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The conveyor belt hypothesis for thymocyte migration: participation of adhesion and de-adhesion molecules

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Abstract

Thymocyte differentiation is the process by which bone marrow-derived precursors enter the thymus, proliferate, rearrange the genes and express the corresponding T cell receptors, and undergo positive and/or negative selection, ultimately yielding mature T cells that will represent the so-called T cell repertoire. This process occurs in the context of cell migration, whose cellular and molecular basis is still poorly understood. Kinetic studies favor the idea that these cells leave the organ in an ordered pattern, as if they were moving on a conveyor belt. We have recently proposed that extracellular matrix glycoproteins, such as fibronectin, laminin and type IV collagen, among others, produced by non-lymphoid cells both in the cortex and in the medulla, would constitute a macromolecular arrangement allowing differentiating thymocytes to migrate. Here we discuss the participation of both molecules with adhesive and de-adhesive properties in the intrathymic T cell migration. Functional experiments demonstrated that galectin-3, a soluble ß-galactoside-binding lectin secreted by thymic microenvironmental cells, is a likely candidate for de-adhesion proteins by decreasing thymocyte interaction with the thymic microenvironment.

Key words
• Thymus
• Thymic nurse cells
• Adhesion molecules
• Extracellular matrix
• Galectin-3

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Introduction

The thymus plays a central role in the development of T lymphocytes. In the process known as thymocyte differentiation, precursors coming from the bone marrow enter the thymus, undergo a series of events which include the sequential expression of differentiation antigens such as CD44, CD25, CD3, CD4, CD8, and the rearrangement of T cell receptor genes. This process culminates with selection of thymocytes that will represent the so-called T cell repertoire (1-3). Selected T lymphocytes will then leave the organ to colonize T-dependent areas in secondary lymphoid organs. Kinetic studies on thymocyte migration favor the idea that these cells leave the thymus in an ordered pattern, as if they were moving on a conveyor belt (4). We have recently proposed that extracellular matrix (ECM) glycoproteins, produced by non-lymphoid cells both in the cortex and in the medulla, would constitute a macromolecular arrangement allowing differentiating thymocytes to migrate (5). Since in order to migrate cells must adhere to their microenvi-

ronment and subsequently de-adhere, the function of molecules with adhesive and de-adhesive properties seems to be important. ECM glycoproteins with adhesive characteristics, such as fibronectin, laminin and type IV collagen, have been described in the human and murine thymus (6,7). Moreover, we have shown that both ECM ligands and receptors, such as the integrins VLA-5 and VLA-6, receptors for fibronectin and laminin, respectively, as well as the CD44 molecule, a receptor for hyaluronic acid, fibronectin and collagen, modulate *in vitro* interactions between thymocytes and thymic microenvironmental cells, such as thymic epithelial cells (TEC) and phagocytic cells of the thymic reticulum (8-10).

Thymic nurse cells as a model for studying thymocyte migration

The thymic nurse cells (TNCs) are lymphoepithelial complexes in which one epithelial cell harbors 2 to 200 differentiating thymocytes (11,12). These complexes seem to work as a special microenvironment where immature thymocytes are presented to class I and II MHC antigens exposed by the epithelial cell, being also exposed to soluble factors such as thymic hormones and interleukins secreted by the epithelial component (12-15). Moreover, TNCs have been shown to participate in positive thymocyte selection and to contain apoptotic thymocytes (16-18). Additionally, TNCs have been used by our group as a model to further evaluate thymocyte/TEC interactions. Since they can be easily isolated from the thymus of different species, from fish to man, TNCs represent a useful tool to study the role of ECM ligands and receptors in intrathymic heterotypic interactions. When plated in culture, freshly isolated TNC adhere and release thymocytes through an active process that is dependent on the integrity of the cytoskeleton and on cell metabolism (19,20). Interestingly, TNC-derived epithelial cells, obtained after complete thymocyte release from TNC complexes, when co-cultured with fetal thymocytes in a hanging-drop system, are able to reconstitute TNC complexes, as if reversing the process of thymocyte release (21). We have shown that ECM ligands and receptors are involved in cell-cell interactions in the TNC model, as they can be modulated by antibodies to these molecules (8,9). Such findings, originally obtained in the murine model, were confirmed in the human and chick models, thus showing the phylogenetic conservation of these events (Figure 1). Our data suggest that ECM ligands and receptors may influence thymocyte differentiation, possibly by stabilizing interactions between thymocytes and the epithelial component of TNC.

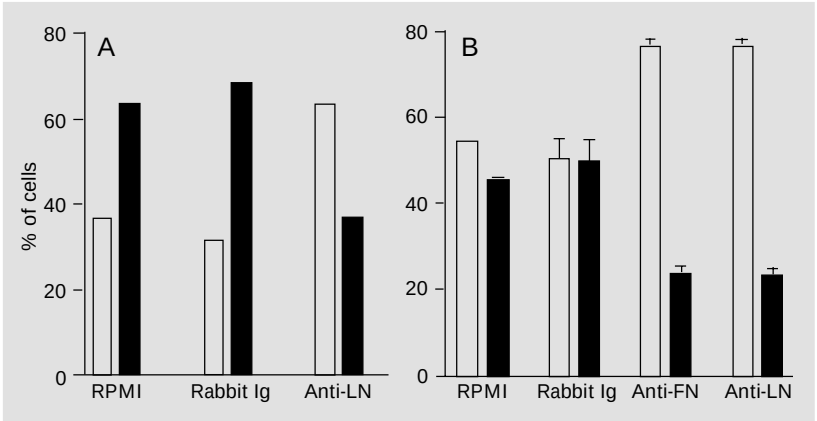

Figure 1 - Extracellular matrix proteins modulate *in vitro* thymocyte release from thymic nurse cells (TNCs) in the human and chick models. Panel A shows that thymocyte release by human TNCs is decreased in the presence of anti-laminin polyclonal antibody. Panel B shows similar inhibitory effects concerning the release of thymocytes by chick TNCs in the presence of anti-laminin (anti-LN) and anti-fibronectin (anti-FN) reagents (Institute Pasteur, Lyon, France). Control cultures were maintained in complete RPMI or treated with rabbit Ig at similar protein concentrations. Black columns, TNC-derived epithelial cells that totally released thymocytes; gray columns, TNCs in which thymocytes are still present.

De-adhesion: a further step in thymocyte migration

As mentioned above, the sequential cellular events leading to thymocyte migration comprise not only their adhesion to the microenvironmental substrate, but also de-adhesion. The latter event can be achieved directly by enzymatic degradation of the

molecular interactions that promote adhesion and/or by eliciting further interactions that induce de-adhesion. Specifically, interactions within the TNC complexes, regarding thymocyte entry into and exit from complexes, could be dependent on the presence of de-adhesion molecules. After a period within the complex, where important steps of thymocyte differentiation seem to occur, these cells must leave TNCs and continue on their way to the periphery. One of the candidates for such de-adhesion proteins is galectin-3, a soluble ß-galactoside-binding lectin which functions as a de-adhesive molecule in other biological systems (22-24) and which has been recently demonstrated to prevent apoptosis in thymocytes (25). In the murine and human thymus, this molecule is secreted by TEC, including the epithelial component of TNCs (Figure 2). Functional experiments demonstrated that incubation of thymocytes with a galectin-3-enriched TEC-conditioned medium decreases thymocyte adhesion to TEC, as well as TNC reconstitution. Moreover, thymocyte release from TNCs is increased in the presence of galectin-3-containing TEC-conditioned medium (Villa-Verde DMS, Silva-Monteiro E, Cotta-de-Almeida V, Jasiulionis MG, Farias-de-Oliveira DA, Brentani RR, Savino W and Chammas R, unpublished results). Since galectin-3 binds to lactosamines expressed on the cell surface and in ECM glycoproteins, such as laminin, which is expressed throughout the thymus, the sequential interaction of thymocytes with these molecules could modulate their adhesion and migration.

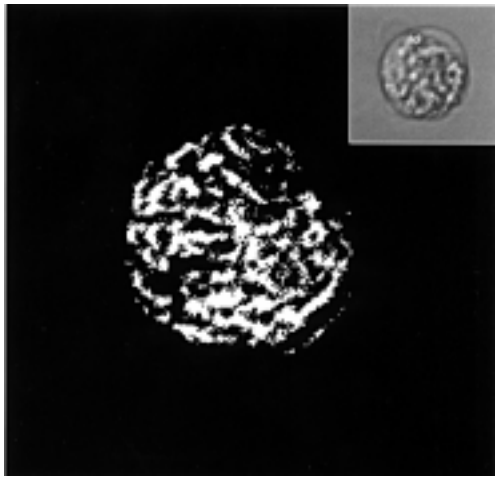

Figure 2 - Expression of galectin-3 by human thymic nurse cells (TNC). A freshly isolated TNC complex stained with anti-galectin-3 monoclonal antibody (B2C10, kindly donated by Dr. F.-T. Liu, La Jolla, San Diego, CA, USA) showed the expression of galectin-3 by the epithelial component of the complex surrounding the thymocytes. No staining was observed in the negative controls, represented by cells treated with isotype-matched immunoglobulins. The insert shows a phase contrast image of a freshly isolated TNC. Images were obtained by laser scanning confocal microscopy (original magnification X63 oil; X40 zoom).

Concluding remarks

The data briefly discussed above indicate that ECM components and their corresponding receptors do play a role in the general process of intrathymic T cell migration. Nevertheless, the precise molecular mechanisms that drive thymocytes to move within the thymus parenchyma are far from being completely understood. Virtually nothing is known concerning the ECM metalloproteinases that are likely to be expressed in the thymus and play a role in the dynamic remodeling of intrathymic ECM.

The ordered migration of thymocytes is also poorly understood: so far nobody can explain why thymocytes necessarily migrate from the cortex to the medulla. Although it is conceivable that such unidirectional movement results from the distinct concentrations of ECM components in the two regions, this issue has not yet been approached experimentally.

Information about these various points will be valuable for a better understanding of intrathymic T cell migration and of how migration is related to death or survival of thymocytes, and consequently of the generation of the T cell repertoire.

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