1-deficient T cells can be detected in the peripheral organs in these mouse models (26, 31). S1P1-deficient T cells also have an abnormal cell-surface phenotype, a markly increased expression of L-selectin^{hi}, β7 integrin and Qa-2 and expressing reduced levels of CD24. CD69 was expressed at intermediate levels on the $S1P^{-/-}$ single-positive thymocytes rather than being fully down-regulated (8, 32). Similarly, constitutive surface expression of CD69 resulted that phenotypically and functionally mature thymocytes accumulated in the medulla of CD69 transgenic mice and failed to be exported from the thymus (31). On the contrary, it is notable that FTY720 treatment was recently found to down-regulate CD69 on single-positive thymocytes and inhibit their egress (24). Two-photon imaging of living T cells in explanted lymph nodes after treatment with S1P1 agonists or antagonists has provided insight into the mechanism by which S1P1 agonists function. The selective S1P1 agonist SEW2871 caused reversible slowing and "log-jamming" of T cells between filled medullary cords and empty sinuses, whereas motility was unaltered in diffuse cortex. Removal or antagonist competition of SEW2871 permitted recovery of T cell motility in the parenchyma of the medulla and resumption of migration across the stromal endothelial barrier, leading to refilling of sinuses, suggesting that S1P1 agonists act mainly on endothelial cell S1P1 receptors to inhibit lymphocyte migration (34).

It was recently shown that chemorepellent signals elaborated by thymic stroma, including the chemokine SDF-1 (or CXCL12), might also contribute to thymic emigration. SDF-1 repels T cells *via* a CXCR4 receptor-mediated, PTX sensitive manner termed chemorepulsion or fugetaxis (25, 35). In CXCR4-deficient C57BL/6 mice, the embryos have the collection of $CD62L^{hi}CD69^{lo}$ recent thymic emigrants, the single-positive (SP) CD4 thymocytes failed to move away from CXCR4-deficient fetal thymus *in vitro*. Moreover, the defect in CD4 SP cell emigration that occurred in the absence of CXCR4 signaling was only partially overcome by the addition of the extrathymic chemoattractant S1P. Blockade of the CXCR4 receptor in normal thymocytes by AMD3100 led to the retention of mature T cells in the thymus *in vitro* and *in vivo*. The addition of extrathymic SDF-1 inhibited emigration of wild-type SP cells out of the thymus by nullifying the chemokine gradient. SDF-1 was also shown to elicit a CXCR4-dependent chemorepellent response from fetal SP thymocytes (25).

The chemokine receptor CCR7 is another crucial lymphoid homing molecule for immune cells. CCR7 ligands (CCL19 and CCL21) in the thymus are predominantly produced by mTEC and are localized in the medulla, whereas TCR engagement of immature cortical DP thymocytes elevates the cell surface expression of CCR7. In the thymus, CCL19 is predominantly localized in the medulla including endothelial venules. CCL19 attracts mature T cells out of the fetal thymus organ culture, but CCL21 fails to show involvement in thymic emigration (26). Pharmacological inhibition of S1P-mediated thymocyte egress in CCR7- or CCR7L-deficient mice results in the accumulation of mature thymocytes in the cortex, suggesting that mature thymocytes may be exported *via* the S1P-dependent mechanism from the cortex in the absence of CCR7 signals (27). Notably, $S1P1^{-/-}$ thymocytes fails to display a chemotactic response to S1P *in vitro*, while continuing to migrate to the CCR7 ligand CCL21 (28). Within the SP thymocytes, the S1P1 expression was predominantly detected in the $CD62L^{hi}CD69^{lo}$ mature sub-population rather than the $CD62L^{lo}CD69^{hi}$ semimature sub-population (30). But CCR7 expression is detected in both $CD62L^{lo}CD69^{hi}$ semimature and $CD62L^{hi}CD69^{lo}$ mature thymocytes, suggesting that the CCR7-mediated medulla migration and the S1P1-mediated thymocyte egress are differently and sequentially regulated during SP thymocyte maturation (Figure 1) (27).

Overall, CCL19, S1P and SDF-1 act as emigration signals, immature intrathymic precursors are insensitive to them, whereas mature thymocytes and peripheral blood T cells are sensitive. But CCL25/CCR9 and CCL22/CCR4 act as important retention factors in thymic emigration. Remarkable expression of CCR9 is induced in the DN to DP transition and DP cells show a strong response to CCL25 in chemotaxis assay. In mice, the down-regulation of CCR9 by mature thymocytes might reduce their responsiveness to CCL25 and facilitate export by freeing cells to immigrate to the peripheral pool (36, 37). Similarly, a remarkable proportion of CCL22-responsive thymocytes are $CD4^{+}CD8^{+}$ cells. Chemotaxis of CCL22/CCR4 is durable on the $CD69^{+}$ $CD62L^{lo}$ stage and disappears when thymocytes evolved into more mature stage of $CD69^{-}CD62L^{hi}$ (38). That means mature thymocytes and peripheral blood T cells lost the responsive capacity to these factors. In one word, thymic emigration is mediated, at least in part, by specific fugetaxis-inducing factors to which only mature cells responsive and some retention factors to which mature cells irresponsive.

Transcription factors

Foxj1

Members of the forkhead family of "winged-helix" transcription factors are important regulators of immune cell development and effector function. Previous studies suggest that Foxj1 inhibits spontaneous autoimmunity in part by antagonizing NF-κB activation in T cells. CD2-Foxj1 transgenic mice exhibited a peripheral T cell lymphopenia, associated with an accumulation of mature single-positive thymocytes. Transgenic thymocytes demonstrated unimpaired lymphoid organ entry in adoptive transfer studies but impaired thymic exodus in response to CCL19, apparently independent of CCR7, S1P1, and NF-κB. In addition, real-time PCR, microarray studies and/or flow cytometry results indicate that transgenic $CD4^{+}Qa\text{-}2^{hi}$ SP thymocytes contain comparable levels of CCR7, CXCR4, LTβR, and S1P1, suggesting that at least their expression is not modulated by Foxj1. These findings confirm the specific role for Foxj1 in regulating thymic egress (39).

KLF2

Mammalian Kruppel-like transcription factors (KLFs) are implicated in regulating terminal differentiation of several tissue types (40-42). KLF2 (also known as LKLF) is expressed in lung, endothelial cells and lymphocytes (43-47), and is essential for normal blood-vessel integrity and lung development (43, 46, 47). Although $KLF2^{-/-}$ thymocyte development is grossly normal, but leads to a massive loss of the peripheral T-cell pool (43), suggests that KLF2 is essential for T-cell trafficking. KLF2-deficient thymocytes show impaired expression of several receptors required for thymocyte emigration and peripheral trafficking, including the S1P receptor S1P1, CD62L and β7 integrin. KLF2 and S1P1 show similar expression patterns in T cells, both being upregulated on thymocyte maturation, down-regulated after T-cell activation and re-expressed in the late effector/ memory pool (28, 43, 48-50). KLF2-null and S1P1-null mice die at similar stages in gestation because of widespread hemorrhaging, probably owing to defective tunica media integrity (28, 45, 47, 51). Furthermore, KLF2 both binds and transactivates the promoter of S1P1 (48). These findings suggest that KLF2 is required for promoting S1P1 expression in endothelial cells and serves to license mature T cells for trafficking from the thymus and recirculation through secondary lymphoid tissues (Figure 2A).

Other influence factors

Egr1

Early growth response gene 1 (Egr1) is a transcriptional regulator whose expression can be induced by multiple signals including the TCR. Egr1-deficient mice have poor accumulation of RTEs in the periphery. The poor accumulation of RTEs in Egr1-deficient mice appears to originate from decreased survival of mature thymocytes and RTE (52).

Ag

T cell development in the thymus involves a series of TCR-mediated control points including TCR-β selection and positive and negative selection. In TCR transgenic mice, Ag injected *i.v.* or intrathymically led to a striking reduction in the number of RTEs in the periphery. This was caused by inhibition of T cell export rather than peripheral deletion, because a cohort of RTEs that were already released before *in vivo* Ag challenge were not depleted, and similar results were observed in Bim-deficient mice, which have impaired T

cell deletion. Within the thymus, the loss of RTEs was associated with the retention of medullary thymocytes rather than increased negative selection (53). In the periphery, TCR-ligation-induced activation of splenic $CD4^+$ and $CD8^+$ T cells from wild-type mice led to more than 95% reduction in the expression levels of endogenous S1P1 (30). Hence, Ag induced thymocyte egress inhibition may be due to the reduction of endogenous S1P1.

LTβR

Thymocytes depend on the interaction with thymic epithelial cells for the generation of a diverse, nonautoreactive T cell repertoire. Thymocytes and medullary epithelial cells (MECs) communicate *via* the lymphotoxin receptor (LTβR) signaling axis. Normal differentiation of thymic MECs requires LTβR ligand on thymocytes and LTβR together with nuclear factor-κB-inducing kinase (NIK) in thymic epithelial cells. Impaired lympho-epithelial cross talk in the absence of the LTβR causes aberrant differentiation and reduced numbers of thymic MECs, leads to the retention of mature T lymphocytes, and is associated with autoimmune phenomena, suggesting an unexpected role for LTβR signaling in central tolerance induction (54).

AHR

The aryl hydrocarbon receptor (AHR) is a ligand-dependent member of the PAS-bHLH family of nuclear receptors. AHR overactivation led to the preferential emigration of DN thymocytes to the periphery and accumulation in the spleen. Some of these recent thymic emigrants had a novel "activated immature" phenotype ($CD3^-TCR\beta^-CD25^{+/int}CD44^-CD45RB^{+/int}CD62L^+CD69^-$) (55).

Laminin-5

Laminin-5 is expressed in the human thymic medulla, in which mature thymocytes are located. Interactions of thymocytes with laminin-5 induced a strong upregulation of active metalloproteinase-14 and then induced the release of a soluble fragment of CD44 cell surface molecule. CD44 led to increased migration of mature medullary thymocytes, whereas it has no effect on cortical immature thymocytes. These data suggest that, *in vivo*, laminin-5 may function in the migration of mature thymocytes within the medulla and be part of the thymic emigration process (56).

Molecular mechanisms

S1P-S1PR signaling

Only a small number of T cells generated in the thymus each day are selected to replenish the peripheral T cell pool. Much is known about thymic selection; however, little is known about the mechanisms regulating medullary maturation and the release of mature T cells into the blood. The majority influence factors referred above are chemokines and their receptors.

Recent studies mainly focused on the regulatory roles of S1P and its analog FTY720 in thymocyte emigration (24, 28, 29). In this review, we tried to elucidate the molecular mechanism of thymocyte egress through S1P-S1PR axis in detail. S1P1 expression levels and signal transduction differ among lymphocytes and vary according to the state of immunological activation. Persistent expression of S1P1 by lymphocytes is mainly attributable to efficient recycling and re-expression through a protein kinase C-ε and activator protein (AP1)-dependent pathway (57, 58). The intracellular signalling that is induced by S1P binding to S1P1 occurs entirely through interactions with Gαi and/or Gαo, which recruit a series of systems for downstream amplification of the signal, such as the phosphatidylinositol 3-kinase (PI3K) and lipid-dependent AKT (also known as PKB) signaling pathway increases the survival of lymphocytes and other immune cells by inhibiting apoptosis, whereas the pathway involving PI3K and the RHO (RAS homologue)-family GTPase RAC is required for the migration of lymphocytes and for their interactions with other cells and with connective-tissue surfaces, and PLC-induced increases in intracellular calcium levels allow the secretion of cytokines and other immune mediators. Studies in bovine aortic endothelial cells (BAEC) identify G protein βγ subunits, Src kinase and the GEF Tiam1 as upstream modulators of S1P-mediated Rac1 activation, and establish a central role for Rac1 in S1P-mediated activation of PI3-kinase/Akt/eNOS signaling in vascular endothelial cells (59). Transgenic expressing the N terminus of the PI3K catalytic subunit (p110ABD; ABD, adaptor binding domain) in thymocytes activates endogenous p110 and results in the accumulation of mature single-positive $CD3^{hi}HSA^{lo}$ thymocytes. Competitive adoptive transfer experiments showed the delayed appearance of peripheral T cells in neonatal transgenic mice, meaning an important role for PI3K activity in the regulation of mature thymocyte exit to the periphery (Figure 2B) (60).

So far, there is no evidence of a direct causal relationship between any individual component of the signalling cascades and a specific alteration in lymphocyte trafficking, though it was reported that the transcription factor KLF2 may regulate S1P1 expression and signalling activity in this setting (48). One report indicates that expression of S1P1 by lymphocytes is required for integrin-mediated firm arrest of lymphocytes in the HEVs of peripheral lymph nodes, but further investigations are required to define the relative role of S1P1-mediated amplification of integrin function in lymphocyte homing (61). By contrast, each specific effect of S1P-receptor signalling in endothelial cells seems to be directly associated with a distinct component in the signal-transduction pathways. For example, signals from S1P1 maintain endothelial-cell integrity by eliciting RAC-dependent reorganization of the actin cytoskeleton, which is essential for normal barrier function of endothelial cells (62-64).

Homeostatic regulation of thymic export

In mice, RTEs begin to seed the peripheral T-cell pool late in embryogenesis and continue to be exported from the thymus at a rate of 1-2% of thymocytes per day throughout life (65).

Studies have shown that RTEs are incorporated regardless of the existing size of the pool (65, 66). This might be a reflection of the importance of RTEs. Even when RTE numbers are dramatically elevated by transplantation of multiple thymuses, the peripheral pool continues to incorporate the additional T cells, rather than exclude them or delete pre-existing peripheral cells to maintain equilibrium. In mice, for example, the grafting of four additional thymuses caused the T-cell pool to grow and remain 80% larger than normal (65). This is indicative both of the elasticity of the peripheral lymphoid compartments and of the potential availability of "niches" to accommodate increased export levels.

However, using an experimental model to allow direct measurement of thymic export, it has confirmed that the thymus failed to vary the export rate or composition of the emigrants in response to changes in the composition or size of the peripheral T-cell pool (66, 67). Regardless of peripheral circumstance, the rate of emigration remained a product of thymus mass, with 1-2% of total thymocytes exported daily. This applied not only in normal mice, but also in instances where the size of the T-cell pool was increased by 80%, or depleted to < 30% of normal. Furthermore, the composition of T cells exported was also unaffected by circumstances in the periphery, despite tight regulation of the CD4 and CD8 T-cell pool sizes (68), approximately two CD4 naïve T cells were exported alongside each CD8 cell (broadly reflecting the ratio seen normally in the thymic medulla and peripheral pool), regardless of induced changes to the peripheral CD4:CD8 ratio (67). Taken together, these experiments showed that the periphery had no detectable homeostatic impact on the export process.

RTEs export by an aged thymus can drop to fewer than 5% of that produced by a young adult. Although the thymus remains functionally competent, the export rate is insufficient to replace the naïve T cells lost daily from the periphery, and as the naïve pool shrinks, homeostatic proliferation is triggered and the memory pool expands (69). With time, these changes become more pronounced and are strongly associated with an increased incidence of autoimmunity, a reduced capacity for immune surveillance, and measurable declines in T-cell function at the level of individual T cells and within the pool as a whole (70, 71). The broader ramifications of these events are illustrated in several studies of elderly patients that demonstrate a strong correlation between the relative severity of age-related immune changes and lowered life expectancy (72-74).

It is not only with increasing age that an over-reliance on peripheral expansion, over thymic output, causes adverse changes in the composition of the T-cell pool. The phenomenon is typical of immune recovery following chemo- or radio-therapy, where "space" created in the T-cell pool provides a strong stimulus to "refill" the pool through proliferation. In these cases, the T-cell deficiency (lymphopenia) is usually so pronounced that homeostatic expansion occurs even in the presence of substantial thymic function. Although the proliferative restoration of cell numbers does improve immunity, full immune recovery is dependent on high thymic output of new RTEs to replenish the naïve pool (74, 75).

The future: therapeutic reversal of thymus atrophy

Many immune problems associated with aging or lymphopenia could, in theory, be alleviated by increased thymic export. The plasticity of the T-cell pool makes it receptive to this form of intervention, but because the rate of thymic export (when measured as a proportion of thymus size) is not responsive to imbalances in the peripheral pool, the only means of producing a sustainable increase in export levels is to increase thymic mass.

Besides of thymus transplantation, several alternatives are also emerging, including *in vitro* systems of *de novo* T-cell differentiation for transplantation of new T cells grown from $CD34^+$ progenitor cells. But for many patients, the preferred alternative would be to increase the rate of export from their own atrophied thymus without surgery. A more complete understanding of the thymic export process might eventually identify factors (e.g., chemokines) capable of inducing short term increases in the emigration rate, but sustainable improvements are likely to require the reversal of thymus atrophy.

One proven strategy is to lower the circulating levels of sex steroids. One variation could be the temporary use of steroid antagonists to maximize thymic export during the most crucial periods of pool recovery, possibly in conjunction with cytokine treatment. For example, even though levels of IL-7 remain stable with age, IL-7 treatment stimulates growth within the thymic stromal compartment and promotes T-cell development by increasing the early stages of thymocyte differentiation (75). On its own, treatment with IL-7 is least effective in the aged and has the side effect of promoting homeostatic expansion among peripheral T cells (76, 77). However, when administered alongside LHRH agonists, IL-7 could optimize the immediate recovery of T-cell numbers through proliferation of residual T cells, and also prompt the longer-term recovery of pool diversity, by restoring thymic function. Growth hormones and growth-hormone secretagogues are also reported to increase thymic mass (13), and the inhibition of thymosuppressive cytokines, such as IL-6, leukemia inhibitory factor, macrophage-colony stimulating factor, stem cell factor and oncostatin M, might have similar effects (78). Targeting these factors could prove useful in future efforts to rejuvenate the thymus. In mice, the aged thymus can be successfully regenerated, and thymic export dramatically increased, by manipulating steroid pathways. The challenge will provide the benefits of thymic regeneration to aged and immunodepleted patients while minimizing the side effects of currently available therapies (79, 80).

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