Developing T-cell migration: role of semaphorins and ephrins

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ABSTRACT **Cell migration is a crucial event for normal T-cell development, and various ligand/receptor pairs have been implicated. Most of them, including chemokines and extracellular matrix proteins, have attractant properties on thymocytes. We discuss herein two further groups of ligand/receptor pairs, semaphorins/neuropilins and ephs/ephrins, which are constitutively expressed by thymocytes and thymic microenvironmental cells. Evidence shows that the corresponding interactions are relevant for developing T-cell migration, including the entry of bone marrow progenitor cells, migration of CD4/CD8-defined thymocyte subpopulations triggered by chemokines and/or extracellular matrix proteins, and thymocyte export. Conceptually, the data summarized here show that thymocyte migration results from a complex network of molecular interactions, which generate not only attraction, but also repulsion of migrating T-cell precursors.—Mendes-da-Cruz, D. A., Stimamiglio, M. A., Muñoz, J. J., Alfaro, D., Terra-Granado, E., Garcia-Ceca, J., Alonso-Colmenar, L. M., Savino, W., Zapata, A. G. Developing T-cell migration: role of semaphorins and ephrins. *FASEB J.* 26, 4390–4399 (2012). www.fasebj.org**

Key Words: thymus · thymic epithelial cells · thymocyte migration · neuropilins · ephs

THE THYMUS, A PRIMARY lymphoid organ essential for the functional maturation of T lymphocytes, does not contain self-renewing lymphoid progenitors. Actually, bone marrow-derived precursors migrate into the organ. Subsequently, developing thymocytes undergo differentiation toward CD4/CD8-defined single-positive cells ($CD4^+CD8^-$ and $CD4^-CD8^+$), migrating throughout distinct thymic epithelial cell (TEC) niches in a key process for proper T-cell functional maturation. Chemokines (CCL19, CCL21, CCL25, and CXCL12) and adhesion molecules (VLA-4, VLA-5, and VLA-6 integrins, CD44, ICAM-1, and VCAM-1) are involved in the migration of progenitors during fetal thymus colonization (1–4), whereas P-selectin/CD62-P, CCL25, CCR9, and CCR7 recruit lymphoid progenitors into the adult thymus (5–9). In addition to classic attractant proteins, other molecules seem to be implicated in thymocyte migration, such as the CDM family members DOCK2 and DOCK180 (10), as well as the product of polysialyltransferase activity, namely polysialic acid (11).

Interestingly, repulsive molecules that guide developing axons (12–16) seem to mediate similar interactions related to thymocyte migration and thymocyte-TEC interactions (17). In this context, we summarize herein current understanding on the role of two families of repulsive molecules, ephrins and semaphorins (Semas), as well as their receptors in the cell migration occurring in the thymus.

Semas AND Sema RECEPTORS

Semas are secreted or membrane-associated glycoproteins initially described as modulators of axon guidance, angiogenesis, and organogenesis (18, 19). They have been grouped into eight classes on the basis of their structural elements and similarities in their amino acid sequences. Class 1 and 2 Semas are found in invertebrates; classes 3 to 7 comprise vertebrate Semas, whereas class V represents viral-encoded Semas.

Class 3 Semas are the only secreted vertebrate Semas, whereas classes 4–7 are transmembrane proteins that can also serve as receptors and interact with other transmembrane molecules (19, 20). High-affinity receptors for

Abbreviations: DC, dendritic cell; E, embryonic day; ECM, extracellular matrix; KO, knockout; NRP, neuropilin; Sema, semaphorin; TEC, thymic epithelial cell; WT, wild type

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doi: 10.1096/fj.11-202952

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Semas include plexins and neuropilins (NRPs). Membrane-bound vertebrate Semas bind directly to plexins, whereas class 3 secreted Semas also bind NRPs (21). Two NRPs have been described to date, NRP1 and NRP2, although NRP2 has several transmembrane isoforms and the two NRPs have several soluble forms. NRP1 binds Semas 3A, 3B, 3C, and 3D, whereas NRP2 binds Semas 3F, 3B, 3C, and 3D (22–24).

As NRPs have a short cytoplasmic domain that lacks signaling activities, NRPs bind class A plexins that act as coreceptors to transduce the intracellular signals (25), with the exception of Sema3E, which binds directly to plexin D1. The plexin A subfamily includes 4 members, A1–A4, and the specific response of NRP-plexin complexes seems to be determined by the combination of different molecules within each subfamily. General structural features of Semas and related molecules are summarized in **Fig. 1**. Sema-triggered signaling pathways, as well as functional aspects in the periphery of the immune system, have been recently reviewed (26).

Eph AND EPHRIN FAMILIES

Eph is the largest family of tyrosine-kinase receptors in animal cells. Their natural ligands are ephrins, which are also extensively represented in numerous cell types, including TEC and thymocytes (17). Both Ephs and ephrins are subdivided into two families, A and B, on the basis of their gene sequence similarities and ligand binding (27), as summarized in Fig. 1. Eph A (10 members) binds GPI-anchored ligands, the ephrin A family (6 members), whereas Eph B (6 members) binds transmembrane proteins, the ephrin B family (3 members). Although each Eph kinase can bind several ephrins and *vice versa*, it exhibits a striking fine specificity; both receptors and ligands transmit cytoplasmic signals (forward and reverse, respectively) to the expressing cells (27). The system is, therefore, very plastic, with different affinities and expression patterns determining a high number of distinct cell–cell interactions, which allow these molecules to play a role in a

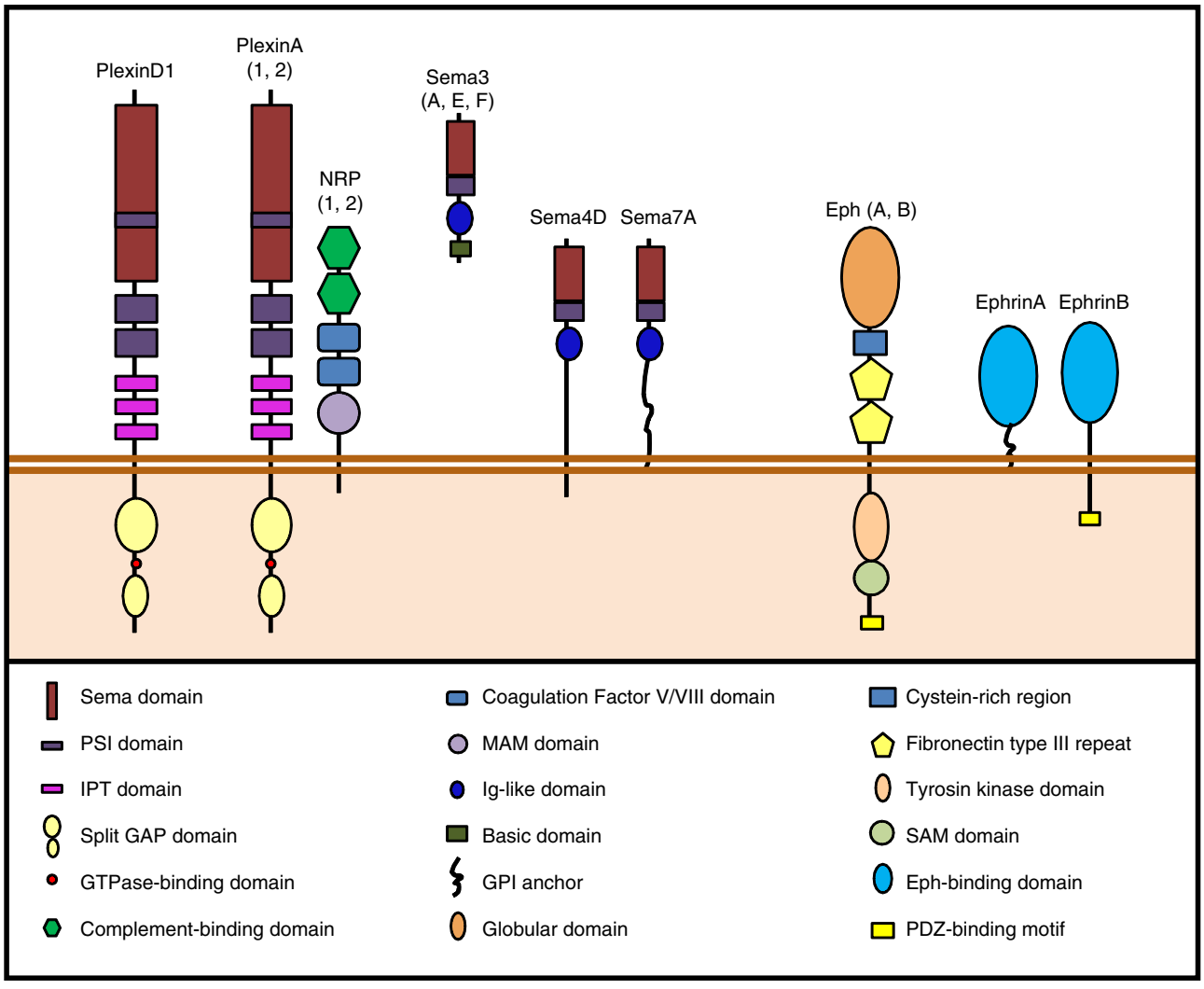

Figure 1. Schematic representations of Semas, NRPs, plexins, Ephs, and ephrins present in the thymus. Domains: PSI, plexin, sema, integrin; IPT, Ig-like, plexin, thrombospondin; GAP, GTPase-activating protein; MAM, meprin, A5, receptor protein-tyrosine phosphatase μ; SAM, sterile α-motif; PDZ, postsynaptic density protein, *Drosophila* disc large tumor suppressor, zonula occludens-1 protein. Compartmentalization of each molecule and the corresponding roles in the thymus are described in Tables 1 and 2.

large number of cell functions. These molecules are involved in defining when and where a given cell type should move, attach or detach itself, contributing to morphogenesis, cell positioning, and cell migration (27, 28). As a consequence, they can specifically restrict cell movement, guide the cells to specific positions, and settle tissue domains and boundaries.

CONSTITUTIVE INTRATHYMIC EXPRESSION OF Semas/NRPS AND Ephs/EPHRINS

The first Sema member described in the thymus was Sema4D, found in both thymocytes and thymic microenvironmental cells (29), thus suggesting a role of this molecule in the maturation-associated thymocyte migration (**Table 1**). Sema7A is expressed by mouse thymocytes in all developmental stages, from embryos to adults, as well as by human thymocytes, mainly in the immature $CD4^+CD8^+$ and mature $CD4^-CD8^+$ subsets (30). Sema3E is highly expressed in the thymic medulla when comparing with the thymic cortex, corticomedullary, and subcapsular zones, whereas the Sema3E receptor, plexin D1, is highly expressed by mouse immature $CD4^+CD8^+$ cells as well as $CD69^+$ maturing thymocytes (31).

Concerning the other class 3 Sema receptors, it has been shown that in the mouse thymus primordium, NRP1 appears later in development, by embryonic day (E) 12.5, and its expression increases with age (32, 33). We have shown that thymocytes from newborn and young adult mice express high levels of NRP1, mainly the immature $CD4^+CD8^+$ and mature $CD4^-CD8^+$ subpopulations (34). Nevertheless, other researchers have reported different results concerning NRP1 expression in thymocytes, ranging from 35 to 78% (32). More recently, Yamamoto *et al.* (35) have shown that the expression of NRP1 varies in mouse thymocytes depending on the cell cycle phase, with NRP1 expression being higher in cycling S-phase cells than in quiescent G_1-phase cells (35). Interestingly, NRP1 membrane levels on lymphoma cells were lower than those on normal thymocytes and did not significantly differ between S and G_1 cells (36).

NRP1 was also described as a marker for mouse $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells (32, 37), although it is not expressed by human Treg cells freshly isolated from the thymus and peripheral lymphoid organs, raising the possibility that in the two species their mechanisms of action may be different (38). Recently, a $NRP1^+$ immature iNKT subpopulation has also been described in the mouse thymus. These cells are exported to peripheral lymphoid organs and comprise the proinflammatory IL-17-producing subset (39).

NRP2 is also expressed in the mouse thymus primordium (from E12.5 on). In later steps, it appears mainly in trabeculae and vessels (33). In adult mice, it has been shown that NRP2 was restricted to the lymphatic vessels (40), consistent with data showing that NRP2-deficient mice present abnormal lymphatic vessel development (41). The expression of the NRP2 ligand Sema3F in the mouse thymic epithelium is observed earlier during development, from E10.5 (33).

Besides the expression of NRPs and plexin D1, plexins A1 and A2 are found in the human thymus, in both thymocytes and TECs (34), which suggests that the interactions mediated by NRPs and class 3 Semas in the thymus are functional in terms of signaling transduction. In

TABLE 1. *Expression and functions of Semas, NRPs, and plexins in the thymus*

Molecule	Expression	Function	References
NRP1	Thymocytes, TECs, stroma, TNCs, iNKT cells, Treg cells	Thymocyte adhesion to TECs; thymocyte migration; inhibition of fibronectin- and laminin-induced thymocyte migration	32–35, 37, 39, 49
NRP2	Trabeculae, lymphatic vessels	ND	33, 40, 41
Plexin A1	Thymocytes	ND	34
Plexin A2	Thymocytes	ND	34
Plexin D1	Thymocytes	Thymocyte migration from the cortex to the medulla; suppression of CCR9/CCL25 signaling by Sema3E interactions	31
Sema3A	Thymocytes, TECs, stroma	Thymocyte adhesion to TECs; thymocyte migration; inhibition of fibronectin- and laminin-induced thymocyte migration; inhibition of CXCL12-induced thymocyte migration	49, 68
Sema3E	Stroma (medulla)	Thymocyte migration from the cortex to the medulla; suppression of CCR9/CCL25 signaling	31
Sema3F	Total thymus	ND	33
Sema4D	Total thymus thymocytes	ND	29
Sema7A	Total thymus thymocytes	ND	30, 77, 78

ND, not determined.

TABLE 2. *Expression and functions of Ephs and ephrins in the thymus*

Molecule	Expression	Function	References
Ephs A1, A2, A3, A4, A7, A8	Thymocytes, TECs	Thymocyte survival and T-cell differentiation; Organization of cortical epithelium (EphA4)	47, 79
Ephs B2, B3	TECs, thymocytes	Migration of lymphoid progenitors into and within thymus; thymocyte survival; T-cell differentiation; Organization of TEC network; TEC survival	42, 44, 45, 80
EphB6	Thymocytes	ND	81
Ephrin A1	Thymocytes	DP thymocyte apoptosis	75
Ephrins A2, A4, A5	TECs, thymocytes	ND	79
Ephrin A3	Thymocytes	ND	79
Ephrins B1, B2, B3	TECs, thymocytes	Thymocyte chemotaxis; modulation of TCR signaling (ephrin B1); Organization of thymic primordium (ephrin B2); TEC organization and thymocyte differentiation (ephrins B1, B2)	48, 61, 81, 82

ND, not determined.

addition to the Sema and NRP protein families, Ephs and ephrins of both A and B families are expressed in TECs and thymocytes, which causes difficulty in defining complementary patterns of expression (17). Thymocytes express all ephrin A and most Eph A members, whereas TECs express Ephs A1, A2, A4, and A8, as well as ephrins A1, A2, and A5. Both TECs and thymocytes express Ephs B2 and B3 and their ligands, ephrins B1 and B2, in fetal and adult mice (see **Table 2**). Thymic dendritic cells (DCs) and macrophages also express some Ephs and ephrins.

Eph/EPHRIN B-MEDIATED INTERACTIONS ARE INVOLVED IN THE MIGRATION OF BONE MARROW PROGENITOR CELLS INTO THE THYMUS

EphB2-deficient bone marrow Lin^- progenitor cells have reduced capacity to colonize wild-type (WT) fetal thymic lobes, as compared with WT $EphB2^+$ progenitors, which suggests an autonomous role for EphB2 in thymus-settling precursors. By contrast, normal migratory capacity was seen when $EphB2^{lacZ}$ bone marrow-derived progenitors were used (42). These progenitors express a truncated form of EphB2 that can transmit reverse signals to microenvironmental cells of fetal thymic lobes but do not receive forward signaling. Therefore, EphB2 expressed in fetal thymic lobes, presumably in the thymic epithelium, also exerts a cell nonautonomous role on the migration of lymphoid progenitors into the thymus (as summarized in **Fig. 2**).

Available thymic niches have been claimed to be essential for controlling the arrival of progenitors into the adult thymus (6, 43). In this respect, we found an important decrease in the immigration capacity of bone marrow precursors, including those derived from WT donors, into the $EphB2^{-/-}$ mutant fetal thymic lobes (42). Under those conditions, the lack of TEC forward and reverse signaling in bone marrow progenitors, together with the absence of forward signals in TECs, have additive inhibitory effects on the coloniza-

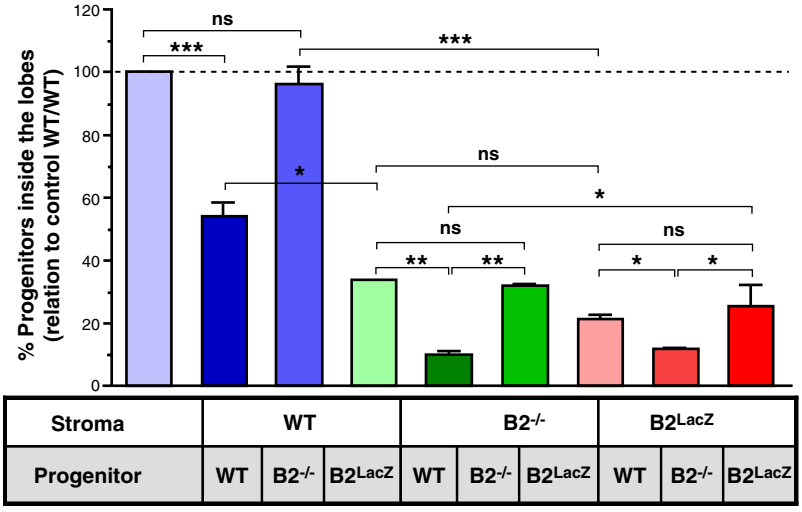

Figure 2. EphB signaling participates in bone marrow progenitor cell migration during thymus settling. Proportions of Lin^- bone marrow-derived progenitor cells inside the alymphoid fetal thymic lobes (E15.5) after 20-h assays in relation to the control condition (WT thymic lobes colonized by WT progenitor cells), considered as 100%. Fetal thymic lobe-derived microenvironmental cells were obtained from WT CD-1 mice, whereas Lin^- bone marrow-derived cells (progenitors) were derived either from WT and mutant mice in a CD-1 background knocked down for EphB2 receptor ($B2^{-/-}$), EphB3 receptor ($B3^{-/-}$) or expressing a EphB2-βgal fusion receptor ($B2^{LacZ}$). The lack of signaling throughout EphB2 or EphB3 significantly reduces the migratory capacity of progenitor cells. Nevertheless, $B2^{lacZ}$ progenitor cells (which do not receive forward signals but activate reverse signals on thymic stromal cells), show control levels of migration. Data are means ± SE representative of 3 independent experiments with ≥3 replicates. ns, not significant. $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$; 1-way ANOVA followed by Newman-Keuls multiple comparison tests. (Adapted from ref. 42.).

tion. Recently, we confirmed the involvement of EphB in the entrance of progenitor cells in fetal thymic lobes by analyzing the migration to and within WT fetal thymic organ cultures (FTOCs) of precursor cells isolated from EphB3$^{-/-}$ mice. As described above for EphB2$^{-/-}$ Lin^{-} bone marrow cells, in both competitive and noncompetitive conditions, EphB3-deficient cells migrate less efficiently than WT progenitors (unpublished results). In other studies, EphB2- and EphB3-deficient thymuses showed similar, but not identical, phenotypic alterations, largely affecting TECs (44). Furthermore, whereas EphB2 could be involved in the double-negative to double-positive progression of developing thymocytes, EphB3 seems to be important in the double-positive to single-positive stage of thymocyte maturation (45).

Moreover, when we tested the ability of different progenitors to colonize the same fetal thymic lobes in competitive conditions, EphB2$^{-/-}$ precursor cells showed the lowest colonization capacity. EphB2^{lacZ} progenitors colonized EphB2-deficient thymic lobes less efficiently than WT progenitors but not WT lobes (42). In addition, both EphB2$^{-/-}$ and EphB2^{lacZ} progenitors remained preferentially in the periphery, and lower proportions of deficient cells appeared in the central area of thymic lobes compared with WT progenitors. Thus, once lymphoid progenitors are inside the thymic lobes, the behavior of EphB2$^{-/-}$ and EphB2^{lacZ}-expressing thymocytes is similar, which suggests that different EphB2-mediated signals are involved in the colonization of thymic lobes as compared to the migration throughout such lobes (42).

Notably, several other intrathymic events that require TEC-thymocyte interactions are mediated by Ephs and ephrins. Ephrin B1-Fc treatment in reaggregate thymus organ cultures (RTOCs) established with double-positive thymocytes and fetal microenvironmental cells prevents thymocyte-TEC interactions, which results in the loss of TEC projections (46). Thymocytes cannot induce proper 3-dimensional organization of cortical TECs in EphA4-deficient thymus (47). Disorganization of epithelial network observed in different EphB mutant mice is a consequence of the lack of proper thymocyte-TEC interactions due to the absence of a correct Eph-ephrin B signaling (44). Likewise, alterations observed in both chimaeras established with EphB-deficient bone marrow cells (45) and of thymocyte-conditioned ephrin B2 mutant mice (48) support the importance of these molecules in the topological interactions of thymocytes and TECs, key for the morphofunctional organization of the organ, likely comprising cell migration events.

SOLUBLE Semas ARE CHEMOREPULSIVE FOR THYMOCYTE MIGRATION

Although data, especially *in vivo*, are not so extensive as those on Ephs and ephrins, class 3 Semas and their associated receptors also seem to be involved in intrathymic cell migration. We have shown that Sema3A is not chemoattractant for human thymocyte migration. On the contrary, it exerts a dose-dependent chemorepulsive effect, which is quite specific since it can be abrogated with anti-Sema3A antibodies (49). Accordingly, Sema3A has deadhesive properties, which can significantly disrupt *in vitro* TEC/thymocyte heterocellular adhesion (49).

Notably, a redundancy seems to exist in Sema3 members, since interactions mediated by Sema3F/NRP-2 are also chemorepulsive for human thymocyte migration (34). In this vein, it has been shown that soluble Sema7A seems to inhibit spontaneous human T-cell migration (50), which suggests that soluble Semas represent a protein group that exert a negative control on thymocyte migration. Nevertheless, this assumption needs further investigation, in view of rather contradictory findings showing that effector T cells can migrate normally into antigen-challenged sites in Sema7A-deficient mice (51). Also, note that in the mouse model, it has been shown that plexin D1-deficient fetal liver cell transplanted mice or Sema3E-knockout (KO) animals present an aberrant accumulation of CD69^{+} thymocytes in the thymic cortex (31). Unfortunately, data in the literature are poor concerning the role of Sema-NRP interactions in the entrance of precursors into and exit of mature thymocytes from the thymus.

Eph-EPHRIN AS WELL AS Sema-NRP INTERACTIONS MODULATE T-CELL MIGRATION TRIGGERED BY CHEMOKINES AND EXTRACELLULAR MATRIX (ECM) PROTEINS

Expression of molecules previously reported to be involved in the migration toward and within the thymus, including ECM ligands such as fibronectin and laminin, as well as the chemokines CCL21, CCL25, and CXCL12, were studied through immunohistochemistry and computer-based quantitation in EphB2-KO animals. In comparison with WT control fetal thymuses, both EphB2$^{-/-}$ and EphB2^{lacZ} E15.5 fetal thymuses revealed a significantly lower staining of all studied molecules, except CXCL12 (42). Notably, except for CCL25, significant differences were not found in the expression pattern of the studied molecules when EphB2$^{-/-}$ and EphB2^{lacZ} thymuses were compared to each other.

Remarkably, the study on the expression (percentage of positive cells and membrane density levels) of ECM receptors (VLA-4, VLA-5, and VLA-6) and chemokine receptors (CXCR4, CCR7, and CCR9) on both Lin^{-} bone marrow-derived progenitors and CD4/CD8-defined thymocyte subsets did not reveal changes in EphB2-deficient compared to control WT mice (42). Nevertheless, it is important to point out that the lack of quantitative changes in the expression of those molecules does not necessarily mean that their activation levels remain unaltered.

In keeping with the proposed role of EphB2 in the progenitor migration to the thymus and in their topological distribution inside the organ, migration through ECM proteins or toward chemokines, assayed in transwell systems, is reduced significantly in both EphB2-deficient thymocytes and Lin^- bone marrow-derived progenitors (42). In these experiments, $EphB2^{-/-}$ cells exhibited even a lower response to the tested chemokines than $EphB2^{lacZ}$ counterparts. Furthermore, a more drastic reduction of migratory capacity of both EphB2-deficient bone marrow precursors and total thymocytes was found in response to CCL25. These results indicate that the lack of EphB2 or its cytoplasmic domain diminishes cell migration and suggest that it could be mediated by cross-regulation of EpB2 and integrins or chemokine receptors. Accordingly, the lack of integrin or chemokine receptor costimulation by EphB2 in the EphB2-defective cells could explain the reduced migration, whereas in the $EphB2^{lacZ}$-expressing cells, the presence of an extracellular domain could induce a certain degree of cross-stimulation despite the lack of kinase activity. This can partially explain why the chemokine-driven migration is not so reduced in those cells as compared to EphB2-KO counterparts. Interestingly, similar Eph/ephrin-mediated phenomena have been described in other cell types (52–54).

We have also demonstrated (42) that Eph stimulation by coated ephrin B1-Fc fusion proteins inhibits laminin- and fibronectin-driven migration responses as well as CXCL12-, CCL21-, and CCL25-induced chemotaxis of both WT bone marrow progenitors and thymocytes (**Fig. 3**). Furthermore, in the same experimental conditions, $EphB2^{lacZ}$ bone marrow progenitors or thymocytes that cannot transmit forward signals do not undergo reduced migration, which thus confirms the specific involvement of EphB2 forward signaling in the process. Ephrin B1 stimulation promotes inhibition of cell migration, presumably by cell repulsion, as it has been previously observed after ephrin B1/EphB2 costimulation in other systems (55, 56) including chemokine-induced lymphocyte migration (57). Accordingly, the physiological migration of progenitor cells and thymocytes driven by different stimuli is mediated by the presence of nonactivated EphB2 molecules (as in the absence of molecule) and negatively modulated by EphB2-ephrin B interactions.

No other information is available regarding the role of Ephs and ephrins in cell migration in the thymus, but the involvement of these molecules, especially those of family A, in the cell migration of diverse immune cells has been reported. EphA2 regulates integrin-mediated adhesion of human DCs to fibronectin (58). Moreover, it has been related with the adhesion of leukocytes to endothelia in inflammatory conditions (59, 60). In addition, the lack of EphA2 signaling alters extravasation of immune cells (57, 61), and $EphA2^{-/-}$ mice accumulate T lymphocytes and DCs in the lungs after injection with *Mycobacterium*

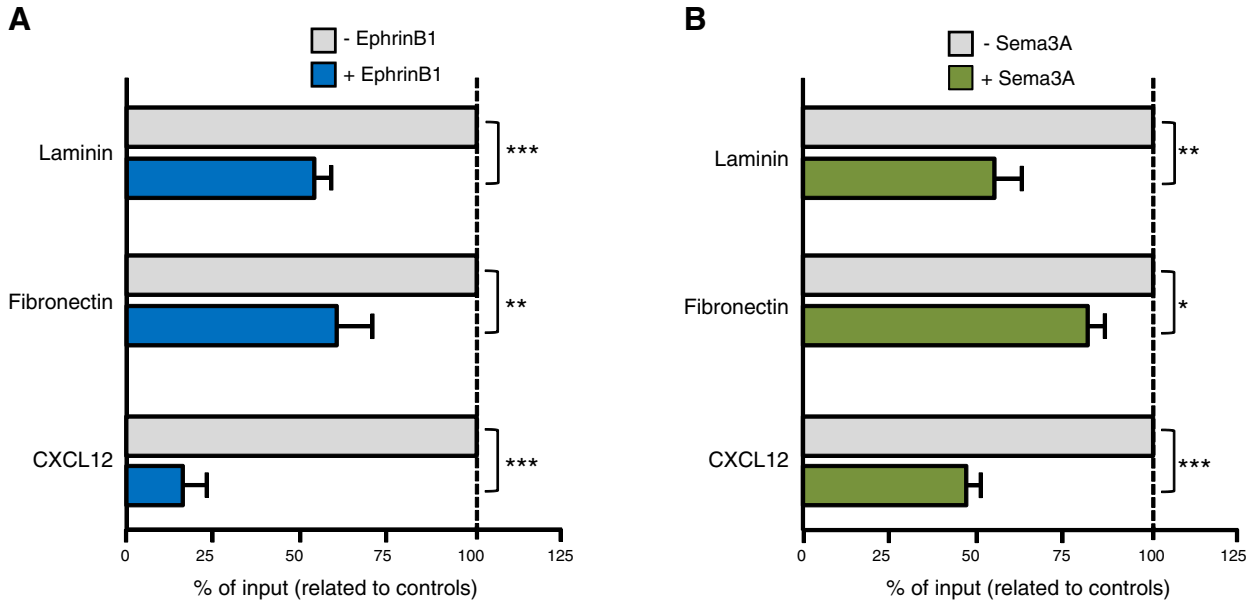

Figure 3. Ephrins and Semas modulate ECM- and CXCL12-driven migration of T-cell progenitors. Essentially, cells were plated in the top chamber of 5-μm transwell inserts in serum-free medium. The cells passing into the bottom chamber after 4 h of incubation were collected, counted, and analyzed by flow cytometry. Spontaneous migration was subtracted from the number of each migrating cell obtained for each specific hapto or chemotactic stimulus. *A*) CD-1 mouse thymocytes were plated in ephrin B1-immobilized transwell inserts coated with fibronectin or laminin. CXCL12 was added to the bottom chambers as a chemoattractant stimulus. *B*) Human thymocytes were plated in transwell inserts coated with fibronectin or laminin. In this case, Sema3A was added to the top chambers as a chemorepulsive stimulus and/or CXCL12 was added to the bottom chambers as a chemoattractant moiety. In each panel, the relative mean response (percentage of input in relation to control without ephrin-B1 or Sema3A) of ≥3 experiments is shown. $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$; Student's *t* test. (Adapted from refs. 42, 49, 68.).

tuberculosis, suggesting the involvement of EphA2 in modulating migration of those immune cell types (62).

Other results are, however, contradictory. Interactions of EphA and ephrin A1 negatively regulate the migration of both murine and human T cells (63), but ephrin A1, in combination with CXCL12, stimulates migration of memory T cells, and ephrin B1-Fc fusion protein stimulates the CXCL12-dependent migration of peripheral blood lymphocytes in a dose-dependent manner (64).

Recently, some of us have reported that certain members of Eph receptors, particularly EphA2 and one of its ligands, ephrin A4, could be involved in B-cell trafficking through lymph node HEVs (65, 66). We demonstrated that critical steps of the extravasation process can be modulated by the interactions of ephrin A4 expressed on circulating lymphocytes and the EphA2 found in the luminal side of $CD31^+$ lymph node HEVs. Thus, ephrin A4 signaling inhibits the CCL19-mediated chemotaxis, but not the migration driven by CXCL12 or CXCL13, of chronic lymphocytic leukemia cells, which express high levels of that ephrin (65, 67). Experiments done using human thymocytes clearly revealed that, in addition to the chemorepulsive effect of Sema3A *per se*, this ligand significantly inhibits fibronectin- and laminin-induced thymocyte migration, illustrating the importance of Sema3A-NRP1 interactions in thymocyte guidance alone or combined with other molecules as ECM ligands (49).

More recently, we have shown that Sema3A can also inhibit human thymocyte migration induced by CXCL12, a chemokine known to mediate cell migration within the thymus (Fig. 3). Sema3A down-regulates CXCR4, the CXCL12 receptor, inhibiting the phosphorilation of kinases involved in the signaling pathways induced by this chemokine (68). Interestingly, in this same study, we demonstrated by confocal microscopy that *in situ*, Sema3A is largely colocalized with CXCL12 in both cortical and medullary regions of the human thymic lobules. Conceptually, such findings corroborate the notion that *in vivo*, such interactions should really take place, ultimately modulating the ordered migration of thymocytes throughout the thymic parenchyma.

CONCLUSIONS AND FUTURE DIRECTIONS

Overall, the data summarized above show that interactions mediated by class 3 Semas and ephrins can down-regulate ECM- as well as chemokine-triggered thymocyte migration (see **Fig. 4**). Thus, they should be considered as further players in the complex process of developing T-cell migration from the entrance of bone marrow-derived precursors to the export of mature T lymphocytes. Further goals will be to determine possible direct associations between Semas and NRPs and Ephs and ephrins in these processes. Although no

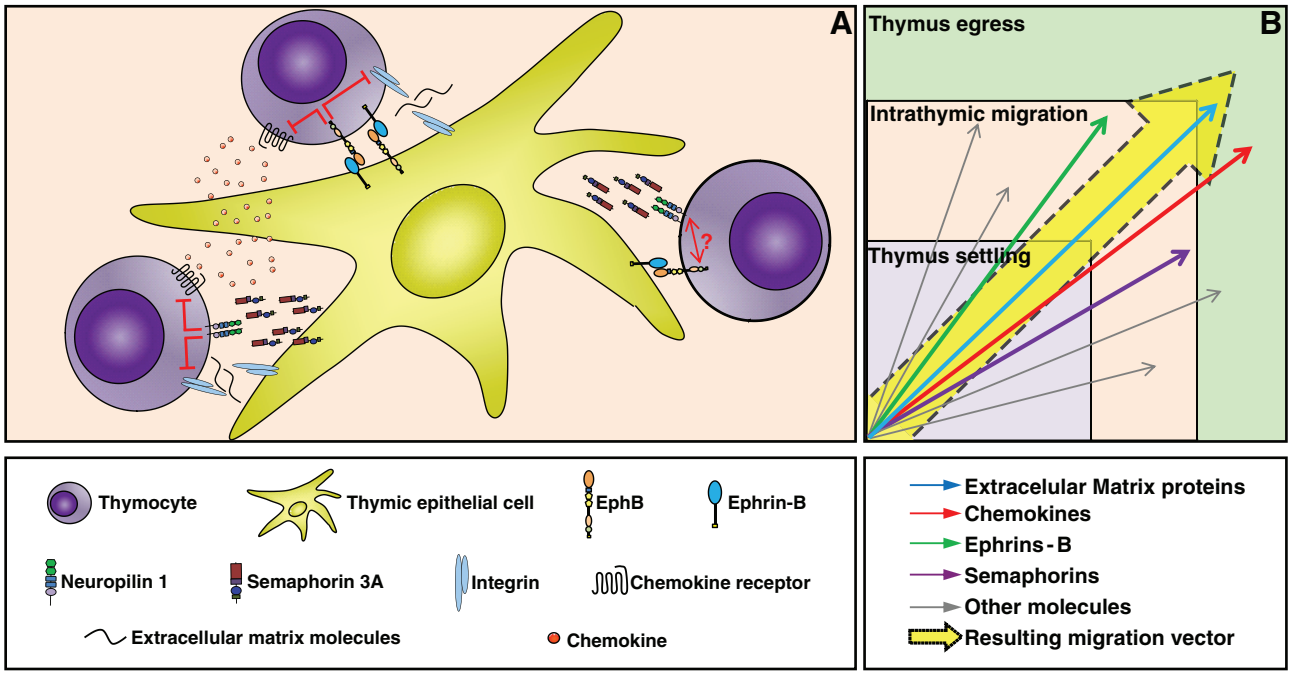

Figure 4. Models of Sema-NRP and Eph-ephrin interactions in developing T-cell migration. *A*) Representative scheme showing that Sema3A/NRP1- and Ephrin B/EphB-mediated interactions are involved in thymocyte-TEC interactions as adhesion and thymocyte migration within the thymus. Interactions mediated by both ligand-receptor interactions can also modulate thymocyte migration by blocking migration induced by ECM molecules and chemokines such as CXCL12. Direct association between Semas and NRPs and Ephs and ephrins in the thymus remains unknown. *B*) Semas and ephrins can be placed in the framework of the concept stating that migration of developing T cells can be described as a multivectorial system, in which the final resulting vector is derived from a balance of several simultaneous and/or sequential ligand/receptor pair interactions, each representing an individual vector. (Modified from ref. 76).

current evidence supports such an association, this hypothesis can be raised (see Fig. 4), since a certain degree of cooperation has been reported in other biological systems. Sema3 and ephrin B2 seem to cooperate for governing growth cone collapse in sympathetic neurons (69). Moreover, it had been proposed an integrated control of TGF-β, Sema, and ephrin signaling in the sorting of cell clusters into distinct rays during the developing male tail of *Caenorhabditis elegans* (70). In addition, ephrin A4 may play a role in the signaling of MAB-20, a class 2 Sema of *C. elegans* involved in the control of axon guidance and epidermal morphogenesis, and this ephrin is coexpressed with plexin PLX-2, another molecule that binds MAB-20, in the late embryonic epidermis, where they could play redundant roles in MAB-20-dependent cell sorting (71).

Since thymocyte migration, as well as thymocyte-TEC interactions, are directly implicated in other processes as positive and negative selection, which result in the generation of the intrathymic T cell repertoire, it would be interesting to determine the possible roles of both Semas/NRPs and Eph/ephrins in these events. Some data have established a relationship between intrathymic selective processes and migration speed using 2-photon laser-scanning microscopy: Positive selection was correlated with rapid and directional migration patterns (72), whereas negatively selecting thymocytes migrate slowly, in a highly confined manner (73). These migratory patterns are dependent on the modulation of chemokine receptors such as CCR7 and possibly integrins (74), which could in turn be modulated by Sema/NRP and Eph/ephrin signaling. In addition, ephrin A-EphA and ephrin B1-EphB seem to regulate thymocyte selection by modulating anti-CD3-induced apoptosis (46, 75), although the role of class 3 Sema/NRP-mediated interactions in this regulation remain largely unknown.

Finally, the data discussed above should be placed in the context of the concept stating that migration of developing T cells can be described as a multivectorial system, derived from a balance of several simultaneous and/or sequential ligand/receptor pair interactions, each representing an individual vector (76). Actually, the model is even more complex, since one given vector can modulate another. In this overall context, each Sema- and each ephrin-mediated interaction should be conceptualized as one individual vector, able to contribute to the resulting migration vector, not only *per se*, but also by its capacities to modulate other individual vectors, such as those represented by ECM and chemokine ligands (Fig. 4). FJ

This work was supported by grants BFU 2007-65520 and BFU 2010-18250 from the Spanish Ministry of Education and Science; RD06/0010/0003 from the Spanish Ministry of Health and Consumption; S-BIO/0204/2006, GR74/910552/2007, and S2010/BMD-2420 CELLCAM-CM from the regional government of Madrid; and also the Oswaldo Cruz Foundation (Fiocruz), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) of Brazil.

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Received for publication March 19, 2012.
Accepted for publication July 10, 2012.