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Lymphoid Seeding in the Thymus: A New Function for Ephb2 and Ephb3

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Abstract

We have found that the lack of EphB2 or EphB3 courses with decreased proportions of early thymic progenitors seeding the adult thymuses of EphB-deficient mice. The origin of these deficiencies is commented on the basis of profound alterations observed in the thymic epithelium of these mutant mice.

Keywords: Eph; Thymus; Lymphoid colonization

Short Communication

Unlike B lymphocytes that differentiate in the bone marrow (BM), T lymphocytes need the special 3D epithelial microenvironment of the thymus to develop properly, in which they establish intimate interactions with Thymic Epithelial Cells (TECs). Thus, whereas B lymphocytes derive from haematopoietic progenitor cells in the BM, T cells need more- or-less committed lymphoid progenitors to colonize the adult thymus through blood vessels because this organ lacks auto-renewing progenitor cells [1]. Mechanisms involved in the selective migration of BM progenitor cells have been largely associated with attracting chemokines [2-4] and molecules involved in lymphoid cell migration through vascular endothelia, such as integrins and selectins [5-7]. However, in recent years other molecules, including members of the CDM family (*Caenorhabditis elegans* CED-5, human DOCK180 and *Drosophila melanogaster* myoblast city (MBC)) [8], polysialic acid [9], Sphingosine-1-phosphate [10] and semaphorins [11] have been reported to be involved in the migration of lymphoid progenitors into the thymus. Furthermore, we have demonstrated that the tyrosine kinase receptors, Eph of subfamily B and their ligands, Ephrins B, also play an important role in the process [12].

Eph are the largest class of tyrosine kinase receptors of animal cells [13]. They and their ligands, Ephrins, are divided into two families A and B, the first one comprising 10 members that interact with Ephrins A (6 members) whereas EphB (6 members) bind largely Ephrins B (3 members). Eph and Ephrins constitute a very plastic system in which each Eph can bind several Ephrins, and vice versa. Furthermore, both molecules send intracellular signals, called forward and reverse signals, respectively [14]. Eph/Ephrin signaling occurs after oligomerization of both receptors and ligands. Eph dimerization courses with phosphorylation of the yuxtamembrane domain, whereas tetramerization phosphorylates other tyrosine residues of the cytoplasmic domains of Eph, beginning the forward signaling cascade through GTPases of family Rho and ERK/MAPK. Ephrin A activation enhances integrin-dependent adhesion through the Src protein tyrosine kinase, Fyn and MAPKs. On the other hand, EphrinB reverse signaling begins with the phosphorylation of cytoplasmic tyrosines that recruit the adaptor Grb4 and regulatory proteins of cytoskeleton or through the PDZ domain that contains a regulatory region of heterotrimeric G proteins (Figure 1) [15].

Over the last few years we have conclusively demonstrated the relationship between some Eph and Ephrins and numerous processes occurring in the thymus [16,17], including the lymphoid seeding of murine adult thymus [12]. Previously, we [18,19] and other authors [20-22] had reported their involvement in cell migration in different experimental models, although there were only few *in vivo* studies.

Thymuses from adult mice lacking EphB2 or EphB3 or expressing a truncated form of EphB2, deficient in forward but not in reverse signals (EphB2-lacZ), showed an important reduction in the percentages of Early Thymic Progenitors (ETP), close to 50% compared to those of WT mice, and of intravascular injected bone marrow (BM) Lin- cells that seed *in vivo* the adult thymus. It is important to remark that, although all studied mutant BM progenitors show a decreased ability to colonize WT thymuses, this decrease was greater when EphB2-/- cells were injected, indicating a more determinant role of EphB2 than of EphB3 forward signaling in thymus seeding. Remarkably, a similar defective colonization occurs after injection of EphB2-lacZ Lin-progenitor cells, indicating that Ephrin B signaling to vascular endothelial cells is also important for a proper cell seeding of the thymus. In previous *in vitro* studies, however, we found that Ephrin B reverse signaling is sufficient to recover BM Lin- progenitor setting of FTOCs [18], supporting a difference between fetal and adult thymus colonization.

Decreased seeding of lymphoid progenitors in these mutant adult thymuses can be attributed to several, non-excluding factors such as: decreased numbers of migrating lymphoid progenitors from bone marrow, altered mechanisms of homing to the thymus and/or accelerated differentiation of mutant ETP.

Both haematopoietic progenitors, especially more primitive ones [23-25], and BM stromal cells express several Eph and Ephrins [26-28]. On the other hand, EphB2-, but not EphB3-deficient adult mice, show important alterations in the proportions of primitive haematopoietic progenitors, including haematopoietic stem cells (HSC), multipotent progenitors (MPP), early lymphocyte progenitor (ELP) and common lymphoid progenitor (CLP), but not in hose more differentiated assumed to be directly involved in thymus homing [12]. In EphB2-lacZ BM, only HSC and MPP cells showed decreased percentages. Differences in the lympho-haematopoietic phenotypes of EphB2 and EphB3 deficient mice have been repeatedly described [16] and BM stromal cells express 20 times more EphB2 than EphB3 or EphB4 [29]. In agreement, EphB4 transgenic mice contain an expanded BM

haematopoietic progenitor cell compartment but show few effects on the lineage committed cell populations [24] and activation of cord blood CD34+ cells by ectopic expression of EphB4 accelerates the transition of primitive cells to lineage restricted precursors [30].

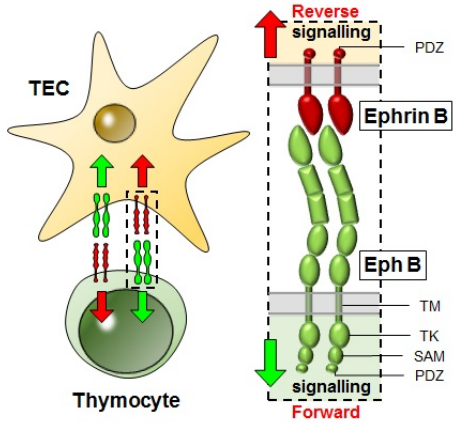

Figure 1: Eph/Ephrin B signaling. Both EphB and ephrins B are expressed in TEC and thymocytes, the most important cell types present in the thymus. They oligomerize and trigger Eph-forward and Ephrin-reverse signaling that mainly depend on specific functional domains such as TM (transmembrane domain), TK (tyrosine kinase domain), SAM (sterile alpha motif) or PDZ, on Eph receptors, and PDZ domains, on ephrin B ligands.

Interestingly, as mentioned there are no changes in the proportions of mutant progenitor cells expressing CCR7, CCR9 and P-selectin glycoprotein ligand-1 (PSGL1) capable of efficiently seeding the adult thymus. In agreement, we have not found changes in the numbers of CD45+ paired-immunoglobulin-like receptor, $PIR^{A/B+}$ lymphoid progenitors that colonize the early thymic primordium of these mutants from the fetal liver [31]. These results suggest that other factors rather than decreased numbers of migrating BM cells could be responsible for the reduced proportions of ETP found in the mutant thymuses.

Independently of the peripheral blood cell population involved in the seeding process, the absence of Eph/Ephrin B signaling in the studied mutants could contribute to its release from the BM. EphB4+ haematopoietic cell migration within BM is inhibited by contact with Ephrin B2 stromal cells [26] and EphA3-Fc protein treatment mobilizes primitive haematopoietic progenitors in the peripheral blood [32]. On the other hand, EphB-dependent mobilization of BM lympho-haematopoietic progenitors could be mediated by CXCL12, a chemokine known to be involved in migration/retention of primitive haematopoietic progenitors in BM [33]. Eph interact with CXCR4, the CXCL12 surface receptor [34], Ephrin B1-Fc fusion proteins stimulate CXCL12-dependent migration of pulmonary lymphocytes [35] and Eph-Ephrin interactions inhibit CXCL12-mediated chemotaxis of cerebellar granular cells [36].

Together, these results suggest that the reduced ETP proportions in EphB-deficient thymuses are presumably due to the altered production and/or expression of molecules known to be involved in lymphoid seeding rather than to variations in the number and/or capabilities of lymphoid progenitors colonizing the organ. These alterations of chemoattractant molecules are presumably a consequence of the severe alterations that affect the epithelial cell component and the morphology of blood vessels (BV) in the mutant thymuses [37,38]. A reduction in CCL21 expression associated with the mouse endothelial cell antigen (MECA) 32+ BV endothelia occurs in the mutant thymuses compared to the WT condition. CCL25, another chemokine involved in thymic seeding, is strongly expressed by TECs but only slightly on BV endothelia. Presumably, CCL25 molecules permeate through the BV to attract circulating CCR9+ progenitor cells into the thymus. There is also an important reduction in P-selectin expression in the thymic endothelia of both EphB2-/- and EphB3-/- mice, but not in EphB2-lacZ ones, that could contribute to the decreased colonization. In supporting, previous studies have demonstrated the close relationships between Eph/Ephrin, chemokines and cell migration in different systems [19,39-41], and activation through EphB4 upregulates PSGL1 expression on endothelial cells [42].

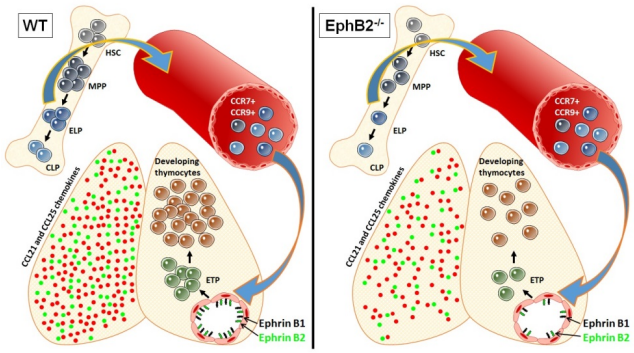

Figure 2: Diagram shows the lymphoid seeding in WT and EphB2-deficient thymuses. The bone marrow of WT mice contains more early haematopoietic progenitors (HSC, MPP, ELP and CLP) than that of EphB2 KO ones, but there are similar numbers of colonizing lymphoid progenitors (circulating CCR7+ and/or CCR9+ cells). Thus, reduced seeding of mutant thymuses seems to be associated with a decreased production of CCL21 and CCL25 and low expression of Ephrin B1 and Ephrin B2 in thymic endothelia.

We had previously demonstrated that chemokine-mediated migration of EphB2-/- Lin- BM-derived precursor cells in Transwell assays was significantly reduced [18] and crosstalk among Eph/Ephrin, integrins and chemokine receptors have been repeatedly reported in other systems [43]. Data on Eph/Ephrin and selectin relationships are less known but EphB4 activation up-regulates PSGL1 expression in endothelial progenitor cells [42]. On the other hand, the expression of both Ephrin B was significantly reduced in the vascular endothelia of EphB2-deficient thymuses, more severely in the case of Ephrin B1. Ephrin B1, but not Ephrin B2, expression was also decreased in EphB3-/- thymic BV endothelia. Since EphB2 is strongly activated by Ephrin B1 rather than by Ephrin B2, we could conclude that EphB2/Ephrin B1 signaling appears to be an important, specific regulatory pathway in thymic seeding.

Finally, the possibility that reduced ETP proportions in EphB-deficient thymuses is related to their accelerated maturation after homing to the organ, must be discarded because these thymuses do not recover the normal numbers of thymocytes, as reported in other mice with defects in thymic seeding [44]. On the contrary, EphB2-/- and EphB3-/- fetal thymuses show a delayed maturation of early

thymocytes [38], increased apoptosis and lower proportions of cycling cells, as compared to WT thymuses [38,45]. These processes could be associated with decreased IL7/IL7R signaling, reduced expression of Dll4, a ligand of Notch involved in early thymocyte maturation, and low numbers of TCRβ transcripts [38].

A summary of these results is shown in Figure 2, in which thymic lymphoid seeding is compared between WT and EphB2-deficient mice, emphasizing decreased proportions of primitive haematopoietic progenitors, but not of more mature committed lymphoid cells, that presumably seed the thymus. It also illustrates an important reduced thymic production of CCL21 and CCL25 and low expression of Ephrin B1 and B2 on the vascular endothelial cells of mutant thymuses.

Acknowledgements

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References

1. Zhang SL, Bhandoola A (2014) Trafficking to the thymus. Curr Top Microbiol Immunol 373: 87-111.
2. Jenkinson WE, Rossi SW, Parnell SM, Agace WW, Takahama Y, et al. (2007) Chemokine receptor expression defines heterogeneity in the earliest thymic migrants. Eur J Immunol 37: 2090-2096.
3. Scimone ML, Aifantis I, Apostolou I, von Boehmer H, von Andrian UH (2006) A multistep adhesion cascade for lymphoid progenitor cell homing to the thymus. Proc Natl Acad Sci U S A 103: 7006-7011.
4. Zlotoff DA, Sambandam A, Logan TD, Bell JJ, Schwarz BA, et al. (2010) CCR7 and CCR9 together recruit hematopoietic progenitors to the adult thymus. Blood 115: 1897-1905.
5. Gossens K, Naus S, Corbel SY, Lin S, Rossi FM, et al. (2009) Thymic progenitor homing and lymphocyte homeostasis are linked via S1P-controlled expression of thymic P-selectin/CCL25. J Exp Med 206 761-778.
6. Kawakami N, Nishizawa F, Sakane N, Iwao M, Tsujikawa K, et al. (1999) Roles of integrins and CD44 on the adhesion and migration of fetal liver cells to the fetal thymus. J Immunol 163: 3211-3216.
7. Wada K, Kina T, Kawamoto H, Kondo M, Katsura Y (1996) Requirement of cell interactions through adhesion molecules in the early phase of T cell development. Cell Immunol 170: 11-19.
8. Lei Y, Liu C, Saito F, Fukui Y, Takahama Y (2009) Role of DOCK2 and DOCK180 in fetal thymus colonization. Eur J Immunol 39: 2695-2702.
9. Drake PM, Stock CM, Nathan JK, Gip P, Golden KP, et al. (2009) Polysialic acid governs T-cell development by regulating progenitor access to the thymus. Proc Natl Acad Sci U S A 106: 11995-12000.
10. Golan K, Vagima Y, Ludin A, Itkin T, Cohen-Gur S, et al. (2012) S1P promotes murine progenitor cell egress and mobilization via S1P1-mediated ROS signaling and SDF-1 release. Blood 119: 2478-2488.
11. Mendes-da-Cruz DA, Stimamiglio MA, Muñoz JJ, Alfaro D, Terra-Granado E, et al. (2012) Developing T-cell migration: role of semaphorins and ephrins. FASEB J 26: 4390-4399.
12. Alfaro D, Garcia-Ceca J, Farias-de-Oliveira DA, Terra-Granado E, Montero-Herradon S, et al. (2015) EphB2 and EphB3 play an important role in the lymphoid seeding of murine adult thymus. J Leukoc Biol 98: 883-896.
13. Barquilla A, Pasquale EB (2015) Eph receptors and ephrins: therapeutic opportunities. Annu Rev Pharmacol Toxicol 55: 465-487.
14. Pasquale EB (2008) Eph-ephrin bidirectional signaling in physiology and disease. Cell 133: 38-52.
15. Kullander K, Klein R (2002) Mechanisms and functions of Eph and ephrin signalling. Nat Rev Mol Cell Biol 3: 475-486.
16. Garcia-Ceca J, Alfaro D, Montero-Herradon S, Tobajas E, Munoz JJ, et al. (2015) Eph/Ephrins-Mediated Thymocyte-Thymic Epithelial Cell Interactions Control Numerous Processes of Thymus Biology. Front Immunol 6: 333.
17. Muñoz JJ, Cejalvo T, Alonso-Colmenar LM, Alfaro D, Garcia-Ceca J, et al. (2011) Eph/Ephrin-mediated interactions in the thymus. Neuroimmunomodulation 18: 271-280.
18. Stimamiglio MA, Jimenez E, Silva-Barbosa SD, Alfaro D, Garcia-Ceca JJ, et al. (2010) EphB2-mediated interactions are essential for proper migration of T cell progenitors during fetal thymus colonization. J Leukoc Biol 88: 483-494.
19. Trinidad EM, Zapata AG, Alonso-Colmenar LM (2010) Eph-ephrin bidirectional signaling comes into the context of lymphocyte transendothelial migration. Cell Adh Migr 4: 363-367.
20. Azab F, Azab AK, Maiso P, Calimeri T, Flores L, et al. (2012) Eph-B2/ephrin-B2 interaction plays a major role in the adhesion and proliferation of Waldenstrom's macroglobulinemia. Clin Cancer Res 18: 91-104.
21. Khounlotham M, Subbian S, Smith R 3rd, Cirillo SL, Cirillo JD (2009) Mycobacterium tuberculosis interferes with the response to infection by inducing the host EphA2 receptor. J Infect Dis 199: 1797-1806.
22. Nikolov DB, Xu K, Himanen JP (2013) Eph/ephrin recognition and the role of Eph/ephrin clusters in signaling initiation. Biochim Biophys Acta 1834: 2160-2165.
23. Ivanova NB, Dimos JT, Schaniel C, Hackney JA, Moore KA, et al. (2002) A stem cell molecular signature. Science 298: 601-604.
24. Nguyen TM, Arthur A, Panagopoulos R, Paton S, Hayball JD, et al. (2015) EphB4 Expressing Stromal Cells Exhibit an Enhanced Capacity for Hematopoietic Stem Cell Maintenance. Stem Cells 33: 2838-2849.
25. Steidl U, Bork S, Schaub S, Selbach O, Seres J, et al. (2004) Primary human CD34+ hematopoietic stem and progenitor cells express functionally active receptors of neuromediators. Blood 104: 81-88.
26. Arthur A, Zannettino A, Panagopoulos R, Koblar SA, Sims NA, et al. (2011) EphB/ephrin-B interactions mediate human MSC attachment, migration and osteochondral differentiation. Bone 48: 533-542.
27. Jung YH, Lee SJ, Oh SY, Lee HJ, Ryu JM, et al. (2015) Oleic acid enhances the motility of umbilical cord blood derived mesenchymal stem cells through EphB2-dependent F-actin formation. Biochim Biophys Acta 1853: 1905-1917.
28. Nguyen TM, Arthur A, Gronthos S (2016) The role of Eph/ephrin molecules in stromal-hematopoietic interactions. Int J Hematol 103: 145-154.
29. Xing W, Kim J, Wergedal J, Chen ST, Mohan S (2010) Ephrin B1 regulates bone marrow stromal cell differentiation and bone formation by influencing TAZ transactivation via complex formation with NHERF1. Mol Cell Biol 30: 711-721.
30. Wang Z, Miura N, Bonelli A, Mole P, Carlesso N, et al. (2002) Receptor tyrosine kinase, EphB4 (HTK), accelerates differentiation of select human hematopoietic cells. Blood 99: 2740-2747.
31. Montero-Herradon S, Garcia-Ceca J, Collado BSD, Alfaro D, Zapata AG (2016) Eph/ephrin-B-mediated cell-to-cell interactions govern MTS20(+) thymic epithelial cell development. Histochem Cell Biol 146: 167-182.
32. Ting MJ, Day BW, Spanevello MD, Boyd AW (2010) Activation of ephrin A proteins influences hematopoietic stem cell adhesion and trafficking patterns. Exp Hematol 38: 1087-1098.
33. Zapata AG (2014) The Adult Hematopoietic Niches-Cellular Composition, Histological Organization and Physiological Regulation. In: S. Wislet-Gendebien, Adult Stem Cell Niches, InTech, Rijeka; 2014. pp. 21-48.
34. Salvucci O, de la Luz Sierra M, Martina JA, McCormick PJ, Tosato G (2006) EphB2 and EphB4 receptors forward signaling promotes SDF-1-induced endothelial cell chemotaxis and branching remodeling. Blood 108: 2914-2922.

35. Kitamura T, Kabuyama Y, Kamataki A, Homma MK, Kobayashi H, et al. (2008) Enhancement of lymphocyte migration and cytokine production by ephrinB1 system in rheumatoid arthritis. Am J Physiol Cell Physiol 294: C189-196.
36. Lu Q, Sun EE, Klein RS, Flanagan JG (2001) Ephrin-B reverse signaling is mediated by a novel PDZ-RGS protein and selectively inhibits G protein-coupled chemoattraction. Cell 105: 69-79.
37. García-Ceca J, Jiménez E, Alfaro D, Cejalvo T, Chumley MJ, et al. (2009) On the role of Eph signalling in thymus histogenesis; EphB2/B3 and the organizing of the thymic epithelial network. Int J Dev Biol 53: 971-982.
38. Montero-Herradon S (2016) Role of EphB2 and EphB3 on the Early Thymic Development, PhD Thesis, Complutense University of Madrid, Madrid.
39. Fuller T, Korff T, Kilian A, Dandekar G, Augustin HG (2003) Forward EphB4 signaling in endothelial cells controls cellular repulsion and segregation from ephrinB2 positive cells. J Cell Sci 116: 2461-2470.
40. Pfaff D, Héroult M, Riedel M, Reiss Y, Kirmse R, et al. (2008) Involvement of endothelial ephrin-B2 in adhesion and transmigration of EphB-receptor-expressing monocytes. J Cell Sci 121: 3842-3850.
41. Sharfe N, Freywald A, Toro A, Dadi H, Roifman C (2002) Ephrin stimulation modulates T cell chemotaxis. Eur J Immunol 32: 3745-3755.
42. Foubert P, Silvestre JS, Souttou B, Barateau V, Martin C, et al. (2007) PSGL-1-mediated activation of EphB4 increases the proangiogenic potential of endothelial progenitor cells. J Clin Invest 117: 1527-1537.
43. Murai KK, Pasquale EB (2003) 'Eph'ective signaling: forward, reverse and crosstalk. J Cell Sci 116: 2823-2832.
44. Zlotoff DA, Bhandoola A (2011) Hematopoietic progenitor migration to the adult thymus. Ann N Y Acad Sci 1217: 122-138.
45. Alfaro D, Muñoz JJ, García-Ceca J, Cejalvo T, Jiménez E, et al. (2008) Alterations in the thymocyte phenotype of EphB-deficient mice largely affect the double negative cell compartment. Immunology 125: 131-143.