Thymic egress: S1P of I000

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F1000 Biology Reports 2009, **1**:60 (doi:10.3410/B1-60)

The electronic version of this article is the complete one and can be found at: <http://F1000.com/Reports/Biology/content/1/60>

Abstract

Recent studies have begun to illuminate the mechanism of T-cell export from the thymus, with the identification of a required lysophospholipid receptor, two upstream transcription factors, and several downstream regulators of cytoskeleton dynamics. This work has generated immediate translational impact, aiding the design of immunosuppressant drugs and the identification of a novel form of human immunodeficiency.

Introduction and context

Human immunocompetence and survival depend on the egress of newly produced T cells from the thymus. Approximately 1% of the thymocyte population emigrates from the thymus each day to populate the peripheral T-cell compartment [1]. Following the arrival of early thymic progenitors, the thymus supports all steps of T-cell development, from the differentiation of progenitors into T-cell receptor (TCR)-expressing CD4 and CD8 double-positive (DP) cells in the cortex to the maturation of DP into CD4 or CD8 single-positive (SP) cells that localize to medullary regions. SP thymocytes initially possess an immature phenotype but over time acquire markers associated with maturation (Figure 1). Egress is thought to occur via blood vessels and lymphatics, but the route that predominates remains unknown [2,3]. An initial insight into the mechanism of thymic egress came with the finding that transgenic expression in thymocytes of pertussis toxin, an inhibitor of G_{α_i} signaling, strongly inhibited egress [4]. A role for $G_{\alpha_{i2}}$ in thymocyte emigration was later suggested [5] though may instead reflect an accelerated transition of $G_{\alpha_{i2}}$ -deficient cells from the DP to SP stage of development [6]. Important further advances regarding the mechanism of thymic egress emerged from a combination of pharmacological and genetic studies that established an essential role for sphingosine-1-phosphate (S1P) and one of its G-protein-coupled receptors.

Major recent advances

The compound FTY720, identified during a screen for immunosuppressants, blocks thymic egress [7]. The active phosphoryl metabolite of FTY720 acts as an agonist for four of five S1P receptors [8], and of these, S1P1 is strongly upregulated in maturing SP thymocytes [9]. Remarkably, genetic deletion of S1P1 in thymocytes phenocopied the pertussis transgenic mouse, indicating that S1P1 is the primary G_i -coupled receptor mediating egress [9,10]. Deficiency in the two kinases that generate S1P led to a similar thymic egress defect [3]. A second small molecule, 2-acetyl-4-tetrahydroxybutyl-imidazole (THI), known to reduce thymic egress when fed to mice [11], was found to inhibit S1P lyase, causing 1,000-fold increases in thymic S1P and down-modulation of thymocyte S1P1 [12]. Similar findings were made when S1P lyase was knocked down using a short hairpin RNA (shRNA) [12] and through analysis of mice lacking the S1P lyase gene [13]. The latter mice showed severe growth abnormalities and poor viability, perhaps reflecting roles of the lyase in sphingolipid metabolism, and thymic function was strongly affected. Introduction of a low-expressing S1P lyase transgene rescued non-lymphoid pathologies while failing to fully restore thymic egress [13]. These studies highlight the importance of appropriately compartmentalized S1P distribution to support normal thymic egress (Figure 1).

Figure 1. Thymocyte maturation and egress via a blood vessel

A thymocyte is depicted undergoing maturation from the cortical CD4 and CD8 double-positive (DP) stage to the medullary CD4 single-positive (SP) stage. The SP cell is initially in an immature state, lacking Krüppel-like factor-2 (KLF2) and sphingosine-1-phosphate receptor-1 (S1P1) and having a characteristic surface phenotype. After a period of a few days, if the cell survives negative selection, it upregulates KLF2 and then S1P1 (green 7-transmembrane structure) and undergoes other changes in surface marker expression. Acquisition of S1P1 allows the cell to respond to S1P (green dot) that is present at approximately 1,000 nM in the vessel lumen (supplied by red blood cells) and locally supplied by radiation-resistant cells, perhaps endothelial cells or pericytes. Thymic pericytes are unusual in being neural crest cell (NCC)-derived. The final egress step triggered by S1P, likely the reverse transmigration step, requires coronin-1A- and mDial1-mediated reshaping of the actin cytoskeleton and thus the cell. Cells that have just exited the thymus are referred to as recent thymic emigrants (RTEs). T cells that are genetically deficient in S1P1 or KLF2 can reach the mature SP stage but then fail to egress and accumulate in the medulla. Treatment with FTY720 or with S1P lyase inhibitors that promote increases in intrathymic S1P causes S1P1 down-modulation from the cell surface and likely degradation (black crosses) and interrupts egress, again causing accumulation of mature SP cells in the medulla. Cells lacking coronin-1A or mDial1 are also egress-defective. imm, immature; mat, mature.

A study aimed at characterizing the altered T-cell compartment in mice lacking Krüppel-like factor-2 (KLF2), a zinc-finger transcription factor, revealed a block in thymic egress associated with reduced S1P1 mRNA in mature thymocytes [14]. KLF2 co-immunoprecipitated with the proximal S1P1 promoter and could transactivate the promoter. However, sufficient S1P1 may remain in KLF2-deficient cells to permit low amounts of thymic egress [15] and further work will be needed to fully define the factors controlling S1P1 expression. Insight into factors that act upstream of KLF2 has come from work on the Foxo family of transcription factors. Foxo1 promotes expression of KLF2

and, likely in turn, S1P1 [16]. Deficiency in Foxo1 led to a slight accumulation of mature SP thymocytes, particularly of the CD8 lineage [17], though the block was less severe than for KLF2 deficiency. Other Foxos appear likely to help drive KLF2 expression. The inhibition of Foxo transcription factors by phosphoinositide 3-kinase (PI3K) signaling [16] might explain the thymic egress block seen in transgenic mice overexpressing PI3K in thymocytes [18].

The factors determining when expressions of Foxo, KLF2, and thus S1P1 are turned on remain undefined, but recent work suggests the involvement of a timer mechanism. In transgenic mice expressing green fluorescent protein (GFP) from the Rag2 locus, the amounts of GFP in the cell can be used as a molecular timer to determine how much a thymocyte has aged since its Rag2-expressing DP stage of development [19,20]. By means of this approach, a medullary dwell time for SP thymocytes of 4 to 5 days was calculated, with egress occurring in a 'conveyor belt' fashion with cells that enter the medulla first exiting the thymus first [20].

To exit the thymus, T cells must reverse-transmigrate across an endothelial barrier. Several recent observations suggest that molecules required for reorganization of the thymocyte actin cytoskeleton are central to this process. One study followed up on the description of a spontaneous mutant mouse line with a thymic egress defect and peripheral T-cell deficiency [21] to identify a role for the Arp2/3 regulatory protein coronin-1A in thymic egress [22]. Notably, although DP cell migration was also affected by the mutation when tested *in vitro*, the *in vivo* thymic defect revealed itself only as an accumulation of mature SP cells, suggesting an especially stringent need for appropriate control of actin branching during the transmigration step. Mice deficient in the formin protein, mDia1, also suffered reduced thymic egress [23]. Since formins support polymerization of unbranched actin, this work further highlights the necessity of fine actin cytoskeleton control to coordinate the cell shape changes required for egress.

In addition to molecules involved in cytoskeletal reorganization, cell adhesion molecules may play a role in thymocyte egress. Recent data suggest that P-selectin glycoprotein ligand-1 (PSGL-1) may be involved [24]. P-selectin marks a subset of thymic endothelial cells, and thymocytes express PSGL-1 [24]. However, the accumulation of thymocytes at the immature SP stage in PSGL-1-deficient mice [24] is unusual for a thymic egress defect (Figure 1). Further study of how PSGL-1 influences SP thymocyte behavior is needed before it can be concluded to function at the egress step.

In addition to endothelial cells, the vascular barrier involves a layer of pericytes and two layers of basement membrane, the latter forming the so-called perivascular space (PVS) [2]. The significance of the PVS in egress remains unclear, though a reconstitution study has correlated the appearance of SP thymocytes in the PVS with the time when cells begin to egress from the thymus [25]. Two recent studies made the striking observation that the pericytes surrounding thymic blood vessels are neural crest cell-derived [26,27]. The significance of this specialized origin for thymic pericytes is unknown but may relate to the barrier properties of thymic vessels [28] or to their unique requirement to support thymocyte egress.

Future directions

Our current understanding of T-cell egress has profited enormously from the discovery of the egress inhibitory drug, FTY720, and the potential of this molecule for treatment of human autoimmune disorders is under ongoing investigation [29]. The immunosuppressive potential of S1P lyase inhibition is also under exploration with a small molecule inhibitor, LX2931, entering clinical trial [30]. An important question in this area will be to define the impact of prolonged inhibition of thymic egress on thymic function.

The importance of normal thymic egress in humans has also been highlighted by the identification of a T⁺B⁺ natural killer (NK)⁺ severe combined immune deficiency (SCID) patient who lacked functional alleles of the coronin-1A gene [22]. Unusual for T⁺B⁺NK⁺SCID but consistent with a thymic egress defect, the patient possessed normal thymic mass [31]. Given that the coronin-1A gene resides in a region of the human genome subject to copy number variation, it seems likely that further cases of SCID resulting from deficiency in this gene will be identified.

Work over the last several years has led to an increasingly detailed model for the essential final step in thymocyte maturation, acquisition of egress competence, but many questions remain. Chief among these are identifying the precise location and cellular dynamics of thymocyte egress, understanding how KLF2 induction is triggered over time, and establishing whether alterations in thymic egress occur as part of the change in thymic function that takes place in certain infectious and autoimmune diseases and during aging. Given the translational advances that have already been made, we can be sure that further research on thymic egress will continue to impact human medicine.

Abbreviations

DP, double-positive; GFP, green fluorescent protein; KLF2, Krüppel-like factor-2; NK, natural killer; PI3K,

phosphoinositide 3-kinase; PSGL-1, P-selectin glycoprotein ligand-1; PVS, perivascular space; S1P, sphingosine-1-phosphate; S1P1, sphingosine-1-phosphate receptor-1; SCID, severe combined immune deficiency; shRNA, short hairpin RNA; SP, single-positive; TCR, T-cell receptor; THI, 2-acetyl-4-tetrahydroxybutyl-imidazole.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

We apologize to colleagues whose work we were unable to cite owing to space limitations. MAZ is in the Biomedical Sciences Graduate Program and Medical Scientist Training Program. JGC is an Investigator of the Howard Hughes Medical Institute.

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