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Everything in Its Place

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In this issue of *Immunity*, Griffith et al. (2009) define the thymic midcortex as a functionally inert zone between subcapsular and cortico-medullary regions, and Ehrlich et al. (2009) infer that structural features of the cortex and medulla regulate migration of thymocytes.

T cell development occurs in the thymus, where it depends on an elaborate stromal microenvironment. The pivotal role of the thymic microenvironment is exemplified by thymic aplasia resulting from lack of the Foxn1 transcription factor that is essential for thymic epithelial differentiation (Nehls et al., 1996) and by a unique autoimmune syndrome caused by defective transcriptional regulator Aire that is required for the presentation of self-antigens to developing thymocytes (Anderson et al., 2002). Despite numerous additional studies addressing various aspects of stromal maturation and the reciprocal interactions of stromal components with developing thymocytes, significant gaps remain in our understanding of thymopoiesis. Two reports in this issue of *Immunity* address, in entirely different yet excitingly complementary ways, two areas of particular interest: (1) regional differences in the stromal landscape and (2) signals governing the navigation of developing thymocytes within and between these different regions. Griffith et al. (2009) describe expression signatures for anatomically distinct stromal compartments in the cortex and the medulla. An unexpected result emerging from their study is an apparent lack of distinctive features in the midcortex. Ehrlich et al. (2009) observed the movement of thymocytes in the cortex and the medulla in slice cultures of thymic tissue. In their system, double-positive thymocytes are excluded from the medulla because of their inability to migrate on medullary substrates, whereas the migration of single-positive thymocytes toward and within the medulla is regulated by chemokine gradients and their ability to move on medullary structures, respectively.

The thymus is a highly organized structure with morphologically distinguishable subcapsular, cortical, corticomedullary, and medullary regions. This anatomy can be observed in all higher vertebrates, from sharks to mammals, suggesting that conserved topology serves essential functions for T cell development and selection of a self-tolerant repertoire. Earlier work has suggested that lymphocyte progenitors settle in the thymus after entry at the corticomedullary junction, migrate across the cortex toward the subcapsular region, turn, and head back toward the medulla to eventually exit from there into the periphery (Petrie and Zúñiga-Pflücker, 2007). These conclusions were reached from analysis of discrete time points rather than from continuous observation in real time. To the latter kind of experiments (Robey and Bousso, 2003), Ehrlich et al. (2009) add their system of observing thymocyte movements in thymus tissue slices by two-photon microscopy. To enable the behavior of specific T cell subpopulations to be followed, the slices are seeded with purified thymocytes before observation commences. Because stroma and thymocytes are labeled with different colors, it is possible to examine how the lymphocytes integrate into the tissue and migrate among the existing structures (Figure 1). As expected, purified CD4,CD8 double-negative (DN) and CD4,CD8 double-positive (DP) thymocytes preferentially settled in the cortex and spared the medulla; correspondingly, CD4 and CD8 single-positive (SP) thymocytes preferentially collected in the medulla. DP cells tend to accumulate near the cortical side of the cortico-medullary junction (CMJ) but never venture into the medulla, despite the fact that it is, in principle, directly accessible in the slice cultures. This behavior is inconsistent with diffusible gradients of repellents and/or attractors but suggests the presence of an incompatible medullary substrate. SP cells exhibit directional movement to the medulla (an effect that is shown by the authors to be mediated by Ccr7) and bidirectional behavior across the CMJ; the net traffic is in the direction from cortex to medulla. This is quite compatible with SP cells responding to a chemokine gradient emanating from the medulla. Indeed, pertussis-toxin-mediated incapacitation of G protein-coupled receptors eliminated medullary bias of movements, slowed cells down (an effect that is partly mediated by Ccr7), and essentially prevented their appearance in the medulla. This unexpected result suggested that, in addition to driving chemotaxis, pertussis-toxin-sensitive but yet unknown mechanisms are important to enable SPs to migrate on medullary substrates. Interestingly, in the tissue slice experiment, Ccr7 was not required for entry into the medulla, unlike the in vivo situation (Ueno et al., 2004). Whether or not this discrepancy is a consequence of the experimental system or indicative of some deficiency of the in vitro assay remains to be elucidated.

At present, the system developed by Ehrlich et al. (2009) requires the purification of thymocyte subsets and their reintegration into a steady-state intrathymic microenvironment. Whether and how this affects the outcome of the experiments is unknown. However, a way forward would be to incorporate into their system the in vivo facility of developmentally regulated expression of different fluorescent colors in a fraction of thymocytes, along with a spectrally distinguishable color expressed by stromal cells. This would make such studies compatible with previous experiments (Robey and Bousso, 2003). As a next step, one might envisage transplanting thymic tissue to an ectopic site more amenable to direct microscopic observation, such as the skin. Additionally,

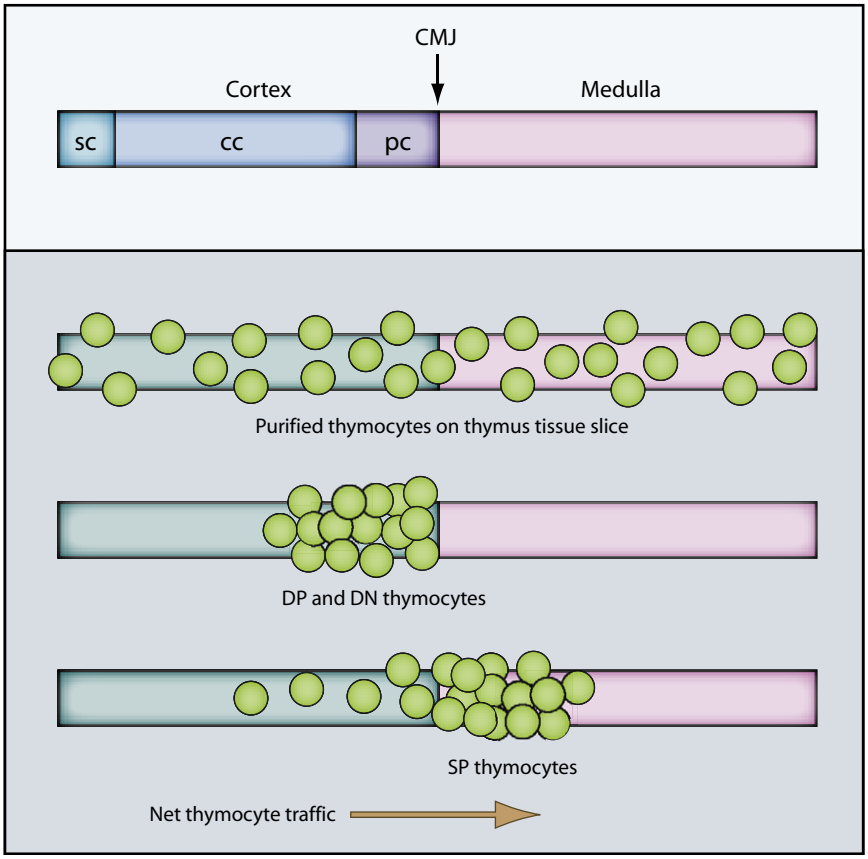

Figure 1. Spatial Organization of Thymus Tissue
The cortex can be subdivided into subcapsular (sc), central (cc), and perimedullary (pc) regions; it is separated from the medullary by the cortico-medullary junction (CMJ). Griffith et al. (2009) have established comprehensive gene expression profiles for stromal components in several subregions of the thymus (top). Schematic of the experimental set-up used by Ehrlich et al. (2009) shown below. Thymus tissue slices are incubated with purified thymocyte subsets such that cells have access to all compartments. After incubation, the distributions of thymocyte subsets relative to the important morphological landmarks are observed by microscopy. CD4,CD8 double-positive (DP) and CD4,CD8 double-negative (DN) thymocytes are found migrating only in the cortex and accumulate in the perimedullary cortex next to the cortico-medullary junction (CMJ). Single-positive thymocytes tend to collect in the medulla but are also found in the cortex; the net traffic occurs in cortico-medullary direction.

there could be a role for alternative animal models, such as fish (Langenau and Zon, 2005). In these systems, extracorporal development enables researchers to observe embryonic development in optically transparent and intact animals. Once transgenic fish expressing different fluorescent colors in the thymic stroma and thymocytes are available, it would be possible to directly observe the enigmatic thymus-homing process, which is beyond the scope of ex vivo studies. Combined with the facile genetic manipulation of embryos via antisense oligonucleotides, the fish conceptually provides a powerful addendum to current mouse models.

The interpretation of the experiments of Ehrlich et al. (2009) and others (Robey and Bousso, 2003) is constrained by our limited insight into the defining functional properties of the thymic stroma. It is remarkable, and perhaps a testament to the difficulty of working with the thymic stroma, that, despite decades of efforts, we still lack a comprehensive picture of stromal differentiation and phenotypic characteristics of its morphologically defined subcompartments. Currently, the most important limitation in working with stromal cells is the paucity of spatially restricted markers that could be used to isolate cells from subcompartments of the cortex and the medulla, respectively. Spatial context is irrevocably lost once stromal cells are isolated from the intact tissue with global markers. Griffith et al. (2009) tackle and partially overcome this substantial problem by using a clever, essentially bioinformatic approach. They first established gene expression profiles by using material taken from microdissected tissue sections, each corresponding to one of the main anatomical landmarks of the thymus, medulla, perimedullary cortex, midcortex, and subcapsular cortex. The resulting profiles are, by necessity, a composite deriving from the stroma and the embedded lymphocytes. In order to extract stromal gene expression patterns, the authors had to find a way of subtracting the thymocyte contribution to the expression profiles. This was done electronically (in two complementary fashions), with expression data from purified thymocyte subsets. Several features of these lists are worth mentioning. Most, but by no means all, known functionally important stromal components are flagged as stromal-specific genes. As expected from previous work, and now confirmed by Griffith et al. (2009), a large number of genes found to be characteristically expressed in the perimedullary cortex appear to be connected to the process of thymocyte movement, whereas the subcapsular cortex is distinguished by an abundance of known or presumptive regulators of cellular differentiation. Apart from validating previous findings and providing a valuable comprehensive resource, the results of Griffith et al. (2009) also offer an intriguing biological clue that is particularly relevant to the initial stages of thymocyte differentiation. The authors find that, surprisingly, the midcortex lacks distinctive features when compared to the other two cortical areas. They raise the possibility that the midcortex functions as an insulator to separate functionally distinct regions of the cortex. Although it will not be trivial to probe the function of the midcortex directly, one possible approach would be to examine whether its dimensions change over time. Perhaps the buffer zone becomes less important as the thymus grows, once the initially well-separated medullary islets and their associated cortical areas have coalesced into larger structures. Now that many researchers will begin to plough through the extensive list provided by Griffith et al. (2009), how should they decide which genes to examine in more detail? For example, perhaps some clues as to

the molecular identity of the exclusive medullary "substrate" invoked by Ehrlich et al. (2009) as supporting the migration of SP but not DP thymocytes are lurking somewhere in this list. Given the striking similarities of thymus morphology and its overall function across different species (Bajoghli et al., 2009), it should be possible to apply a kind of evolutionary filter to the expression profiles obtained by Griffith et al. (2009) in order to determine which of the many genes in the region-specific groups are most likely to be functionally important.

The findings of Griffith et al. (2009) and of Ehrlich et al. (2009) nicely illustrate how new and unconventional approaches could blaze the trail to describing and understanding the myriad of cellular interactions in a primary lymphoid organ and its genetic underpinnings.

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