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# Thymocyte migration: an affair of multiple cellular interactions?

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## Abstract

Cell migration is a crucial event in the general process of thymocyte differentiation. The cellular interactions involved in the control of this migration are beginning to be defined. At least chemokines and extracellular matrix proteins appear to be part of the game. Cells of the thymic microenvironment produce these two groups of molecules, whereas developing thymocytes express the corresponding receptors. Moreover, although chemokines and extracellular matrix can drive thymocyte migration *per se*, a combined role for these molecules appears to contribute to the resulting migration patterns of thymocytes in their various stages of differentiation. The dynamics of chemokine and extracellular matrix production and degradation is not yet well understood. However, matrix metalloproteinases are likely to play a role in the breakdown of intrathymic extracellular matrix contents. Thus, the physiological migration of thymocytes should be envisioned as a resulting vector of multiple, simultaneous and/or sequential stimuli involving chemokines, adhesive and de-adhesive extracellular matrix proteins, as well as matrix metalloproteinases. Accordingly, it is conceivable that any pathological change in any of these loops may result in the alteration of normal thymocyte migration. This seems to be the case in murine infection by the protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas' disease. A better knowledge of the physiological mechanisms governing thymocyte migration will provide new clues for designing therapeutic strategies targeting developing T cells.

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## Introduction

Cell migration is a crucial event in intrathymic T-cell differentiation. From the entrance of T-cell precursors into the thymus to the exit of mature cells from the organ, developing thymocytes encounter cortical and medullary microenvironments through distinct types of interactions. Herein we will discuss data indicating that thymocyte migration throughout the organ is a result of different cellular interactions. Yet, before entering this issue, it seems worthwhile to provide a general background to the relationship between developing thymocytes and the nonlymphoid compartment of the thymus.

## The thymic microenvironment and T-cell differentiation

The thymus is a primary lymphoid organ in which bone marrow-derived T-cell precursors undergo differentiation, ultimately

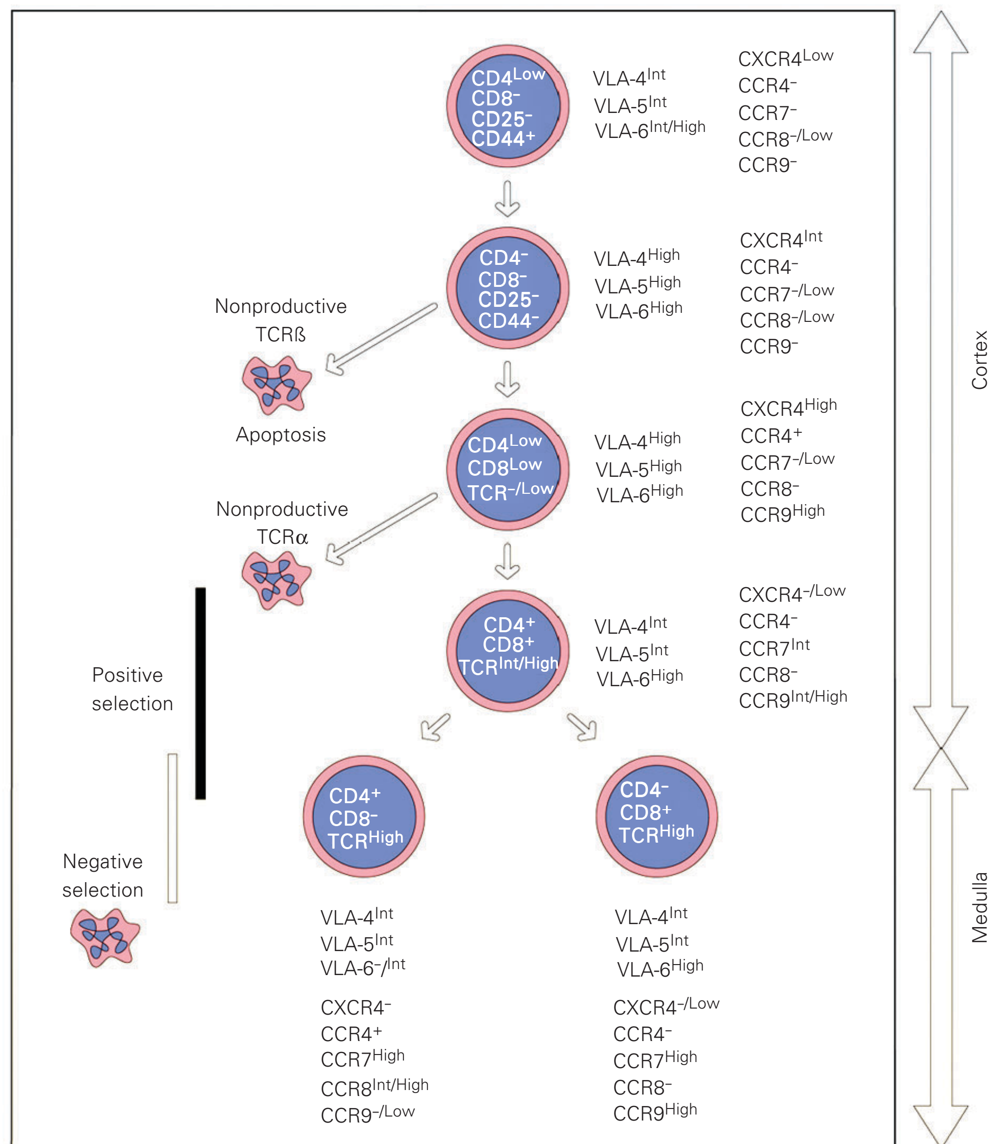


Figure 1. Expression of chemokine and extracellular matrix receptors in differentiating thymocytes. This scheme shows the expression of extracellular matrix receptors (herein exemplified by VLA-4, VLA-5 and VLA-6) and chemokine receptors (CXCR4, CCR4, CCR7, CCR8 and CCR9) as a function of the four TCRαß/CD4/CD8-defined stages (and CD25/CD44-defined subsets of double-negative cells) of thymocyte differentiation. Also, we illustrate apoptosis points secondary to nonproductive TCR gene rearrangements as well as negative selection. The patterns depicted are derived from a compilation of data in which VLAs or chemokine receptors were traced independently, i.e., experiments looking for the co-expression of these two kinds of receptors have not yet been reported. Although some chemokine receptors can be subset specific, VLAs can be found in all CD4/CD8-defined subsets, from double-negative → double-positive → single-positive stages. High = high density; Int = intermediate density; Low = low density; + = positive; – = negative; TCR = T-cell receptor; VLA = very late antigen.

leading to the export of positively selected thymocytes towards the T cell-dependent areas of peripheral lymphoid organs. This process consists of the sequential expression of various proteins and rearrangements of the T-cell receptor (TCR) genes.

The most immature thymocytes express neither the TCR complex nor CD4 or CD8 accessory molecules, being called double-negative thymocytes, and corresponding to 5% of all thymic lymphocytes. In mice, this stage can be further characterized by the differential expression of the CD25 and CD44 markers.

Maturation progresses with the acquisition of CD4 and CD8 markers, generating the $CD4^+CD8^+$ double-positive cells, which comprise 80% of the whole population. In this stage, TCR genes are rearranged, and productive rearrangements yield the membrane expression of TCR in low density ($TCR^{low}$). Thymocytes that do not undergo a productive TCR gene rearrangement die by apoptosis, whereas those expressing productive TCR will interact with peptides presented by molecules of the major histocompatibility complex (MHC), expressed on microenvironmental cells. This interaction will determine the positive and negative selection events crucial for normal thymocyte differentiation. Positively selected thymocytes progress to the mature $TCR^{high}$ $CD4^+CD8^-$ or $TCR^{high}CD4^-CD8^+$ single-positive stage, comprising 15% of thymocytes that ultimately leave the organ to form the large majority of the peripheral T-cell repertoire (reviewed in Refs. 1,2). Figure 1 summarizes the sequential steps of intrathymic T-cell differentiation.

Thymocyte differentiation occurs as cells migrate within the thymic lobules: $TCR^-$ $CD4^-CD8^-$ and $TCR^+CD4^+CD8^+$ are cortically located, whereas mature $TCR^+CD4^+$ $CD8^-$ and $TCR^+CD4^-CD8^+$ cells are found in the medulla. Along this journey, thymocytes encounter various components of the thymic microenvironment, a three-dimensional network formed of thymic epithelial cells (TECs), macrophages, dendritic cells, fibroblasts and extracellular matrix (ECM) components (1,2). One key interaction involves the TCR/peptide-MHC, in the context of CD4 or CD8 molecules. Additionally, thymocytes interact with TECs via the ECM, an interaction that modulates thymocyte migration (3).

Microenvironmental cells also modulate thymocyte differentiation by soluble polypeptides comprising: a) cytokines, such as interleukin-1 (IL-1), IL-3, IL-6, IL-7, IL-8 and stem cell factor; b) chemokines, such as SDF-1$\alpha$, IP-10 and thymus expressed chemokine (TECK) (respectively named CXCL12, CXCL10 and CCL25 according to the international nomenclature), as well as c) thymic hormones, including thymulin, thymopoietin and thymosin-$\alpha$1 (2,4).

The TEC network is heterogeneous in terms of morphology and phenotype. Notorious in this respect is the thymic nurse cell (TNC), a lymphoepithelial complex formed by one epithelial cell and variable numbers of thymocytes, which is typically located in the cortical region of thymic lobules (5).

## Experimental models used to study cell migration in the thymus

In order to facilitate the comprehension of the concepts further developed below, we briefly comment on some of the existing strategies to evaluate thymic cell migration. The entry of T-cell precursors into the thymus can be evaluated *in vitro* by means of deoxyguanosine-treated fetal thymus organ cultures (1). These are cultures of the thymic microenvironment devoid of hematopoietic-derived cells, where we can seed T-cell precursors in order to study their entry into the microenvironmental anlagen. Alternatively, T-cell precursors can be trapped *in vivo* by injecting hematopoietic stem cells into irradiated adult mice and tracing their engraftment into a thymus that has been depleted of

autologous lymphoid cells by irradiation. Similar data can be obtained after injecting cells into SCID mice. Using these protocols, coupled to immunohistochemistry, one can trace injected cells within the thymic lobules.

As mentioned before, migration of immature thymocytes can be investigated *in vitro* by studying TNCs, as illustrated in Figure 2A. In culture, TNCs spontaneously release thymocytes, and TNC-derived epithelial cells can reconstitute lymphoepithelial complexes after being co-cultured with fetal thymocytes, thus corresponding to an *in vitro* model of thymocyte migration within the TEC context.

Figure 2B shows cell chemotaxis, another strategy to evaluate immature as well as mature thymocyte migration *in vitro* under distinct stimuli (6). Last, the exit of thymocytes can be evaluated by organ chemotaxis or by direct *in vivo* study of recent thymic emigrating cells, either by the original technique of intrathymic injection of fluorescein isothiocyanate (FITC) followed by tracing FITC$^+$ cells in peripheral lymphoid organs (7), or by the detection of TCR excision circles derived from the rearrangement of the TCR genes (8).

## Chemokine-driven thymocyte migration

Thymic microenvironmental cells produce various chemokines, and their differential roles in thymocyte migration have been recently reviewed (4). One chemokine, the CXCL12 (SDF-1α) is secreted by TECs, particularly in the subcapsular and medullary regions, and preferentially attracts immature CD4$^-$CD8$^-$ and CD4$^+$CD8$^+$ cells. Accordingly, the corresponding CXCR4 receptor is expressed in these stages of thymocyte development (4).

The chemokine CCL25 (previously named TECK) is expressed by TECs and dendritic cells, and also attracts immature thymocytes, although all thymocyte subsets are responsive (9). Accordingly, the CCR9 receptor for CCL25 is expressed at various stages of murine thymocyte differentiation, particularly in CD4$^+$CD8$^+$ and CD8$^+$ single-positive cells (4).

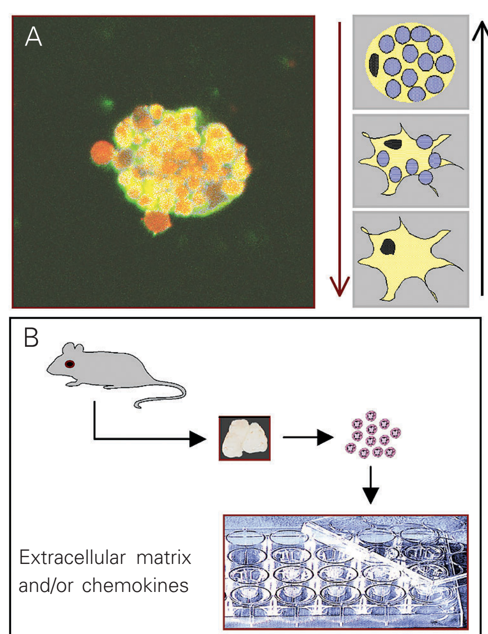


Figure 2. Two strategies to study cell migration in the thymus. *A*, The migration of immature thymocytes can be investigated *in vitro* by studying lymphoepithelial complexes, the thymic nurse cells (TNCs). The left panel depicts one of these complexes, in which thymocytes can be seen (original magnification: 600X). In culture, TNCs release thymocytes spontaneously, as illustrated by the left arrow. In short-term cultures, we can evaluate the percentages of round-shaped TNCs (i.e., prior to thymocyte release), lymphocyte-containing spread complexes that partially release thymocytes, and thymocyte-free epithelial cells. Additionally, TNC-derived epithelial cells can reconstitute lymphoepithelial complexes after being co-cultured with fetal thymocytes (right arrow), and we can count the percentages of reconstituted lymphoepithelial complexes. *B*, Cell chemotaxis is one strategy to evaluate immature, as well as mature, thymocyte migration *in vitro* in response to distinct stimuli. Cells are freshly obtained from a thymus, placed in a transwell chamber previously treated with the given chemoattractant and led to migrate towards the bottom of the chamber. Migrating cells can be counted and phenotyped.

The chemokine CCL19 is preferentially chemoattractive for mature thymocytes, being found in the medullary TECs and corticomedullary junction of thymic lobules (4). Recent data strongly indicate that CCL19 is involved in the emigration of thymocytes from the neonatal thymus (10).

Medullary TECs also produce other chemokines such as CXCL9, CXCL10 and CXCL11, involved in the migration of mature human TCRαß$^+$CD8$^+$ thymocytes, as well as TCRγδ$^+$ and natural killer cells through their common receptor CXCR3 (reviewed in Refs. 4 and 11).

Developing thymocytes differentially express the corresponding chemokine receptors according to the expression of distinct chemokines by thymic microenvironmental cells (see Figure 1).

## Extracellular matrix-mediated thymocyte migration

ECM glycoproteins, such as laminin and fibronectin, are distributed heterogeneously within the thymic parenchyma, being produced by distinct microenvironmental cells. We have shown that cultured TECs produce laminin, fibronectin and type IV collagen, as revealed in various TEC preparations, including TNCs (see Figure 3). Further results suggest that fibroblasts and MHC class II$^+$ phagocytic cells of the thymic reticulum also produce these ECM components (see Ref. 3).

Isoforms of ECM glycoproteins have been reported in the thymus. For example, the fibronectin isoform recognized by very late antigen-5 (VLA-5) through its RGD motif, is located throughout the thymic parenchyma, whereas the isoform derived from alternative splicing of fibronectin mRNA (and recognized by VLA-4 through the REDV motif) appears to be restricted to the medulla (12). Isoforms of laminin (generated by the transcription of distinct genes, and not alternative mRNA splicing) have been reported. The first laminin isoform characterized in the mouse thymus was laminin-2 (also named merosin, formed by the α2ß1γ1 heterotrimer) and thymocytes bind to this laminin isoform. Interestingly, aberrant thymocyte development was detected in the *dy/dy* mutant mouse, which lacks laminin-2. Such alterations include thymic atrophy with decreased relative numbers of CD4$^-$CD8$^-$ thymocytes and an increase in the number of apoptotic cells bearing this phenotype, suggesting that the CD4$^-$CD8$^-$ → CD4$^+$CD8$^+$ progression requires laminin-mediated interactions (13).

Laminin-5, formed from the α3ß3γ2 heterotrimer, was detected also in the human thymus, being produced by medullary TECs and able to trigger outside-in signals to thymocytes (14). In mice, anti-laminin-5 antibodies blocked thymocyte expansion, as well as CD4$^-$CD8$^-$ → CD4$^+$CD8$^+$ differentiation (15).

Several laminin isoforms are present in TEC preparations. In the human thymus, laminin-1 seems to be distributed widely within lobules and septae, whereas laminin-2 is restricted to cortical TECs (16). Although it remains undetermined whether all laminin isoforms are functional in the physiology of the thymus, we further defined that laminin-1 and laminin-2 can modulate thymocyte migration within TNC complexes (Ocampo P, Brito JM, Corrêa-de-Santana E, Borojevic R, Villa-Verde DMS and Savino W,

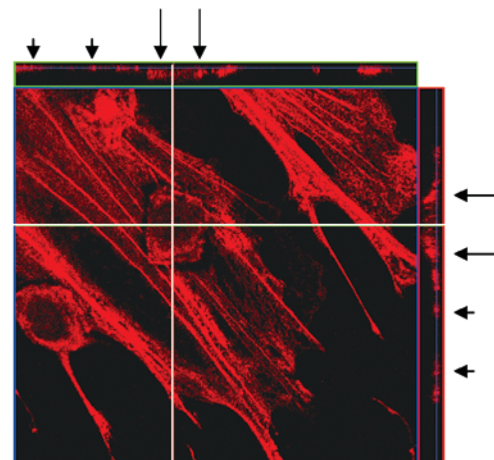

Figure 3. Detection of laminin in thymic nurse cells. In this image obtained by confocal microscopy, we can see cultured thymic nurse cells immunostained with an anti-laminin polyclonal antibody (that does not discriminate a given isoform of the molecule). The main image is the result of superposition of several confocal sections. The white lines in the middle of the figure indicate the vertical sectioning of the whole image, so that we can see in both the vertical and horizontal edges the corresponding images in depth. In such edges, long arrows indicate labeling in the cell bodies, whereas short arrows point to labeled spots corresponding to the extracellular laminin-containing fibers. Original magnification: 1000X.

unpublished results). Since most intra-TNC thymocytes are $CD4^+CD8^+$ cells, it is conceivable that, in addition to the double-negative → double-positive progression, the migration of $CD4^+CD8^+$ thymocytes is also influenced by laminin(s).

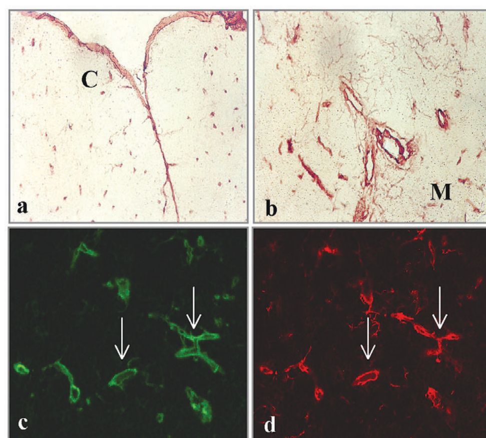


Figure 4. Intrathymic distribution of nidogen and of the molecule recognized by the ER-TR7 monoclonal antibody. In these frozen sections of young adult mouse thymus, we can see in the upper panels immunoperoxidase assays showing the presence of nidogen in the cortical (a) and medullary (b) regions of thymic lobules. Note that nidogen content is higher in the medulla (M), compared to the cortex (C). The molecule recognized by the ER-TR7 monoclonal antibody is seen in the bottom panel (c), and its co-localization with laminin is well known (d), as indicated by the arrows. Original magnification: 250X.

Table 1. Extracellular matrix molecules produced by thymic microenvironmental cells.

| Molecule | Intralobular localization[a] | Cellular sources | References |
|---|---|---|---|
| Fibronectins | | | |
| Conventional | C-M | TEC, PTR, Fb | 12, 22-24 |
| Alternative splice-derived | C-M | ND | 12 |
| Laminins | | | |
| Laminin-1 | C-M | TEC, Fb | 25 |
| Laminin-2 | C | TEC | 16, 26 |
| Laminin-5 | C or M | TEC | 14, 16 |
| Nidogen | C-M | TEC, Fb | Our unpublished data |
| Collagens | | | |
| Type I collagen | C-M | Fb | 22, 23, 27 |
| Type III collagen | C-M | Fb | 22, 23, 27 |
| Type IV collagen | C-M | TEC, PTR, Fb | 22-24 |
| Galectins | | | |
| Galectin-1 | C-M | TEC, | 28 |
| Galectin-3 | C-M | TEC, | 18 |
| Galectin-9 | C-M | TEC, PTR | 29 |
| Glycosaminoglycans | | | |
| Heparan sulfate | ND | TEC | 20 |
| Hyaluronic acid | C-M | TEC | 21, 30 |

[a]Distribution within the thymic lobules. C = cortex; C-M = cortex and medulla; Fb = fibroblasts; M = medulla; ND = not determined; PTR = phagocytic cells of the thymic reticulum; TECs = thymic epithelial cells. When only TECs are assigned, it means that the molecule has been positively demonstrated in TEC, but it does not necessarily mean that other microenvironmental cells do not produce a given molecule.

We have characterized nidogen as a further ECM ligand of the thymic microenvironment (Figure 4). This ECM molecule is frequently associated with laminin, and both molecules are co-localized in the thymus. Furthermore, we defined that one cellular source of nidogen is the thymic epithelium. Functionally, nidogen also appears to influence TEC/thymocyte interactions (17).

Galectins are also ECM-related molecules produced by thymic microenvironmental cells. In particular, we showed that both TECs and phagocytic cells secrete galectin-3 (18).

It is worth mentioning that other ECM proteins are likely to be expressed in the thymus. Accordingly, the ER-TR7 monoclonal antibody recognizes a molecule that co-localizes with laminin (Figure 4). Although this molecule is distinct from a variety of well-defined ECM proteins (19), it remains to be identified. We attempted to characterize *in vitro* the cell type responsible for the intrathymic production of the ER-TR7 molecule, but apparently the molecule or the epitope recognized by the ER-TR7 antibody is lost in culture. Also, immunoblotting experiments failed to determine the molecular weight of this molecule.

Finally, it should be noted that TECs also produce and secrete glycosaminoglycans such as heparan sulfate and hyaluronic acid, which apparently modulate TEC/thymocyte adhesion *in vitro* (20,21). Production of ECM components by the thymic microenvironment is summarized in Table 1.

In addition to producing ECM ligands, thymic microenvironmental cells express ECM receptors such as the fibronectin receptor VLA-5, as well as the laminin receptors, VLA-3 and VLA-6 (3). Moreover, we showed that cultured TECs are sensitive to fluctuations in laminin: TECs grow faster on a 10 µg/ml laminin substrate (31), which also enhances the expression of focal adhe-

sion kinases by these cells, as illustrated in Figure 5.

Integrin-type ECM receptors are also expressed by differentiating thymocytes in a pattern that is illustrated schematically in Figure 1. Accordingly, ECM proteins form molecular bridges between thymocytes and microenvironmental cells, a biological event that can provide signals to both cell types.

One essential event in cell migration is the adhesion of migrating cells to the substrate. We have demonstrated that thymocyte adhesion to cultured TECs is enhanced in the presence of ECM components, such as fibronectin and/or laminin, whereas antibodies against these adhesive molecules promote an opposite effect. Moreover, pretreatment of TECs with anti-VLA-5 or anti-VLA-6 antibodies promoted the same effects (Figure 6). Interestingly, the density of laminin receptors is enhanced at TEC boundaries bearing adherent thymocytes (32).

In other experiments, the adhesion of enriched populations of immature thymocytes (derived from sublethally irradiated mice) to TEC monolayers was shown to be largely blocked by pretreatment with anti-VLA-4 monoclonal antibody or with the fibronectin 1-25 IIICS peptide, which contains the critical LDV motif seen by VLA-4 (33).

Thymocyte release from TNCs is enhanced by fibronectin and laminin and diminished by the corresponding anti-ECM or anti-ECM receptor antibodies. Similar blocking effects can be observed in the *de novo* formation of TNC complexes when co-culturing TNC-derived epithelial cells with fetal thymocytes (5). These data show that ECM-mediated epithelial/thymocyte interactions affect both the entry to and exit of lymphocytes from this particular microenvironmental niche. Consistent with these data, both fibronectin and laminin exert a haptotatic effect on thymocytes since they induce migration of these cells *ex vivo* (Figure 7).

In addition to the role played by adhesive ECM glycoproteins, such as fibronectin, laminin and collagens, thymocyte migration might be influenced by molecules that favor de-adhesion. We have demonstrated that galectin-3 enhances thymocyte de-adhesion to microenvironmental cells and modulates thymocyte transit into and out of TNCs (18).

We recently attempted to modulate thymocyte migration *in vivo* by injecting intrathymically an RGD-containing peptide, that in principle could interfere with VLA-5-mediated interactions. Simultaneously, we injected FITC so as to trace recent thymic emigrants in peripheral lymphoid organs. We noticed that, under these experimental conditions, although the CD4/CD8-defined thymocyte subsets did not significantly change, recent thymic emigrant numbers were re-

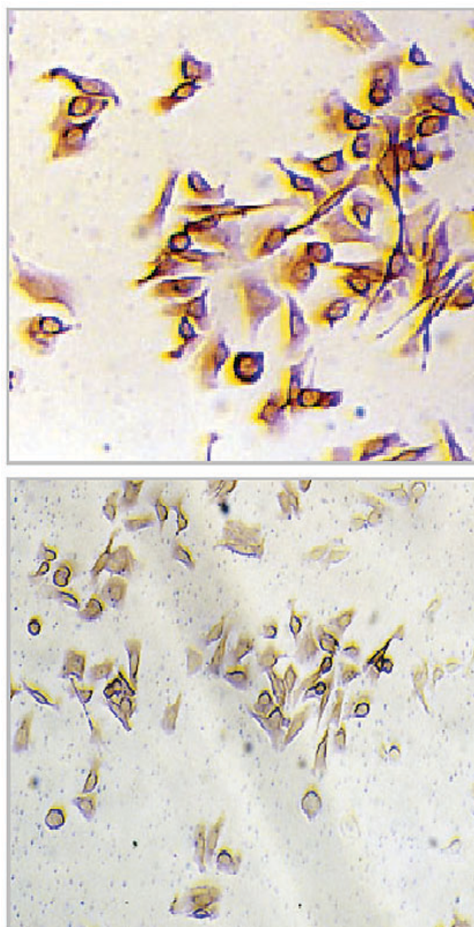

Figure 5. Laminin enhances focal adhesion kinase by thymic epithelial cells. The enzyme is visualized herein by immunocytochemistry, and its density is increased when this murine thymic epithelial cell line grows on laminin (upper panel), compared to untreated control (bottom panel). Original magnification: 250X.

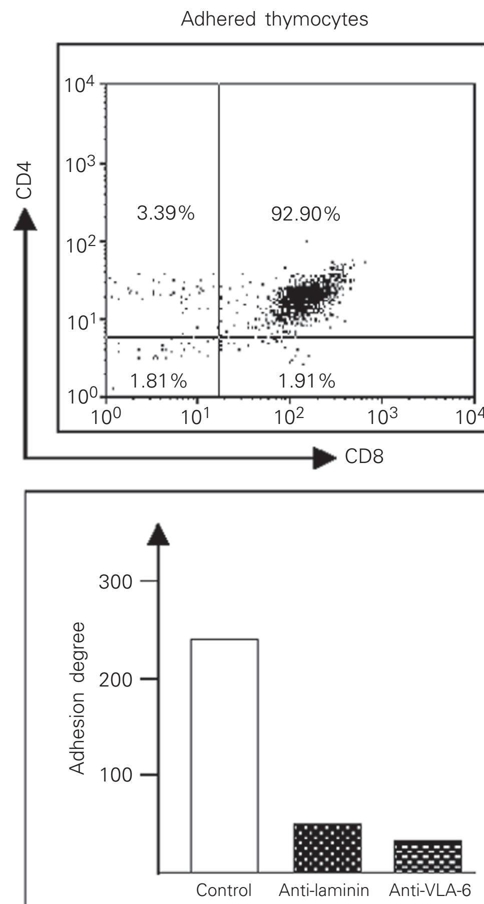


Figure 6. Disruption of laminin-VLA-6 interaction abolishes thymocyte adhesion to thymic epithelial cells. Freshly isolated mouse thymocytes were adhering to a thymic epithelial cell line. The upper panel is a double-labeling cytofluorometric profile showing that the immature CD4/CD8-defined thymocyte subsets preferentially adhere to the thymic epithelial cell culture. This adhesion is largely blocked by anti-laminin or anti-VLA-6 antibodies (bottom panel). VLA = very late antigen.

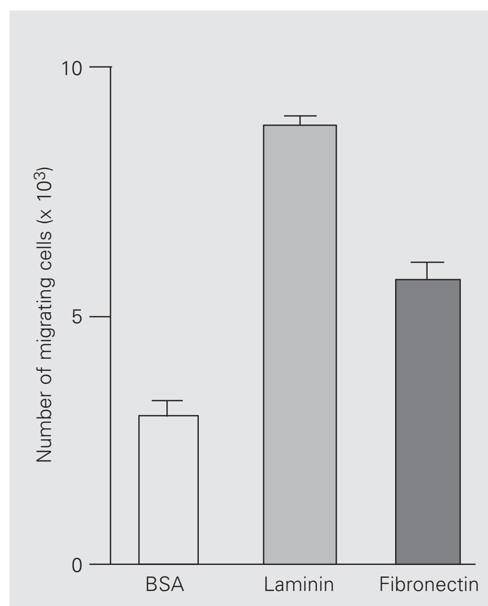


Figure 7. Extracellular matrix-driven thymocyte migration *ex vivo*. Freshly isolated mouse thymocytes were stimulated to migrate in transwells to which fibronectin or laminin had been added previously. Both extracellular matrix proteins enhance cell migration as compared to bovine serum albumin (BSA) used as negative control.

duced in the spleen (but not in lymph nodes), as seen in Figure 8.

## Potential role of matrix metalloproteinases in intrathymic extracellular matrix-mediated interactions

A further and relevant aspect regarding the role of the ECM in thymocyte migration concerns the regulatory mechanisms involved in the control of ECM production and degradation. Concerning the expression of ECM ligands and receptors by TECs, *in vitro* data indicate that cytokines, hormones and growth factors are involved (2,34). Much less is known about the physiological breakdown of thymic ECM. Matrix metalloproteinases (MMPs) are obvious candidates and recent data have indicated that some MMPs are expressed in the human thymus (35), a finding that we have confirmed, at least regarding MMP-9 (Figure 9).

A potential regulatory function of MMPs in intrathymic ECM-mediated interactions is also likely, since the colonization of fetal thymus by T-cell precursors is significantly reduced *in vitro* in the presence of an MMP inhibitor (4), and a strong colonization of adult mouse thymus is seen with lymphoma cell lines expressing high levels of MMP-9 (36).

## Is there an extracellular matrix-chemokine interplay in the thymus?

The existence of an intrathymic ECM-chemokine interplay derives from data showing that: a) CXCL12 enhances VLA-4 expression on myeloma cells and hematopoietic progenitors, resulting in a higher degree of binding to fibronectin (37), an effect that is abolished by anti-VLA-5 antibodies (38); b) CXCL12 binds to and is presented by fibronectin to a T-cell line, resulting in a greater degree of migration compared with the soluble chemokine alone (39), and the CXCL12-induced migration of $CD34^+$ pre-

cursors is enhanced by fibronectin (40). Accordingly, CXCL12 plus fibronectin- (or laminin) induced thymocyte migration in transwell chambers is greater than that elicited by the chemokine alone (41). Moreover, we showed that thymocyte migration induced by CCL19 plus fibronectin is higher than the migration induced by CCL19 or fibronectin alone (Figure 10).

Interestingly, chemokines appear to enhance T-cell adhesion to ECM and to activate ECM integrin-type receptors, as well as focal adhesion kinase (reviewed in Ref. 11). In this respect, we recently observed that CXCL12-driven migration of thymocytes in transwells is partially blocked by preincubation of the cells with anti-VLA-6 antibody (Smaniotto S, Mello-Coelho V, Villa-Verde DMS, Pléou JM, Postel-Vinay MC, Dardenne M and Savino W, unpublished results).

## Conclusions and perspectives

The first and obvious conclusion from the data discussed here is that the molecular circuits governing thymocyte migration are complex and comprise distinct types of cellular interactions. In this respect, at least chemokines and ECM proteins appear to act in combination. Furthermore, synergy occurs between chemokines and ECM, even at the level of receptor regulation. In this respect, the simultaneous detection of distinct ECM and chemokine receptors during thymocyte differentiation is important, and is currently being studied by our group.

Moreover, this dynamic ECM-chemokine system may be regulated by at least one group of degrading enzymes, the MMPs. Thus, the physiological migration of thymocytes should be envisioned as a vector resulting from multiple, simultaneous and/or sequential stimuli, involving chemokines, adhesive and de-adhesive ECM proteins, as well as MMPs. If we accept this hypothesis, it is conceivable that any pathological change in any of these loops might result in the alteration of normal thymocyte migration. This seems to be the case in the murine infection by the protozoan *Trypanosoma cruzi*, the causative agent of Chagas' disease. In mice acutely infected with this para-

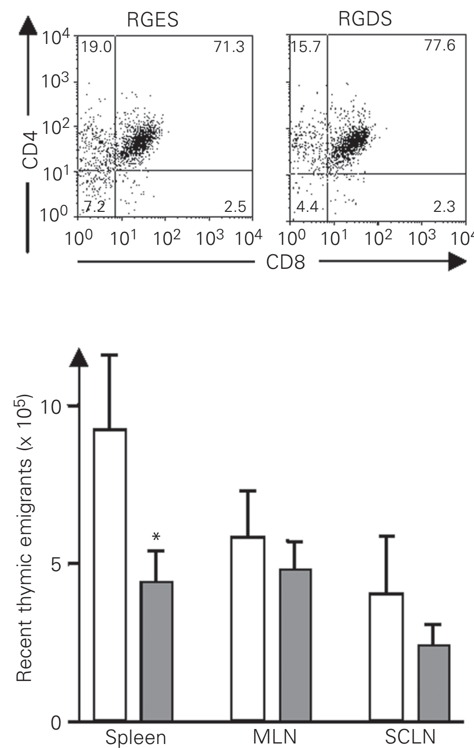


Figure 8. RGD-containing peptide modulates recent thymic emigrants. This figure depicts experiments in which young adult mice were injected intrathymically with RGDS- (filled columns) or RGES- (open columns) containing peptides. FITC was simultaneously injected in order to label thymocytes, so as to allow tracing the recent thymic emigrants in peripheral organs. In the upper panel, CD4/CD8-defined cytofluorometric profiles reveal that the injection of the VLA-5-blocking peptide containing the RGD sequence did not promote any phenotypic change in thymocyte subpopulations, as compared to the negative control (RGES). Nevertheless, the numbers of recent thymic emigrants (lower panel) that homed to the spleen (but not to the subcutaneous or mesenteric lymph nodes) are reduced in the RGDS group. MLN = mesenteric lymph nodes; SCLN = subcutaneous lymph nodes; VLA = very late antigen. $^*P < 0.05$ (Student *t*-test).

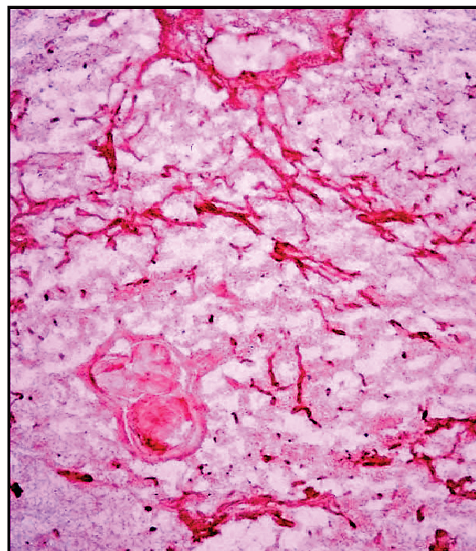

Figure 9. Distribution of matrix metalloproteinase-9 (MMP-9) in the human thymus. In this histological section, MMP-9 distribution is seen in the medullary region of a thymic lobule and surrounding blood vessels, similar to the pattern of laminin or fibronectin. Original magnification: 250X.

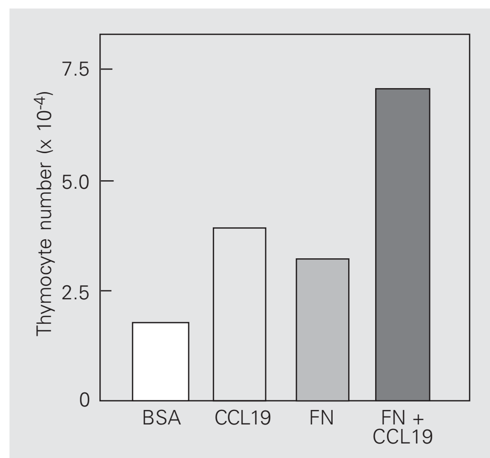


Figure 10. Combined role of fibronectin (FN) and CCL19 (macrophage inflammatory protein-3ß) in inducing thymocyte migration. In this figure, thymocytes were stimulated to migrate in transwells to which fibronectin or CCL19 had been previously added separately or together. The migration degree observed where fibronectin plus CCL19 was added was higher than when each molecule was applied separately. BSA = bovine serum albumin.

site, we found severe thymic atrophy with depletion of $CD4^+CD8^+$ cortical thymocytes, accompanied by an increase in the intrathymic ECM content and changes in the expression of fibronectin and the laminin receptors VLA-4, VLA-5 and VLA-6 in the remaining thymocytes. Additionally, cultured TNCs, infected *in vitro* or derived from infected mice, exhibited increased ECM contents correlated with an enhancement of thymocyte release. In this respect, we found abnormal numbers of $CD4^+CD8^+$ cells in lymph nodes of infected animals (reviewed in Ref. 3).

On the basis of the data discussed above, it is clear that a better knowledge of the mechanisms governing intrathymic T-cell migration will provide new clues for understanding how the thymus works in normal conditions and for designing therapeutic strategies targeting developing T cells.

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