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The thymus microenvironment in regulating thymocyte differentiation

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The thymus plays a crucial role in the development of T lymphocytes by providing an inductive microenvironment in which committed progenitors undergo proliferation, T-cell receptor gene rearrangements and thymocyte differentiate into mature T cells. The thymus microenvironment forms a complex network of interaction that comprises non lymphoid cells (e.g., thymic epithelial cells, TEC), cytokines, chemokines, extracellular matrix elements (ECM), matrix metalloproteinases and other soluble proteins. The thymic epithelial meshwork is the major component of the thymic microenvironment, both morphologically and phenotypically limiting heterogeneous regions in thymic lobules and fulfilling an important role during specific stages of T-cell maturation. The process starts when bone marrow-derived lymphocyte precursors arrive at the outer cortical region of the thymic gland and begin to mature into functional T lymphocytes that will finally exit the thymus and populate the peripheral lymphoid organs. During their journey inside the thymus, thymocytes must interact with stromal cells (and their soluble products) and extracellular matrix proteins to receive appropriate signals for survival, proliferation and differentiation. The crucial components of the thymus microenvironment, and their complex interactions during the T-cell maturation process are summarized here with the objective of contributing to a better understanding of the function of the thymus, as well as assisting in the search for new therapeutic approaches to improve the immune response in various pathological conditions.

Key words: thymus, T-cell maturation, thymic microenvironment, thymocyte differantiation, chemokines, extracellular matrix, thymic nurse cells, metalloproteinases

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Introduction

The thymus is the specialized central lymphoid organ where precursors of T cells undergo differentiation, selection and proliferation processes. The primary function of the thymus is to develop immature T cells into cells that will be able to carry out immune functions.[1]

The organ, located in the anterior mediastinum, consists of two encapsulated lobes that are divided by numerous septa into multiple lobules. Each lobule presents two different regions, i.e., cortex and medulla. The outer cortical portion is densely populated by pre-T lymphocytes (or thymocytes while in the thymus), and the inner medullary portion contains few, but fully mature, T lymphocytes. Alongside the thymocytes, at different stages of maturation, the thymic environment is formed by epithelial cells, which form a meshwork to provide mechanical support and stimuli for the proliferation and development of thymocytes and by macrophages, dendritic cells, fibroblasts and matrix molecules.[2] Also importantly, this cellular network involves blood vessels in the cortex and so effectively isolates developing lymphocytes to preclude the possibility of contaminating these cells with antigens (blood-thymus barrier).[3]

T lymphocytes are generated from bone marrow-derived lymphocyte precursors that enter the thymic cortex through blood vessels. Currently, little is known of the mechanisms that attract precursors to the thymus or facilitate their migration

through the surrounding tissues. Also, the phenotype of T-cell precursors remains unclear but some markers, such as c-Kit (CD117) and CD34 have been reported to be associated with them.[4] More recently, signaling through the Notch receptors and the Notch ligands named Delta-like and Jagged have also been implicated during T-cell development because T cells fail to develop from Notch1-deficient bone marrow precursors.[5-8]

The development of T cells within the thymus is a complex process that involves four main stages based on their expression of CD4 and CD8 co-receptors. At early stage of development, T-cell precursors have a $CD34^+CD4^-CD8^-$ "double negative" (DN) phenotype. This phase is also characterized by differential expression of the CD44 and CD25 molecules and these cells represent 5% of the total thymic lymphocytes. At the end of this phase, when the CD3 expression increases, the thymocytes present the $CD3^{low}CD4^-CD8^-$ phenotype and a first step toward the expression of a functional T-cell receptor (TCR) takes place. Immature single-positive (ISP) cells ($CD3^{low}$ $CD4^+$ $CD8^-$ or $CD3^{low}$ $CD4^-$ $CD8^+$) start to rearrange the *TCRB* gene. Subsequently, the TCRβ chain becomes assembled into the pre-TCR complex with the invariant pre-Tα chain. Pre-TCR signaling confers survival and allows development to proceed through a $CD4^+CD8^+TCR^{low}$ double-positive (DP) subset of thymocytes, which represent about 80% of the total cells in the organ. The thymocytes that were not able to generate a functional TCR die through apoptosis, whereas those expressing functional TCR will be exposed to endogenous peptides presented by self-major histocompatibility complex (MHC) molecules present on thymic microenvironmental cells. These interactions will determine the positive and negative selection events that will be decisive for the selection of a mature T-cell repertoire.[9]

In the positive selection, double-positive thymocytes ($CD4^+CD8^+$) interact with self-major histocompatibility complex (MHC) molecules present on the cortical epithelium and this event leads cells to lose one of the co-receptor molecules (CD4 or CD8). Thus, a self MHC-restricted T-cell repertoire is generated. Then, the "single-positive" (SP) thymocytes ($CD4^+CD8^-$ or $CD4^-CD8^+$) undergo negative selection in which those whose TCR recognizes self-peptides present in the thymic microenvironment are eliminated. Cells that fail the positive or negative selection die through apoptosis. On the other hand, the selected cells survive and migrate as mature T lymphocytes to the peripheral lymphoid tissues where they will mount and regulate cell-mediated immune responses.

Although it has long been known that interactions between the thymocytes and thymic environment are crucial during T-cell development, the molecular nature of such interactions that lead to positive and negative selection are yet unknown. The medullary thymic epithelial cells (mTEC), which correspond to the vast majority of cells in the thymus, and cells of the monocyte/dendritic cell lineage of the thymus are considered to play a major role in the establishment of self tolerance by eliminating auto-reactive T cells (negative selection) and/or by producing immune-regulatory T cells, which prevent CD4 T-cell mediated organ specific autoimmune diseases.[10,11] mTECs express the autoimmune regulator (AIRE) gene that regulates the expression of tissue-specific antigens (TSAs) to the developing thymocytes in a dosage-dependent manner.[12,13] So, a slight decrease in AIRE gene function can lead to a decrease in thymic protein expression, allowing the emigration of auto reactive T-cell clones to the periphery.

The successful development of mature T cells depends on the constant migration of the thymocytes through the thymic microenvironment. Such migration is essential for thymic stromal cells to provide signals to thymocytes that lead to proliferation, differentiation and generation of diversity.[14] Although the mechanisms directing this migration are poorly understood, clear evidence has been obtained showing that the thymic microenvironment, collectively, influences the process of T-cell development through surface molecules and by secreting soluble polypeptides as cytokines, chemokines and hormones.

Although it is well established that the interaction between thymocytes and thymic epithelial cells is essential for the development of thymocytes, the influence of thymocytes on thymic epithelial cell development, maintenance and regeneration in both the embryonic and the adult thymus remains unclear.[15,16]

This commentary addresses the critical role of the thymic microenvironment exploring the contribution of some of its components in regulating the intrathymic events of adhesion and migration during the thymocyte differentiation.

Chemokines and Their Receptors

It is now apparent that chemokines (chemotactic cytokines), a superfamily of small polypeptides that first had their role recognized in the leukocyte migration from the blood stream to the inflammation sites,[17] also contribute to the functionality of the thymic microenvironment. Based on N-terminal cysteines, chemokines are classified into four major subfamilies: CC, CXC, C and CX3C (where x can be any amino acid).[18,19] Most chemokines are secreted from cells as soluble forms, some of which are afterward immobilized via binding to various ECM components to produce a chemokine gradient.[20] In contrast, CXC chemokine ligand (CXCL) 16 and fractalkine (CX3CL1) are membrane-anchored chemokines, and have been shown to possess an extracellular chemokine domain fused to a mucin-like stalk, transmembrane-spanning region and intracellular part.[21,22] It is believed that these transmembrane chemokines could function not only as chemokines but also as adhesion.[23] Chemokines act through chemokine receptors (CXCR1, CCR1-11, CXCR1-5, CX_3CR1), which are a subfamily of G protein-coupled 7-transmembrane receptors.[24,25]

It was shown that the fetal thymus produces several chemokines, including CCL21, CCL25 and CXCL12 that are vital chemoattractants at different stages of thymocyte maturation, not only addressing the flow but also propitiating the correct location of the immature T-cell precursors in the cortical layer.[14,26] Indeed, at different stages of maturation,

thymocytes present receptors for chemokines produced in the thymus.[14]

The chemokine stromal cell derived factor-1α (SDF-1α)/CXCL12 is highly expressed in the adult thymus particularly in the sub capsular and medullar areas attracting preferentially $CD4^-CD8^-$ and $CD4^+CD8^+$ cells that express its cognate receptor CXCR4.[1,27] The CXCL12-CXCR4 biological axis is important in mediating the flow of thymocytes from the sub capsular region to the corticomedullar region, as well as the entrance of single-positive thymocytes into the medullary region. Thymus specific deletion of CXCR4 in vivo results in failed cortical localization together with arrest of the developmental process.

The thymus-expressed chemokine (TECK)/CCL25 is produced by medullary dendritic cells and thymic epithelial cells in both cortex and medulla.[28-30] CCR9 mediates chemotaxis in response to CCL25 and is expressed in all stages of T-cells differentiation, with maximum expression in $CD4^+CD8^+$ (DP) cells. The CCR9 expression is downregulated in mature thymocytes and the loss of responsiveness to CCL25 occurs just before the T-cell emigration.[31] However, Uehara et al. investigating the role of CCR9 during lymphocyte development by using CCR9-deficient ($CCR9^{-/-}$) mice observed normal development of αβ-lineage T cells and increased numbers of peripheral γδ-T cells but reduced numbers of $γδTCR^+$ intraepithelial lymphocytes (IEL) in the small intestine.[32] As CCL25 is also produced in the epithelial layer of the small intestine,[30] the authors suggest that CCR9 can be involved in regulating the migration of progenitor cells to the thymus or in retention of T progenitor cells in the thymus but plays an important role in regulating the development and/or migration of γδ-T lymphocytes.

Wurbel et al. have reported a 3-fold decrease in total thymocyte numbers in CCR9-deficient mice at embryonic day from 14.5–17.5.[33] However, it is not clear whether CCR9 deficiency impairs colonization of T-precursor cells to the thymus primordium, or the absence of CCR9 causes defects in intrathymic survival or proliferation of immature thymocytes or both. Indeed, it was shown that anti-CCL25 antibody did not prevent thymus colonization. However, it is clear that CCL25 intracellular signaling is mediated by the integrin α4β1.[28,29]

A recent work has demonstrated that signaling through CCR7, the chemokine receptor for CCL19/CCL21, is essential for proper differentiation of DN thymocytes since CCR7-deficient mice present impaired development of these cells, altered thymic architecture and decreased absolute numbers of thymocytes.[34] In the same way, experiments with mice lacking expression of the chemokines CCL19/CCL21 reveal mutations in thymic architecture and reduced numbers of circulating T cells in the periphery. This fact strongly suggests that the CCL19/CCR7 axis must be important for the emigration of mature T cells from thymus to the peripheral lymphoid organs.[35]

CCR8, the receptor for the chemokine CCL1, is preferentially expressed in the thymus and Kremer et al. have recently investigated its expression in different murine thymocyte subsets.[36] The authors show that the expression of CCR8 protein is modulated along the process of thymocyte maturation, with two transient expression waves. The first one takes place in $CD4^-$ $CD8^-$ (DN) thymocytes, which showed a low CCR8 expression, whereas the second wave occurs after the TCR activation by the Ag-dependent positive selection in $CD4^+$ $CD8^+$ (DP) cells. From that maturation stage, CCR8 expression gradually increases along the pathway leading to $CD4^+$ single-positive (SP) cells, in which CCR8 expression is maximal. Conversely, no significant amounts of CCR8 protein were found in $CD8^+$ SP thymocytes. These results suggest a relevant role for CCR8 in T-cell maturation, and the possibility of using this molecule as a marker for the identification of thymocyte subpopulations recently committed to the $CD4^+$ lineage.

Other chemokines, like CXCL16 and CX3CL1 were also found at higher levels in fetal thymus than in nonthymic epithelial/mesenchymal rudiments, suggesting a possible role to them in the development of T cells.[26,37]

Besides the role in thymocyte conduction, other functions have been attributed to chemokines such as thymocyte proliferation, survival and involvement in negative selection.[38] Recently, it was observed that the autoimmune regulator (Aire) can modulate the production of thymic chemokines involved in corticomedullary migration and thus play a role in intrathymic thymocyte migration and maturation.[39] These authors have shown that Aire deficiency results in reduced gene expression and protein levels of three ligands for CCR4 (CCL5, CCL17 and CCL22) and two ligands for CCR7 (CCL19 and CCL21), altering the coordinated maturation and migration of thymocytes.

Certainly, both the differential expression of chemokines in distinct thymic microenvironments and the time- and stage-specific regulated expression of chemokine receptors on developing cells are implicated in the regulation of T-cell proliferation and movement within the thymus. Probably, a more complete understanding of the significance of chemokines/chemokines receptor interactions concerning their potential role during T-cell maturation will arise in the next years from experiments using mice deficient in either chemokines or their receptors.

Extracellular Matrix Components

Of most importance to the intrathymic migration are the events of adhesion and de-adhesion that makes possible the constant contact between maturating cells and different microenvironmental cells. In this context, an important component of the thymic microenvironment is the extracellular matrix (ECM), which interacts with thymocytes driving their traffic inside the organ. TECs produce fibronectin, laminin and collagen type IV that interacts with specific receptors expressed on thymocytes and this interaction seems to be crucial in the thymocyte flow through the thymus.[16] Many adhesion molecules and respective ligands have been postulated as important in the thymic limphopoiesis including α1β2/ICAM, CD18, CD2/LFA-3 and E-cadherin. However, the more deeply studied components in thymocyte development are fibronectin, laminin and galectins.[40,41]

Isoforms of ECM glycoproteins have been reported in the thymus. Some

isoforms of fibronectin that are recognized by the receptor VLA-5 (CD49e/CD29) have been described to be located throughout thymic parenchyma, whereas an isoform, which is derived from alternative splicing of fibronectin mRNA and recognized by the receptor VLA-4 (CD49d/CD29), is restricted to the medulla of the organ.[16]

Different isoforms of laminin generated by transcription of different genes and the adhesion of thymocytes to these isoforms, like laminin-2 for example, have been reported.[42,43] In laminin-2-deficient mice an aberrant thymocyte development could be observed. The animals exhibited a precocious thymic atrophy with a decrease in the relative number of $CD4^-CD8^-$ thymocytes and an increase in the number of apoptotic DN cells.[44] These findings suggest that laminin is required to the progression from $CD4^-CD8^-$ to $CD4^+CD8^+$ thymocytes. The isoforms laminin-5 that is detected in thymus is also involved in thymocyte proliferation and differentiation from DN to DP stage.

The expression of ECM components in the thymus correlates with the expression of fibronectin receptors (VLA-4 and VLA-5) and laminin receptors (VLA-3 and VLA-6) on differentiating thymocytes and cells of the thymic microenvironment. According to the model proposed by Cotta-de-Almeida and colleagues this fact provides molecular bridges among thymocytes and microenviromental cells in such a way that both cell types can benefit from ECM signals.[45]

It has been also proposed that the activation state of integrins is essential to induce signals to developing thymocytes. In this sense, the guanine nucleotide-binding protein Rho is identified as a key signaling molecule in the thymus to control integrin function and cell motility. Rho action on the integrin function might influence the thymocyte ability to attach to ECM elements, and subsequently, to detach from it to move forward. Vielkind and colleagues have shown that thymocytes lacking Rho function show reduced ability to activate integrins and inability to migrate efficiently.[46]

Certainly, the importance of ECM elements in thymocyte development stems from their ability to mediate the organized chemokine-induced migration.

The Interplay Between ECM Components and Chemokines

Because tissue ECM and chemokines are key biological mediators in cell migration, it is reasonable to suppose that interplay between these elements is occurring during the thymocyte maturation process. Yanagawa et al. have demonstrated that CXCL12 with fibronectin (or laminin) induces higher thymocyte migration in transwell chambers than that elicited by the chemokine or ECM molecules alone.[47] Savino et al. also demonstrated that thymocyte migration induced by CCL19 plus fibronectin is higher than the migration pattern induced by these molecules alone.[35] Results from Sanz-Rodrigues and his group showing that MMP-9 is able to cleave several chemokines, such as CXCL8, CXCL5 and CXCL6, add to the concept of ECM-chemokine interplay in thymocyte migration.[48]

Galectins and Thymic Function

Galectins, a family of carbohydrate-binding proteins with an affinity for β-galactosides, are widely distributed from fungi to mammals.[49,50] Fifteen mammalian galectins have been identified to date, from which 12 are present in humans.[51] Galectins can be detected in the cytoplasm, nucleus, at the cell surface, or in the extracellular matrix, and their localization is changed in different developmental stages and physiological conditions. Their biological significance is not yet fully understood because they are involved in too many phenomena, such as adhesion to extracellular matrix (ECM) glycoproteins, migration, chemiotaxis, inflammation, immune system homeostasis and control of tumorigenesis.[51,52] In thymus, three members of this family that positively and negatively regulate T-cell death have been described: galectin-1 (gal-1), galectin-3 (gal-3) and galectin-9 (gal-9).[52-55]

Cell death is critical for proper T-cell development in the thymus since it prevents the production of nonfunctional and auto-reactive T cells. Gal-1, gal-8 and gal-9 are secreted by TEC and can induce death in human and murine T cells during development. Although the gal-9-induced death mechanisms remain unclear, literature has shown that the gal-1-induced T-cell death occurs by cross-linking cell surface receptors.[56] Gal-1 binds to T-cell-surface glycoproteins CD2, CD3, CD4, CD7, CD43 and CD45. The immature double-positive (DP) thymocytes are the principal targets for gal-1-induced apoptosis and this process involves the CD7, CD43 and CD45 receptors. CD7 is essential for triggering the death signal and its co-localization with CD43 suggests that these two receptors may act in concert to initiate cell death. The CD45 receptor has been identified as a regulator of cell death since some studies using two CD45 negative cell lines demonstrated an essential role for CD45 in signaling gal-1-induced T-cell death.[56-58] Apparently, the CD45 function is related to its state of glycosylation.[53,56] CD2, CD3 and CD4 are not required for T-cell death but, most probably, must be important for mediating other biological effects of gal-1 on these cells. In fact, there have been several reports on the regulatory growth[59] and/or immunomodulatory activities of galectin-1.[51,52,60] Recently, the gal-8 isoform was found in rat and mouse thymus. This galectin induces apoptosis in the CD4(high)CD8(high) thymocytes through caspases pathway activation, suggesting the gal-8 participation in T-cell maturation. However, the complete mechanisms remain unclear.[61] While T-cell maturation is the net result of migration through thymic environments, the presence and/or modulation of galectins modifies this maturation-related process. It is now known that interactions between cells and ECM are necessary for the maintenance of the thymus architecture and intracellular signal transduction, requisitions for maturation of T cells. As gal-1 has been shown to increase the adhesion of so many cells to the ECM via the cross-linking of glycoproteins (integrins) exposed on the cell surfaces with carbohydrate moieties of ECM components such as laminin and fibronectin, it is reasonable to state that gal-1 must be a role in the modulation of cell adhesion and signaling during maturation of T cells.[35,62-64]

Gal-3 is a multifunctional protein implicated in a variety of biological

functions, including tumor cell adhesion, proliferation, differentiation, angiogenesis, apoptosis, cancer progression and metastasis. This galectin is the only member of the galectin family that has an anti-apoptotic function.[56,64,65] Gal-3 was found to have significant sequence similarity with Bcl-2, a well-characterized suppressor of apoptosis. Intracellularly, gal-3, like Bcl-2, preserves mitochondrial integrity and cytochrome *c* release.[56] On the other hand, recent work has demonstrated that while intracellular galectin-3 blocks T-cell death, the extracellular galectin-3, like gal-1, directly induces death of human thymocytes (preferentially the double-negative ones) and T cells.[55] However, CD7 and CD43 receptors that are required for galectin-1-induced death are not required for death triggered by galectin-3, suggesting that gal-3 and gal-1 induce death of T cells through distinct T-cell surface events.[64] It has also been reported that gal-3 is involved in many immunoregulatory processes, such as cell–cell adhesion and adhesion of cells to matrix glycoproteins.[65,66]

In the thymus, Villa-Verde and coworkers[62] have showed that gal-3 is predominantly found in the medullary area and that distinct microenvironmental elements, such as TEC and phagocytic cells produce, secrete and accumulate this galectin on the cell surface. Besides, the same authors postulate that the intrathymically produced galectin-3 disrupts thymocyte/microenvironmental cell adhesive interactions and, in contrast to the adhesive role of gal-1, would act as a de-adhesion molecule.[62,63]

Because interactions at the cell surface are of prime importance for migration and selective processes, growing attention will be paid in coming years to the role of galectins during T-cell development in the thymus.

Metalloproteinases and Thymocyte Migration

Matrix metalloproteinases (MMPs) are a family of extracellular matrix-degrading and processing enzymes that have been implicated in many physiological and pathological conditions.[67] Based on their substrate specificities they can be divided into a number of groups, which include the collagenases (MMP-1, -8, -13 and -18), capable of cleaving fibrillar collagen, the gelatinases (MMP-2 and -9), which can further degrade these collagens and basement membrane collagen type IV, the stromelysins (MMP-3, -10 and -11), which can cleave fibronectin, laminin and proteoglycans, the matrilysins (MMP-7 and MMP-26), also called endometases, which processes ECM components and cell surface molecules, and the membrane-associated MMPs with poorly defined substrate specificities, although some group members display collagenolytic activity and can activate other MMPs.[68,69]

MMP activity is tightly regulated in several ways: at the transcriptional level, where they are modulated by cytokines/chemokines and growth factors, at the level of posttranscriptional processing, where they need activation in the extracellular space by other proteases, and at the level of inhibition, where they are regulated by their specific tissue inhibitors named TIMPs.[70-72]

Particular interest has been focused on MMP-2 and -9 (also known as gelatinase A and B), because they preferentially degrade type IV collagen, a major component of basement membrane. MMP-2 and MMP-9 are dominant MMPs in the vascular tissue and related to tumor invasion and metastasis by their capacity for tissue remodeling via extracellular matrix as well as basement membrane degradation and induction of angiogenesis.[72]

Evidences have showed that thymic microenviromental cells produce MMP-9 the largest and most complex member of MMPs family.[73] It has been reported that MMP-9 is important for the remodeling of the extracellular matrix and the migration of cells.[74] Both of these functions are essential components of T-cell maturation, so it would be reasonable to suppose that MMPs have a crucial role in thymocyte migration.

In fact, the importance of MMPs in the development of T lymphocytes is known since the late 90s. Studies using explanted fetal thymus organ cultures (FTOC) have shown that T-cell development was inhibited beginning at the DN thymocytes stage when inhibitors of metalloproteinases were supplied to the system.[75,76] This impairment of T-cell development was related to modifications in *TCRB* gene rearrangement and TCR-β protein expression, implying a role of MMPs in Notch1 signaling since a role in promoting *TCRB* rearrangement and cellular expansion in response to pre-TCR signals has been attributed to this receptor.[77,78]

ADAMs (*a d*isintegrin *a*nd *me*talloprotease), a newly discovered family of integral membrane and secreted glycoproteins, have also raised considerable interest because of their ability to perform both function, adhesion and extracellular matrix degradation.[79] Besides, it has been demonstrated that Kuzbanian (ADAM 10), an ADAM protease, may also fulfill important functions in the Notch signaling.[80,81] Recently, Manilay et al. have showed that T-cell development is blocked between DN and DP stages of T-cell development in dominant-negative form of Kuzbanian transgenic (dnKuz Tg) mice.[82] This block correlates with premature downregulation of CD25 expression and reduced *TCRB* expression, similar to the effect of Notch-1 deletion.[77] Recent evidence further indicates that the cell surface expression of the chemokines CXCL16 and CX3CL1 can be regulated, through proteolytic release from the cell surface (also termed ectodomain shedding), by the activity of ADAM10 and ADAM17.[83-85] Shedding is believed to constitute an important regulatory mechanism for cellular signaling by either reducing the amount of distinct receptor proteins present on the cell surface leading to reduced cellular responsiveness to determined stimuli or by releasing soluble ectodomains of growth factors and cytokines capable of stimulating other cells. It is plausible to suppose that by this mechanism, ADAMs could participate in the T-cell development.

Because matrix-disrupting enzymes are believed to play an important role in the degradation of the ECM during cellular migration in several systems, more detailed studies must be carried out to define the expression profile of MMPs, TIMPs and ADAMs, as well as their spatial distribution in the thymus, and to

determine the precise role of these proteins on thymocyte maturation.

Thymic Nurse Cell: A Lymphoepithelial Complex

Within the thymus cortex, we can also find the so-called thymic nurse cells (TNCs), which are lymphoepithelial complexes formed by thymic epithelial stroma cells (TEC) enveloping two to 200 thymocytes in different stages of maturation.[86] TNCs were described in mice in 1980 by Wekerle and Ketelson[87,88] and since then they had been isolated from the thymus of different animal groups and species such as fish, frogs, chickens, sheep, horses, pigs, rats and humans.[89]

Since the 1980s, TNCs have been considered a specialized model of microenvironment for studies involving T-cell development. However, the events that take place in TNCs remain unclear, with some evidence of its participation on T-cell maturation and on positive/negative selection through apoptosis. TNCs express both class I and class II MHC antigens allowing the interaction with the T-cell receptor (TCR) and the participation in negative or positive selection.[90,91] In accordance with these properties, TNCs have been related to maturation of T cells, although it is still not clear what kind of immature phenotypes can be found in TNCs complexes, mainly due to difficulty in preventing contamination of non involved thymocytes in culture.[86,88] Anyway, due to the easy isolation of thymic tissue and culture ex vivo, TNCs have been considered a good specialized model for studies involving T-cell differentiation, maturation and negative or positive selection.

These complexes are formed in vivo by the uptake of early thymocyte immigrants from the bone marrow by TEC in the cortical thymus region. In vitro, and recently in vivo, studies showed that during the thymocyte uptake production of the finger like projections with participation of the many membrane and cytoskeletal proteins of both TEC and T cells takes place.[92] This event ends with the formation of the vacuole surface surrounding engulfed thymocyte.[86] Moreover, it has been shown that immature thymocytes are able to adhere onto TNC-derived epithelial cells in an ECM-dependent manner.[35,56,62,93] This process can be explained by the presence of the intercellular cell adhesion molecule 1 (ICAM-1), one of many molecules involved in the process of cellular adhesion, on the surface of the TNCs and vacuoles surrounding enclosed thymocytes and, by the constitutive production of extracellular glycoproteins such as fibronectin, laminin and type IV collagen by TNCs.[62,86] As mentioned earlier, in addition to the adhesive role of ECM moieties, thymocyte migration or motility might be influenced by gal-1, gal-3 and gal-9 that may favor adhesion or de-adhesion events. Some papers also demonstrated that these molecules, mainly gal-1 and gal-3, modulate thymocyte adhesion to microenviromental cells and thymocyte transit into and out of TNC.[56,62,63] Villa-Verde et al. using the in vitro model recently demonstrated that gal-3 is able to inhibit thymocytes/TEC interactions, to accelerate thymocyte release from TNCs complexes and to decrease TNCs reconstitution by fetal thymocytes.[62] This same group has also shown antagonist effects with exogenous gal-1 in TNCs culture.[63]

The thymocytes, despite being completely enclosed by TNCs membranes, remain fully intact and metabolically active, displaying also a high mitotic activity.[86,88] The maintenance of cell activity only occurs due to the favorable microenvironment that is assured during the formation of these complexes which therefore can be considered true microenvironments inside the cortical thymic environment.

Recently, it was demonstrated that $CD3^{low}CD4^{-}CD8^{+}$ thymocytes (the so-called immature single positives, ISP) can be facilitated, in vitro, to differentiate into $CD4^{+}CD8^{+}$ T cells within RWTE-1 cells, a rat TNCs clone.[94] However, some authors argue that the presence of ISP thymocytes is due to a kinetic of double-positive cell ($CD3^{low}CD4^{+}CD8^{+}$) development suggesting that ISP is only a transient state and not a real subpopulation.[86,94] Still, in accordance with other recent papers, the TNCs complex mostly bears $CD4^{+}CD8^{+}$ immature cells. Such inconsistent results and conclusions can be caused by different experimental conditions or by a selective uptake of immature T-cell sub-types by TNCs.[88]

Despite some controversies involving thymocyte phenotypes and functions, there is almost a consensus about the participation of TNCs in thymocyte maturation and the relevance of this in vitro model for opening new possibilities for analysis of stroma cell-thymocyte interactions concomitant with studies on early events of intrathymic T-cell differentiation.

Thymic Atrophy in Physiological and Pathological Conditions

Despite its importance for the immune system, the thymus undergoes involution in both physiological and pathological conditions.

In humans, physiological thymic involution starts after puberty and complete involution is expected at the age of 25 years. It is characterized by gradual reduction in the size of the gland caused by involution of the epithelial tissue.[95]

Moreover, during this age-related thymic deterioration the expansion of PVS (perivascular space) and increase of adipose tissue occurs. Conjointly, these histological alterations affect the thymic function and are associated with an increased susceptibility to infections, autoimmune disease and cancers.[96] Thymic involution is also observed in several pathological conditions. In humans, for example, it has been shown that HIV infection leads to shrinkage of the thymus with a reduction of Hassal's corpuscles and loss of cortex-medullary delimitation.[97] In experimental models, the thymus involution has been observed in different conditions such as alloxan-induced diabetes, malnutrition, *Trypanosoma cruzi*, *Candida albicans, Paracoccidioides brasiliensis* and *Plasmodium berghei* infection.[98-101]

Recently, our group have reported that *T. cruzi* and *P. berghei*-related thymic involution is correlated with loss of cortical-medullary delimitation and is associated with increased density of fibronectin and laminin and reduced numbers of DP thymocytes.[99,101] These observations are also correlated with high migratory capacity of thymocytes that probably brings consequences for the peripheral immune response. Similar alterations can be observed in drugs-induced diabetes.[98,99,101] Other parasites such as virus,

mycobacterium and bacteria also colonize the thymus, but to evaluate thymus damage more studies need to be done.

Because of the important role of the thymus in maintaining a healthy immune system, it is reasonable to assume that age- and/or pathological-related thymic involution must change markedly the immune response of subjects. Thus, several endogenous molecules that are involved in the maturation and development of T lymphocytes are now being studied in order to be used as therapeutic proposals aiming at the reversal of thymic involution. Experimentally, some promising results were obtained using hormones and cytokines: after administration of ghrelin, a peptide correlated with adipogenesis, thymopoiesis was increased and growth hormone (GH), IL-7 and keratinocyte growth factor (KGF) have also been shown to be thymostimulatory in old mice.[96] Recently, it was demonstrated that leptin, a hormone linked to satiety, has potential therapeutic value in a systemic model of endotoxin-induced thymic involution since the results suggest that leptin administration augments thymopoiesis in the setting of LPS-induced shock.[102]

Conjointly, the data obtained with these new experimental models offered by us and by other laboratories support the crucial role that the thymus plays in immune system homeostasis and so the search for endogenous molecules that have potential stimulatory effects on the thymus is an open field.

Conclusion

It is now known that T-cell development depends on constant migration of bone marrow progenitors through the complex thymus microenvironment in order to find signals for survival and proliferation. However, the molecular and cellular events associated with T-cell maturation are in the process of being identified and many unanswered questions remain in this exciting area. Understanding the thymocyte interactions within the thymus will be beneficial in discerning the process of T-cell development and for future strategies aimed at thymic function.

Only now is the complexity of thymus microenvironment beginning to be understood, but there is little doubt that the enormous potential value of the knowledge about generation of T cells mandates its continued investigation.

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