Signals for lymphocyte egress

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Lymphocyte egress from lymph nodes is thought to require signals on T cells for the initiation of exit movement. Another possibility is that signaling instead opens endothelial 'gates' through which lymphocytes pass.

Lymphocyte egress from both primary and secondary lymphoid tissue requires the lipid mediator sphingosine 1-phosphate (S1P), which is thought to induce this effect through binding sphingosine 1-phosophate receptor 1 ($S1P_1$) on lymphocytes. Understanding this mechanism is particularly important because certain $S1P_1$ agonists function as immunosuppressants. How they accomplish this is not clear, but one hypothesis suggests that these agonists cause rapid internalization of SIP_1 on lymphocytes that then cannot respond to endogenous S1P. In this issue of *Nature Immunology*, Cahalan and colleagues explore the effects of $S1P_1$ receptor agonists and antagonists on T cell migration in explanted mouse lymph nodes and offer a different explanation for how the $S1P_1$ agonists function[1]. Their conclusion is that these agents are in fact agonists that act on endothelial cells rather on lymphocytes. The effect of agonism on endothelial cells (in the lymph node, for example) is to close endothelial 'gates', preventing lymphocyte egress and hence mediating immunosuppression.

Using two-photon microscopy, Cahalan and colleagues elegantly demonstrate that the pharmacological $S1P_1$ agonist SEW2871 reduces T lymphocyte migration in the lymph node medulla, but not the cortex. Approximately 5 min after application of SEW2871 to the lymph node, migration velocity of the lymphocytes fell from 6.5 μm/min to about 2 μm/min, and washout of SEW2871 restored migration velocity within about 30 min. In contrast, T cells in the cortex migrated at a velocity of 10–12 μm/min and were completely unaffected by the presence or absence of SEW2871. SEW2871 had its strongest effect on medullary T cell migration toward the lymphatic sinuses, the lymphatic vessels draining each lymph node (**Fig. 1**). Visualizing the sinuses and lymphocytes in the presence of SEW2871, Cahalan and colleagues noted that the cells were 'repelled' or prevented from entering the sinus space. They conclude that endothelial stromal 'gates' that normally allow T cells access to the lymph node sinus are closed by the action of SEW2871 on the endothelial cells themselves; however, they also noted effects of SEW2871 on the movement of medullary T cells (as reflected by the reduced migration speed). Both effects were reversed by the addition of W123 or VPC23019, two $S1P_1$ antagonists that have no apparent effect on T cell migration when administered alone. Consistent with the observation of a preferential effect on medullary T cells, a previous study[2] failed to show effects of another $S1P_1$ agonist, phosphorylated FTY720 (ref. 3), and other S1P receptors such as $S1P_3$, $S1P_4$ and $S1P_5$, on the migration of cortical T cells.

A series of papers has proposed that $S1P_1$ is downregulated after binding to strong agonists such as phosphorylated FTY720 (refs. 4,5). Because the concentration of S1P, the physiological ligand of receptors $S1P_1$–$S1P_5$, is high in blood, S1P receptors are normally downregulated in blood lymphocytes[6]. This could account for other data showing that $S1P_1$-deficient T cells have migration patterns similar although not identical[5] to those of wild-type T cells treated with a strong $S1P_1$ agonist. After entering lymph nodes, where a specific S1P lyase keeps the amount of S1P low[7], lymphocytes re-express $S1P_1$ within a few days. Re-expression of $S1P_1$, according to this model, allows the T cells to respond to the relatively low endogenous S1P in the lymph node and

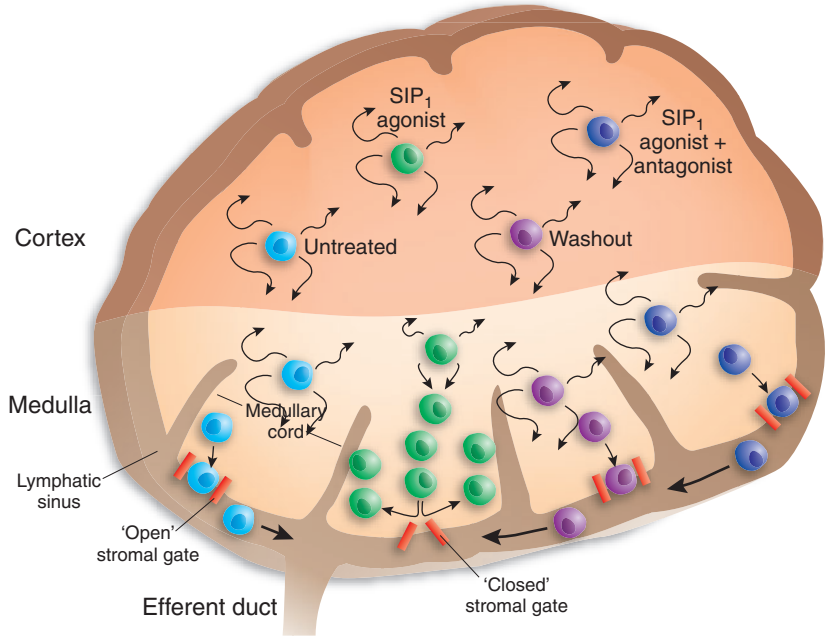

Figure 1 In untreated lymph nodes (blue cells, left), random lymphocyte migration proceeds at a velocity of 10–12 μm/min (arrows) in the cortex (top; reddish shading) and 6.5 μm/min in the medulla (light tan). Adding the $S1P_1$ agonist SEW2871 (green cells) slows migration in the cortex to 2 μm/min, but leaves cortical migration unaffected. In SEW2871-treated nodes, cortical lymphocytes can no longer enter the sinus ('curved-back' arrows), presumably by a closure of endothelial 'gates' that normally allow access. After washout of SEW2871 (purple cells), medullar migration and sinus entry are restored. The same results are obtained when an $S1P_1$ antagonist such as W123 or VPC23019 is added (blue cells).

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then migrate out of the medullary sinus into the efferent lymphatic[6]. One caveat of those studies is that S1P is highly bound to $S1P_1$ and therefore the effective concentrations at the receptor are unknown. The free concentration of this nearly insoluble lipid molecule is certainly the result of many competing equilibria between local formation, elimination, binding and compartmentalization.

At first glance, the two hypotheses — closing of 'gates' in the sinus endothelium by $S1P_1$ agonists[1] or downregulatiion of $S1P_1$ expression on T cells by $S1P_1$ agonists[4–6] — seem to be at odds with each other. However, the effects described by Cahalan and colleagues[1] are rapid (10–30 min) and transient, whereas the $S1P_1$ receptor downregulation occurs over a longer time scale of several hours[4,5]. Moreover, although the opening of endothelial 'gates' is a likely hypothesis[1], no direct evidence for this mechanism is provided, although the imaging studies provided are consistent with this interpretation. Furthermore, SEW2871 had a substantial effect on T cell migration in the medulla. And finally, the hypothesis that stromal 'gates' are closed by SEW2871 cannot explain published data suggesting that $S1P_1$-deficient lymphocytes traffic in a way similar to cells treated with a strong $S1P_1$ agonist[5].

The study by Cahalan and colleagues used explanted lymph nodes, in which the natural distribution of S1P may be changed because of the cessation of blood and lymph flow, although the authors did use concentrations of agonists and antagonists that are likely to be as much as ten times higher than the possible endogenous S1P concentrations. Also, SEW2871 has a lower affinity for $S1P_1$ than do both S1P and phosphorylated FTY720 (refs. 8,9), and its ability to downmodulate $S1P_1$ receptors was not tested here. The phosphorylated FTY720 used in other studies not only has 30-fold higher affinity for $S1P_1$ than SEW2871 but also activates $S1P_3$–$S1P_5$. In fact, it has been suggested that FTY720 downregulates $S1P_1$, whereas SEW2871 induces the same signaling cascades induced by S1P[10].

So what does all this mean for T cell trafficking in lymph nodes? The paper presented here has demonstrated short-term and reversible effects of SEW2871 that slow migration and prevent entry into medullary sinuses[1]. These effects are, in addition, readily blocked by a $S1P_1$ antagonist. Both effects are produced relatively quickly, which to the authors represents evidence of little to no $S1P_1$ downregulation and therefore support for the stromal 'gate' interpretation. One way to evaluate these data alongside those already published on $S1P_1$ agonists is that the short-term effects of $S1P_1$ agonists shown here[1] may actually complement longer-term effects of $S1P_1$ agonists on receptor expression, as has been proposed[5,6]. Thus, the two mechanisms by which $S1P_1$ agonists affect lymphocyte trafficking may not be mutually exclusive, but instead represent two ways these compounds can, as with the agonists being evaluated as immunosuppressants, prevent lymphocytes from leaving lymph nodes.

What more should be done to improve the understanding of lymphocyte egress from lymph nodes? Quantitative studies that account for all lymphocytes in the system over time may help[11]. Also, two-photon microscopy in a live lymph node perfused by its natural blood supply is more likely to preserve the natural S1P gradients, which might alter the results obtained with explanted lymph node (a point that is still debated by the imaging community). It remains to be determined, furthermore, whether the addition of exogenous S1P or FTY720 has the same effect as SEW2871. To address the function of possible endothelial effects of $S1P_1$, a conditional, (lymphatic) endothelium-specific $S1P_1$ knockout would be very useful. The endothelium-specific $S1P_1$ knockout mouse dies *in utero*[12], thus an inducible system would be needed. Finally, receptor-specific agonists and antagonists that act on single S1P receptors will be helpful in further dissecting this system. The study by Cahalan and colleagues takes an important step in this direction by using three $S1P_1$-specific agents. The discovery of endothelial gates and the apparent effects of $S1P_1$ agonists and antagonists on these gates adds a new and important layer to our understanding of lymphocyte egress.

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