

Tug of War at the Exit Door

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The lipid sphingosine-1-phosphate has been identified as a key exit signal for lymph nodes. In this issue of *Immunity*, Pham et al. (2008) show that its action can only be understood in the context of retention signals transduced by CCR7.

The molecular rules that control lymphocyte egress from lymph nodes are still poorly understood compared to the multi-step paradigm that controls entry through high endothelial venules. The Cyster lab has been leading the charge in studying the role of the lipid sphingosine-1-phosphate (S1P) in this process, exploiting T cells from S1P receptor-1 (S1P₁)-deficient mice, and in this issue of *Immunity*, Pham et al. (2008) demonstrate that the effects of S1P₁ deficiency and pharmacological agents that target S1P₁ are dependent upon the amount of the homeostatic chemokine receptor CCR7. These data suggest a tug of war between S1P exit signals and CCR7 ligand retention signals for the attention of the T cell.

CCR7 is a chemokine receptor expressed on naive and central memory T cells, and it is critical for efficient lymphocyte entry into secondary lymphoid tissues through high endothelial venules (Sallusto et al., 1999). CCR7 also contributes to the localization of T cells within T cell zones and makes a quantitative contribution to the speed of T cell migration within secondary lymphoid tissues, but other Gi-linked G protein-coupled receptors also contribute to this process, based on experiments with pertussis-toxin-treated T cells or mice (Okada and Cyster, 2007). Here, Pham et al. (2008) show that CCR7 also plays a critical role in T cell retention within lymph nodes, working in opposition to S1P exit signals. They perform a side-by-side comparison of wild-type and mutant T cell egress in vivo by adoptive transfer and steady-state experiments with the collection of thoracic-duct lymph. T cells overexpress-

ing CCR7 show a relative egress defect, whereas cells lacking CCR7 egress more rapidly. The most dramatic effect is that CCR7 deficiency partially restores egress in S1P₁-deficient T cells, and treatment of the T cells with pertussis toxin is even more effective in restoring egress in the absence of S1P₁. Treatment of mice with the drug FTY720 largely phenocopies the S1P₁-deficient phenotype by downregulating S1P₁ on the T cells (Mandala et al., 2002). Remarkably, CCR7-deficient or pertussis-toxin-treated T cells show restoration of egress in FTY720-treated mice. Thus, exit through cortical sinusoids, at least, appears to be a default T cell behavior in the absence of G α i-mediated signaling. How this works physically is not entirely clear because pertussis-toxin-treated T cells move very slowly in the parenchyma (Okada and Cyster, 2007). Direct imaging of this process by two-photon laser-scanning microscopy will be interesting to see in the future, but these experiments will need to have better markers for tissue context (stroma, lymphatics) because it is clear that not all cortical and paracortical regions are equivalent.

Pham et al. (2008) interpret these results in terms of a direct competition or a tug of war between CCR7 ligands, the major one being CCL21, and S1P that would be played out near the cortical sinusoids. S1P is at high concentrations in the blood and lymph and it is a chemotactic agent for T cells via S1P₁ coupling to G α i, which would thus also be inhibited by pertussis toxin. Stromal cells in the T cell zones produce CCL21, as well as CCL19, a second ligand for CCR7. The

superficial paracortex regions immediately around high endothelial venules are likely sites in which S1P and CCL21 gradients dovetail (Figure 1). The concept of competing chemotactic signals has been explored previously for neutrophils and B cells responding to antigen. Hierarchies of competing stop and go signals have also been invoked in control of immunological-synapse formation during antigen recognition (Dustin, 2004). However, Pham et al. (2008) add new data to our understanding of chemotactic control after antigen recognition. Specifically, they show that after antigen-driven proliferation, effector T cells downregulate CCR7 and increase S1P₁ expression, which should favor egress through cortical sinusoids, based on their genetic data. This process of egress to allow effector dissemination might be the key therapeutic target of FTY720 and related drugs.

The authors demonstrate these effects histologically in cortical sinusoids, an exit compartment of lymph nodes that has been largely overlooked, at least since the 1970s. Most egress studies focus on the medullary cords, which are closer to the efferent lymphatics and are the place where T cells accumulate in FTY720-treated animals (Mandala et al., 2002); however, the relative percentages of T cell egress mediated by the cortical sinusoids versus the medullary cords is not known. In the lymph node, the subcapsular sinus, cortical sinusoids, and the medullary cords are lined by lymphatic endothelium. Because T cells are closer to the cortical sinusoids in the steady state, it is possible that much of the homeostatic T cell egress takes place via the cortical

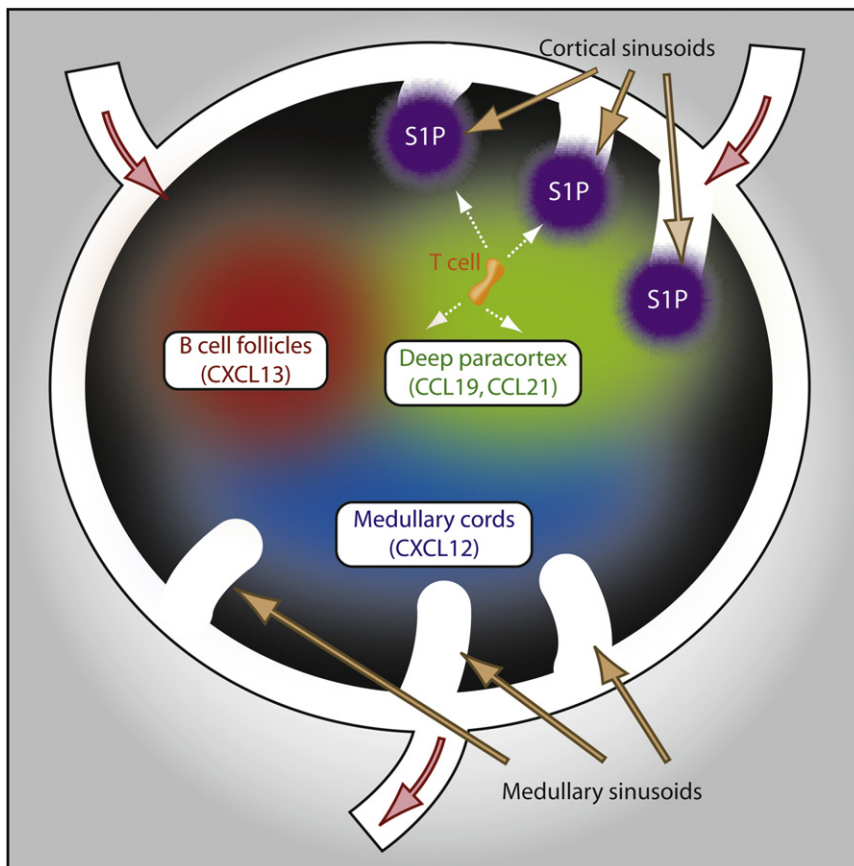


Figure 1. Chemoattractant Gradients in Lymph Nodes

The schematic shows a three-compartment model for a lymph node, in which overlapping gradients of chemokines CCL19 and CCL21 (green), CXCL13 (red), and CXCL12 (blue) define three parenchymal compartments, the deep paracortex, B cell follicles, and medullary cords, respectively. These chemokines are captured on stromal cell surfaces but could also form gradients, particularly in boundary regions like those around the cortical sinusoid exit sites. The cortical and medullary sinusoids are bounded by lymphatic endothelial cells, which might have different properties. Pham et al. (2008) provide evidence for functional S1P gradients that engage in a tug of war with CCL19 and CCL21 gradients around cortical sinusoids (purple), which are always permissive for T cell exit, whereas Wei et al. (2005) provided evidence for control of exit at medullary sinusoids, in which S1P₁ signaling closed exit doors. This suggests the possibility that S1P is not constitutively present at medullary cord exit sites but might be increased to prevent egress under some conditions.

sinusoids. The medullary cord parenchyma is occupied by plasma cells and macrophages, and thus it is possible that lymphatic endothelium in this region are specialized for antibody transport and might regulate cellular traffic in response to innate immune signals from the macrophages to limit spread of infection.

Although this paper adds to the impressive genetic data from the Cyster lab demonstrating that S1P₁ on T cells is critical for egress from lymph nodes, it still does not explain the discrepancy with data from Cahalan and colleagues in explanted lymph nodes that clearly demonstrates an important gate-keeper role for medullary sinusoid endothelial cells in

controlling the egress of wild-type T cells in an S1P₁-dependent fashion (Wei et al., 2005). The only hypothesis that could bring these two stories together, short of experimental artifacts with gene-deficient T cells or explanted lymph nodes, is that the gate-keeper roles of cortical and medullary sinusoid endothelial cells are differently programmed, as suggested above. Intravital microscopy and genetic studies dynamically dissecting the different role of these two lymphatic compartments will be important for the full understanding of the mechanics and biology of egress control in lymph nodes.

This is a complex problem in that there are multiple molecular components that

act cooperatively in multiple compartments to control egress. The output signal—T cell in efferent lymph—is relatively limited in resolution. Although direct imaging might provide some insights, the high-level complexity due to cooperative dynamic events that span a wide range of length scales (molecular receptor-ligand interactions to cell populations in lymph nodes) makes intuition of the underlying mechanisms difficult. Such problems in immunology can benefit from synergistic *in vitro*, *in vivo*, and *in silico* studies (Chakraborty et al., 2003). For example, one could begin by asking how gradients in different ligands (e.g., S1P, CCL21) and temporal patterns of receptor expression (e.g., S1P₁, CCR7) control egress. Models for random migration of T cells in lymphoid tissues (Beltman et al., 2007) would have to be augmented by the incorporation of chemotactic motion and combined with models (or measurements) that describe signaling stimulated by receptor-ligand binding. A computational model to examine the veracity of the mechanism suggested by the data reported by Pham et al. (2008) could be set up as follows. T cells move in the paracortex via a default random process that is biased by chemokine gradients and receptor expression. Upon encountering an egress portal (cortical sinusoid), signals due to S1P₁-S1P interactions could dominate over signals due to CCR7-CCL21 interactions with a certain probability, leading to egress. Theoretical and computational methods based on Langevin and Master equation approaches (Van Kampen, 1992) could interrogate such a model to understand how the pertinent variables compete to modulate egress versus retention, as well as whether physiologically relevant values of quantities (such as receptor expression) can affect experimentally observed outcomes. In addition to data in Pham et al. (2008) and other studies on response as a function of ligand amount, such modeling efforts would also require dose-response curves as a function of receptor expression. This type of data (or sophisticated signaling models) is required for the determination of the probability with which one type of signal (e.g., stimulated by S1P₁-S1P interactions) can dominate over competing ones. For the system examined by the Cyster lab, such data could be obtained

from in vitro studies. Synergy between computational studies of increased sophistication, plus genetic and imaging experiments, might shed light on mechanistic principles that could then enable an understanding of which factors determine the compliance or resistance of gatekeeper lymphatic endothelium in different compartments.

Pham et al. (2008) adds two key elements to future exit models. First, you need to know the exits. Cortical sinusoids appear well positioned to be the most important exit sites for T cells. Second, unlike the unidirectional paradigm for entry through high endothelial venules, exit signals from tissue can only be appreciated in the context of competing retention

signals. Tissue T cells could often be held in a tug of war between opposing signals with shifting dominance, as responses progress, underlying patterns of movement. Identifying of the opposing teams and how they are organized will be important in advancing our understanding of in vivo T cell function.

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