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Editorial

Editorial: **Semaphorins: a further chemotropic family expressed in the thymus**

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Developing thymocytes complete their T cell-lineage differentiation through the intricate migratory pathway that they follow to the thymus [1]. The molecular control of thymocyte trafficking, such as the homing of T cell progenitors to the thymus, their intrathymic migration, and exit to the periphery, involves chemokinetic agents, including members of the superfamily of chemokines. In the thymus, chemokines that are produced by thymic stromal cells play a pivotal role in guiding the direction of migrating thymocytes, which sequentially express different chemokine receptors [2]. Recently, it is postulated that intrathymic migration of thymocytes is not only a result of the active movement of these immune cells toward an agent (chemoattraction) but also away from a chemotactic factor (chemorepulsion) [1, 2]. Although the repulsion of neurons and their axons has emerged as an important process in patterning the nervous system [3], a physiological role for chemorepulsion in directing movement in the immune system has yet to be revealed. Therefore, any evidence that chemorepulsion regulates immunocyte migration demands close attention.

It is established that molecules typically discovered in the nervous system are also found in the immune system and vice versa. For example, the chemokine CXCL12 is able to drive neuron migration, whereas the stem cell factor controls neuron migration through CD117/c-kit activation. Accordingly, data presented by Garcia et al. [4] in this issue suggest that Sema3A, a soluble member of the semaphorin family, is involved in thymocyte migration and chemorepulsion. The semaphorin family, through interaction with multimeric receptor complexes, is important for the homeostasis and morphogenesis of many tissues and is widely studied for its role in neural connectivity, cancer, cell migration, and immune responses [5]. There are eight major classes of semaphorins. Each class of semaphorin has many subgroups of different molecules that share similar characteristics. For example, semaphorin Class 3 ranges from Sema3A to Sema3E. Each one of the Class 3 semaphorins is expressed in different regions of the body during development, and although some encourage the growth of axons, others inhibit it. Different semaphorins use different types of receptors. Most semaphorins use receptors in the group of proteins known as plexins. Class 3 semaphorins use a group of proteins known as NRPs as coreceptors with plexins, and Class 7 semaphorins are thought to use integrins as their receptors.

Sema3A/NRP-1/Plexin-A signaling has emerged recently as an important contributor to T cell homeostasis [6]. Sema3A is widely expressed by a variety of cells in central and peripheral immune compartments, including thymic epithelial cells, DP thymocytes, antigen-primed T cells, macrophages, and activated B cells. Sema3A inhibits the proliferation of mitogen-activated T cells and reduces the clonal expansion of human leukemia T cells [7]. The study by Garcia and colleagues [4] provides experimental evidence showing that DP thymocytes are most susceptible to Sema3A-mediated effects. This susceptibility is regulated by the controlled expression of NRP-1. As NRP-1 is also expressed after T cell activation in the periphery [8], the study by Garcia et al. [4] raises the interesting possibility that regulated NRP-1 expression might control the Sema3A susceptibility of peripheral T cells as well.

The thymus is an established source of the chemokine SDF1/CXCL12, and antibodies to the SDF1/CXCL12 receptor, CXCR4, could partially inhibit cell emigration from the thymic cultures [9]. Garcia and et al. [4] first reported evidence for molecular cross-talk between Sema3A and chemokine-driven migration. In a Transwell chemotaxis chamber assay, they showed that Sema3A inhibits DP thymocyte migration promoted by SDF1/CXCL12. This chemokine binds CXCR4 with high- and low-affinity sites, and the binding to the lower-affinity site is required to induce a chemorepulsive signal [9]. Of note, the chemorepulsive role of CXCL12 on thymocytes was also abrogated significantly by Sema3A [4]. These observations suggest that chemoattraction and chemorepulsion exert a cooperative action during intrathymic migration of T

Abbreviations: DP=double-positive, NRP-1=neuropilin-1, SDF1=stromal cell-derived factor 1, Sema3A=semaphorin-3A

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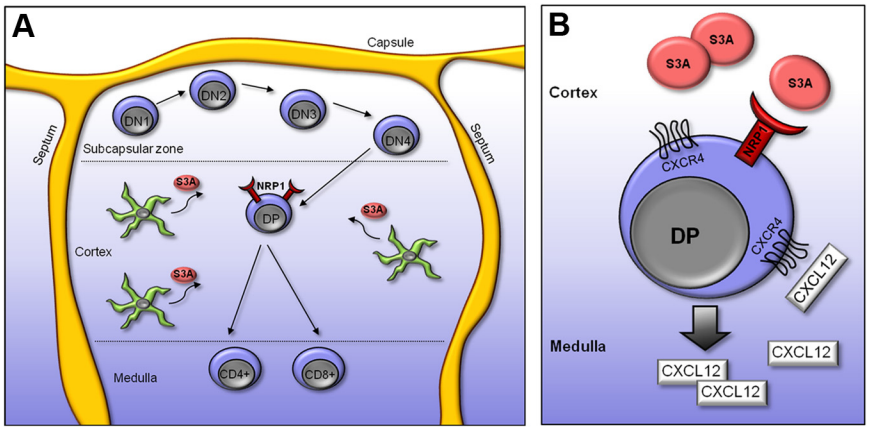

Figure 1. T cell migration and development in relation to Sema3A expression. (A) Differentiation of thymocytes first occurs within the subcapsular zone of the thymus. At the DP stage, thymocytes migrate to the cortex and express the Sema3A receptor NP-1 (NRP1). Moreover, they encounter cortical epithelial cells that release chemokines and Sema3A in the thymic microenvironment. This stage may be important for MHC class restriction and negative selection. Thymocytes then migrate to the medulla, where CD4 or CD8 lineage commitment occurs. Finally, mature T cells exit the thymus from the medullary region and enter the peripheral circulation. DN, CD4 and CD8 double negative T cell; S3A, semaphorin-3A. (B) As Sema3A inhibits migration signals mediated through CXCR4 via CXCL12, DP thymocytes migrate rapidly toward the medulla in response to CXCL12, rather than responding to the influence of CXCL12 in the cortex.

cells (**Fig. 1**). Accordingly, one can predict that pathological changes in any of these loops may result in abnormal thymocyte migration and the failure of central tolerance. For example, an increase in the number of single-positive cells in the thymic cortex of *CCR7*$^{-/-}$ mice is associated with autoimmune phenomena, a pathology attributed to the loss of CCR7-directed migration of maturing thymocytes to the medulla. This is a task not accomplished in the present study by Garcia and colleagues [4]. It is also unclear, based on their results, whether other semaphorins could play a similar role in thymocyte migration. In this regard, it is notable that DP thymocyte chemotaxis can also be influenced by a Sema3E/Plexin-D1-dependent pathway [10]. In fact, *Plxnd1*$^{-/-}$ and *Sema3e*$^{-/-}$ mice present a thymic organization and phenotype similar to that of *CCR7*$^{-/-}$ mice [10]. This suggests that in all likelihood, other plexins and semaphorins can contribute to the fine positional coordination and migratory paths of cells within the thymus.

The matter is complicated further. In fact, thymocytes also regulate the development of cortex and medulla, suggesting that thymocytes and thymic epithelial structures develop in a codependent manner [1, 2]. At present, the role of semaphorin signaling in thymus development is unknown.

In summary, the understanding of the molecular mechanisms governing intrathymic T cell differentiation poses a great challenge to immunology. The recent progress in the thymocyte migration underscores the notion that the molecular circuits governing thymocyte development are complex, comprising distinct types of cellular interactions. In this respect, at least SDF1/CXCL12 and Sema3A appear to act in a combined way. Nevertheless, the precise cross-talk between these two ligand/receptor interactions is far from being unraveled completely and thus, needs to be investigated. The use of genetically engineered mice, bearing conditioned knockout genes coding for distinct chemokines and semaphorins and their corresponding receptors, will represent important tools to go deeper into the understanding of the relative contribution of each molecule for the entire process of intrathymic T cell migration. The second issue, urging to be dissected, corresponds to the expression and role of the various semaphorins in the thymus, as well as how they are regulated. This shall provide new clues to understand how T cell precursors enter the thymus and mature thymocytes leave the organ.

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