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Retroviruses and Thymus Nonlymphoid Cells

J. Boniver, C. Humblet, A.M. Rongy, C. Delvenne, P. Delvenne, R. Courtoy, A. Thiry, M.P. Defresne

Laboratory of Pathological Anatomy and Cytopathology,
Department of Pathology, University Hospital of Liège, Belgium

The thymus is a critical organ in the T lymphoid system: it is the site where the T cell precursors acquire the characteristics of mature T cells and thus the capacity to react to antigens after migration to the periphery [for review, see 1,2]. We will summarize here the major features of the role of the thymus.

The functions of the thymus include the immigration of hematopoietic precursors, originating in bone marrow, their maturation into antigen (Ag)-reactive T cells and the selection of the appropriate Ag specificities, and then release to the periphery.

The maturation process requires the acquisition of functions such as help, killing and suppression and the induction of several T cell lineage-restricted genes, which encode for membrane molecules. Some are related to the T cell antigen receptor complex, such as the TCR γ, δ, β, and α genes and the CD3 gene or they are involved in the MHC restriction process, such as the CD4 and CD8 molecules encoding genes. Others code for surface molecules which act as cell surface homing receptors required for the migration of T lymphocytes from the thymus towards the peripheral lymphoid tissues.

The maturation of T cells follows a sequence of events, starting after the immigration of marrow-derived precursors. The progeny start dividing as lymphoblasts in the outer cortex, in the so-called subcapsular zone. This proliferative compartment is most likely the source of all maturing thymocytes. Most cortical thymocytes die in situ. A minority of them, after acquisition of the major characteristics of T cells, migrate to the peripheral lymphoid tissues. The most mature thymocytes, displaying the cell surface molecules and the functional capacities of peripheral T cells, are located in the medulla and reside therein for a long time.

The maturation of T cells requires specific interactions between thymocyte precursors and nonlymphoid cells that provide specific maturational microenvironments. The nonlymphoid compartment of the thymus is composed of several cell lineages: (a) epithelial cells (EC), which derive from pharyngeal pouches and form a heterogenous population composed of several subclasses of EC, with different localizations, phenotypic characteristics and functions; (b) macrophages (MP) which are bone marrow derived and located mostly at the cortex-medullary junction and in the medulla; (c) interdigitating dendritic cells (IDC) which are also bone marrow derived and are mostly found in the medulla, and (d) fibroblasts and endothelial cells which derive from the mesenchyma.

These stromal cells establish close interactions with the maturing T cells. Let us summarize the most important interactions:

(1) The incoming hemopoietic T cell precursors penetrate into the thymic parenchyma through the vessels of the corticomedullary junction. They appear first in association with MHC class II determinant (Ia)-negative MP. Little is known about the direct consequences of these interactions; as a result, lymphoid cells migrate towards other areas in the thymus. Besides this interaction with the incoming T cell precursors, some MP might also act in the destruction and phagocytosis of the thymocytes that have just died in the deep cortex.

(2) Two or 3 days after penetrating the thymus, the immature thymocytes are found aggregated in the outer cortex in contact with a particular type of EC, which have been designated as 'thymic nurse cells' (TNC). The lymphoid cells are a distinctive lymphocyte population: they are mainly lymphoblasts and are dividing. The interaction between lymphocytes and the nurse EC most likely leads to a proliferative stimulus on the former and thus contributes to the expansion of the T cell pool within the thymus. Whether or not this lymphoepithelial interaction also acts as a maturing stimulus is still controversial. There are data suggesting their role in positive selection.

(3) A third type of lymphostromal interaction involves a subset of EC in the deep cortex. These EC have long branching processes connecting one cell to another by desmosomal junctions and surrounding lymphocytes. These EC express very high levels of class II MHC determinants, but no detectable class I MHC molecules. Many of the lymphocytes interacting with these dendritic epithelial cells express TCR molecules and bind Ia antigens of the EC. These interactions between small cortical lymphocytes and these EC are most likely involved in the selection of T cells, leading to the survival of a minority of lymphocytes, capable of recognizing self-MHC determinants and

the death of cells, with inappropriate reactivities. These interactions may also play a role in the acquisition of the homing receptors for peripheral lymphoid tissue, since the emigrating thymocytes derive from this thymic area.

(4) Medullary T lymphocytes are in contact with EC expressing high levels of class I MHC determinants or with bone marrow derived IDCs, which are both class II and class I positive. If the role of medullary EC is still unknown, it is likely that medullary IDCs are involved in negative selection of mature thymocytes bearing high affinities anti-self TCRs.

The substrate of the interactions between maturing lymphocytes and stromal cells are still poorly known. It is possible that stromal cells produce cytokines, which then act on lymphocytes. 'Thymic hormones' are to be classed among such cytokines. Their role in vivo is, however, still partially unknown. Cell-to-cell contacts also seem to be important: some subsets of maturing lymphocytes most likely bind class I or class II determinants which are expressed by various kinds of stromal cells. Adhesion molecules are also involved.

It is very clear that if thymic stromal cells are so important as inductive microenvironments of T cell maturation, any pathological events which concern them, such as virus infections, might disturb T cell lymphopoiesis and hence the integrity of immune responses.

Among the retroviruses which are able to infect thymic cells, two systems will be described below: (1) the murine leukemia viruses, which induce thymic lymphosarcomas in mice, and (2) the HTLV and HIV types of retroviruses in humans.

Murine Leukemia Viruses and Thymus Nonlymphoid Cells

Thymic lymphomas develop spontaneously in some mouse strains such as AKR, i.e. more than 90% of animals die of leukemias by 1 year of age. In some other strains, the incidence of spontaneous thymic lymphomas is low but a high incidence can be induced by exogenous agents such as radiation, chemical carcinogens and retrovirus [for review, see 3, 4]. The development of lymphomas is effectively prevented by thymectomy, and the implantation of thymuses into thymectomized mice restores their susceptibility to lymphoma development [4].

The essential role of the thymus in these systems is not due to the fact that this organ would be the unique source of target cells for the lymphoma-

genic agents. In fact, such targets can be found, besides the thymus, in other lymphohemopoietic organs such as bone marrow, spleen, fetal liver or lymph nodes [5]. The specific role of the thymus seems more specifically due to the nonlymphoid stromal cells, the above-described inductive microenvironment. In fact, transplantation studies have clearly shown that target cells of extrathymic origin can progress towards lymphoma transformation *only* within the thymus [5].

Studies on spontaneous lymphomas in AKR mice and induced lymphomas in C57BL/Ka mice have been devoted to the analysis of the microenvironmental thymic factors contributing to the development of lymphomas. In AKR mice, the development of lymphomas is dependent on the early production of endogenous N-ecotropic retroviruses [6, 7]. These viruses are proposed to serve as a source of parental viruses, together with a xenotropic virus, in the generation of recombinant mink cell focus-forming (MCF) viruses, which are lymphomagenic [8]. These MCF viruses are thymotropic, meaning that they infect thymocytes in vivo and replicate therein, but require the presence of ecotropic virus in order to facilitate their replication and spread in thymic tissue [9].

The site of the spontaneous generation of MCF virus within the thymus of these mice has been studied by many authors. By using thymus grafting methodology, evidence was provided of the central role of *thymic stroma* in the expression and amplification of MCF viruses, resulting in the infection of thymocytes and induction of preneoplastic changes among them. Of course, these studies could not identify those subsets of thymic stromal cells which are involved in these processes [10–12].

Kaplan and co-workers at Stanford University and our group in Liège have been studying for a long time the pathogenesis of experimentally induced thymic lymphomas in C57BL/Ka mice. In this mouse strain, a high incidence of such lymphomas can be induced by inoculation of a retroviral isolate, the radiation leukemia virus (RadLV), which was initially extracted from X-ray induced thymic lymphomas of the same mouse strain [for review, see 4]. Target cells for infection are found in thymus, bone marrow, spleen, fetal liver, but their transformation into lymphoma is possible only within the thymus [5]. The substrate for the thymus dependency of this leukemogenic process has been the subject of many investigations.

First of all, we demonstrated that thymic EC can be infected in vitro or even in vivo by RadLV and then sustain viral replication [13, 14]. Secondly, some EC obviously play an essential role in the early events following virus inoculation [15]. In fact, virus replication is found very early in the lympho-

on the immune system is a controversial issue'. Several autopsy reports have described a severe thymus atrophy in patients with AIDS. Signs of an active destruction process and degeneration have been claimed, whereas plasma cellular infiltrates have been reported.

As an example, Savino et al. [26] showed strong alterations to the pattern of cytokeratin and differentiation antigens of thymic EC in situ and, in addition, regions of EC necrosis, with loss of cell limits. Thymulin (a thymic hormone produced by thymoepithelial cells) was decreased. Seemayer et al. [27] described particularly the disappearance of Hassall's corpuscules. More recently, however, Schuurmann et al. [25] compared thymus specimens taken at autopsy from 8 AIDS patients with those taken from patients with congenital immunodeficiency and other patients after allogenic bone marrow transplantation. In all cases, the authors observed similar features, i.e. a severely involuted architecture. There was no major difference between thymuses in AIDS patients and the other patients studied.

There is, however, evidence that thymic epithelium can be infected by HIV. This was reported by Schuurmann et al. [25] who used immunocytochemistry and in situ hybridization. Furthermore, Numazaki et al. [28] succeeded in infecting human thymic epithelial cells in vitro by HIV. These cells even showed evidence of virus replication, as assessed by the presence of reverse transcriptase in the culture supernatants.

Interestingly, some anti-HIV antibodies, such as anti-gag p17 and p24 antibodies, react with EC in the involuted thymuses of normal subjects. The positive cells were found in the subcapsular zone and in the medulla, where a subpopulation of thymic hormone-producing cells has been described [29]. In fact, there is a sequence homology between thymosin α_1 and HIV p17 protein [30] and an octapeptide T from gp120 protein [31]. However, the cells labeled with anti-p17 and anti-p24 reagents in the normal involuted thymuses do not contain thymosin α_1, nor with the gag/pol probes used to detect HIV related RNAs. Thus, the cross-reactivity observed is not fully understood.

Thus, if it appears very clearly that HIV can infect thymic cells, there is presently no proof that thymic involution in AIDS patients is related to that infection rather than to a reaction to the debilitating disease.

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J. Boniver, Laboratory of Pathological Anatomy and Cytopathology, Department of Pathology, University Hospital of Liège (B35), B–4000 Liège (Belgium)