**Editorial: Ephs, ephrins, and early T cell development**

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**Introduction**

The development of self-tolerant, MHC-restricted T cells occurs in the thymus throughout the life of vertebrates. However, the thymus lacks precursors with self-renewal ability and is normally, continuously replenished with progenitors imported from the bone marrow [1]. The cross-talk between T cell progenitors and nonhematopoietic thymic stromal cells (thymic epithelial cells, endothelial cells, and mesenchyme) is essential for T cell development [2]. Similar interactions are also likely to be important for homeostasis of mature lymphocytes in peripheral lymph nodes and tissues. However, many of the molecular determinants mediating these signaling events in vivo have yet to be elucidated. Recently, ephrins and their cognate receptors, Ephs, have emerged as potentially important mediators of thymic seeding. In this issue of the Journal of Leukocyte Biology, Alfaro et al. [3] report requirements for the Eph family members EphB2 and EphB3 for efficient generation of the ETP.

**DIVERSE ROLES OF EPH-EPHRIN**

Nearly 20 yr after Drescher and colleagues [4] identified ephrins by their function as repulsive axon guidance signals, diverse roles for Ephs and ephrins have been described in practically every mammalian tissue [5, 6]. Ephs have contextual functions depending on the specific tissue in which they are expressed and local physiologic conditions. Ephs are the largest class of receptor tyrosine kinases and are involved in myriad physiologic processes. They are unusual, as they do not recognize soluble ligands as do most receptor tyrosine kinases, and therefore, they lack the ability to participate in long-range signaling [5, 6]. Rather, Ephs exclusively bind membrane-bound ligands, ephrins, expressed by neighboring cells. Following engagement, both Eph-expressing cells (forward signaling) and ephrin ligand-expressing cells (reverse signaling) transduce intracellular signals [5, 6]. Eph forward signaling, initiated following ephrin binding, involves phosphorylation of tyrosine residues on the cytoplasmic tails of Ephs [5, 6]. This results in recruitment and activation of PDZ and SH2 domain-containing binding partners and effector proteins, including Rho/Ras GTPases and Src kinases, which modulate actin cytoskeletal dynamics [6]. Eph forward signaling has been implicated in cell repulsion or de-adhesion, particularly in neuronal growth cones during axon guidance, although adhesive properties have also been described [5]. Ephrin reverse signaling requires ligand engagement with Ephs and results in phosphorylation of serine and tyrosine residues on the intracellular tails of ephrins and subsequent recruitment of PDZ and SH2 family proteins [6]. Prominent roles for ephrin reverse signaling in cell adhesion via integrin modulation are well described, although signaling outcomes are often cellular context dependent [5]. Additionally, Eph-ephrin interactions have been shown to result in cell proliferation, migration, tissue organization, and spatial neuronal patterning [5, 6].

**EPH-EPHRIN SIGNALING IN T CELL MIGRATION**

In normal mice, bone marrow progenitors and thymic stromal cells express both EphB2/EphB3 and ephrinB1/ephrinB2 ligands (Fig. 1A). EphBs are activated on each cell (forward signaling) when progenitors engage ephrins on thymic stromal cells. Accordingly, signals are transmitted into ligand-expressing cells when progenitors and thymic stromal cells interact (reverse signaling). Bidirectional signaling is disrupted in EphB mutants, such that EphB2<sup>−/−</sup> and EphB3<sup>−/−</sup> progenitors, and thymic stromal cells are incapable of receiving forward or sending reverse signals (Fig. 1B–D). However, cells expressing the hypomorphic EphB2LacZ receptor only lose the capacity to receive forward signals, but reverse signaling is preserved (Fig. 1C).

A role for Ephs and ephrins in progenitor homing to the thymus had been suggested earlier from fetal RTOC experiments, in which EphB2<sup>−/−</sup>, EphB2LacZ, or WT T cell progenitors were cultured with thymic stromal cells, devoid of lymphocytes [7]. Notably, in RTOC experiments, EphB2 mutant T-lineage progenitors colonized the thymus with reduced efficiency compared...
with WT progenitors. Furthermore, in vitro cell migration assays indicated that EphB2\(^{2/2}\) T-lineage progenitors migrate poorly in response to CCL25 compared with CCL21 and CXCL12 [7]. Interestingly, compared with EphB2\(^{2/2}\) cells, EphB2LacZ T-lineage progenitors, as well as EphB2LacZ mature T cells, exhibited enhanced migratory capacity in response to chemotactic stimuli, suggesting a role for reverse Eph-ephrin signaling in T-lineage progenitor migration [7].
**EPHB2−/− SCID CHIMERAS DEFINE A BLOCK AT THE DN TO DP TRANSITION**

Previous work defined a requirement for EphB signaling in T-lineage progenitor progression from the early CD4−CD8− (DN) to CD4+CD8− (DP) stage in SCID chimera transplanted with EphB2, EphB3, and EphB2−/−/EphB3−/− bone marrow progenitors [8]. The defect in DP generation observed in SCID mice injected with EphB3−/− progenitors was less severe than that observed in EphB2−/− chimeras. Interestingly, in EphB2lacZ-SCID chimeras, in which bone marrow precursors are deficient in forward signaling but retain reverse signaling, the DN to DP block was partially rescued (Fig. 1C) [8]. These data suggested an additional role for EphB2 reverse signaling in DP survival. Although proliferation of DN, DP, CD4+, and CD8+ cells in EphB2−/−, EphB3−/−, and EphB2lacZ-SCID chimeras was normal, the proportion of apoptotic cells in the DN, DP, CD4+, and CD8+ compartments in EphB2−/− and EphB3−/− chimeras increased (Fig. 1B and C). Intriguingly, cell death in EphB2lacZ-SCID chimeras was not increased but was comparable with controls (Fig. 1A and C), further supporting a role for EphB in T cell survival.

**DEFECTS IN ETP GENERATION**

In the present study, Alfaro et al. [3] describe forward signaling requirements through the EphB2 and EphB3 in blood cell progenitors and stromal cells for efficient progenitor colonization of the adult murine thymus. With the use of EphB2−/−, EphB3−/−, and EphB2lacZ mice, the authors found the percentage of ETP in all EphB mutants reduced (Fig. 1). The results indicate that EphB2 and EphB3 are required for entry of hematopoietic progenitors into the thymus and/or response to Notch signaling and subsequent proliferation after entry into the thymus. To assess whether EphB2 was required on bone marrow precursors or thymic stromal cells, Eph mutant bone marrow progenitors were transplanted into WT mice and the thymus assayed 3 wk later for donor-derived ETP. Significant reductions in ETP were observed in all Eph mutant animals, suggesting a progenitor colonization defect. The competitive fitness of Eph mutant progenitors was assayed in mixed bone marrow chimeric mice, and EphB mutant progenitors were outcompeted significantly by WT progenitors in the generation of ETP.

**HOMING DETERMINANTS REDUCED ON THYMIC STROMAL CELLS IN EPHB−/− MICE**

Recent reports suggest that Eph-ephrin signaling is critical for development of a functional thymic microenvironment [9]. As Ephs and ephrins are widely expressed, the authors tested the hypothesis that stromal cells deficient in Eph-ephrin signaling might also influence progenitor seeding of the thymus. Indeed, both normal and EphB mutant progenitors showed reduced T cell development when transplanted into EphB mutant mice, suggesting a requirement for forward signaling from progenitor cells to thymic stromal cells for efficient thymic colonization (Fig. 1D). As stromal cell-derived CCL21/CCL25 and P-selectin mediate thymic homing under normal physiologic conditions [10], Alfaro et al. [3] reasoned that the molecular determinants of thymic homing might be reduced in EphB mutant mice. Interestingly, all EphB mutants expressed reduced thymic CCL21/CCL25, whereas only EphB2−/− and EphB3−/− mice expressed a reduced percentage of P-selectin+ cells compared with control animals. When analysis of chemokine expression was restricted to mouse pan-endothelial cell antigen+ thymic blood vessels, reductions in chemokine expression were observed in EphB2 and EphB3 mutant thymi only.

Therefore, the new results from Alfaro et al. [3] indicate that Ephs and ephrins are required at early stages of T cell development. These stages can be modeled in vitro by use of RTOC or the OP9 stromal coculture system [11]. Hence, such in vitro models of T lymphopoiesis can be exploited to understand if homing per se is defective or if proliferative expansion or differentiation in response to Notch signaling is altered in Eph-ephrin-deficient progenitors. In the future, agents that target Eph forward signaling and ephrin reverse signaling could conceivably be used to control progenitor homing and boost T cell development. These include agents that target the Eph extracellular domain and the ephrin extracellular domain, Eph-inhibitory antibodies, Eph-activating antibodies, Eph peptide agonist/antagonist, Eph small-molecule antagonist, and Eph kinase inhibitors, which are currently available or under development [6]. Agonist drugs may be particularly useful for enhancing T cell regeneration to alleviate the T cell lymphopenia that occurs after conditioning regimens [10].

**REFERENCES**


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