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# THE THYMIC MICROENVIRONMENT IN INFECTIOUS DISEASES

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## 1. INTRODUCTION

There is now increasing evidence placing the thymus gland as a target organ in a variety of infectious diseases. In some viral affections, as the lymphochoriomeningitis, thymic lymphocytes can be directly infected (Lohler & Lehman-Grube, 1981). Conversely, in a series of viral and parasitic infections, thymocytes are not infected. In any case, a massive death of these cells is a common finding. Suprisingly however, relatively few data foccuse the consequences of a given infection upon the microenvironmental compartment of the thymus.

In the present review, we shall summarize a number of recent data, coming out from our and other laboratories, showing that the thymic microenvironment, particularly its epithelial component, is pleiotropically affected in distinct infectious diseases, caused by viruses or parasites. Furthermore, we compiled evidence suggesting that the increase in extracellular matrix production occurring in a variety of models of infectious diseases may be somewhat associated with thymocyte death.

Nonetheless, before going into the results obtained on this subject, it might be worthwhile to briefly discuss some general features of the thymic microenvironment.

## 2. THE THYMIC MICROENVIRONMENT: AN INTRODUCTORY COMMENT

It is currently accepted that key events of intra-trymic T cell differentiation are driven by influence of the socalled *thymic microenvironment*. This later actually corresponds to a tridimentional network composed of distinct cell types as well as extracellular matrix elements.

The thymic epithelium is the major component of the thymic microenvironment and plays important and multifaceted influences in early events of T cell differentiation. This is accomplished by at least two distinct ways: a) secretion of a variety of polypeptides as thymic hormones (see review Bach, 1983), interleukin 1 (Le et al., 1988a) and granulocyte-macrophage colony stimulator factor (Le et al., 1988b), and b) cell-to-cell contacts, including those occurring through classical adhesion molecules (Nonoyama et al., 1988) and, most importantly, the key interactions with differentiating thymocytes that are mediated by the major hostocompatibility complex products, highly expressed on thymic epithelial cell (TEC) membranes (Janossy et al., 1980; Jenkinson et al., 1981; Savino et al., 1985; van Ewick et al., 1988).

Although collectively TEC can be characterized by the presence of cytokeratin-containing intermediate filaments and desmosomes (Singh, 1986), the thymic epithelial reticulum is a heterogeneous tissue in which distinct cell types have been defined on the basis of their ultrastructural differences (Lampert & Ritter, 1988). Moreover, TEC subsets in both cortical and medullary regions of the thymic lobules have been evidenced immunohistochemically by means of monoclonal antibodies (MAb) raised againt human or murine thymic fragments (Haynes et al., 1983, 1984; MacFarland et al., 1984; van Vliet et al., 1984; De Maad et al., 1985; Lobach et al., 1985; Kaneshina et al., 1987; Takacs et al., 1987). In addition, and using a different MAb-based strategy, we succeeded in demonstrating a group of TEC subpopulations based on their CK specificities (Savino & Dardenne, 1988a, 1988b). Interestingly, although the physiological significance of these MAb-defined TEC subsets remains unknown, they revealed to be useful markers in the study of thymic pathology.

In addition to these TEC markers, we noticed that extracelullar matrix (ECM) components could be altered in some pathologi-

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cal conditions, particularly myasthenia gravis (Savino et al., 1988), Down's syndrome (Fonseca et al., 1989) and diabetes (Savino et al., 1990), thus placing these molecules as further tools in the study of the thymus along with human or experimental infectious diseases.

## 3. THYMIC ATROPHY: A COMMON FEATURE IN ACUTE INFECTIOUS DISEASES

It is largely known that one of the most common characteristics of the thymus in a series of immune deficiency states, including acute infectious diseases is a severe loss of thymic weight (see review Dourov, 1986). This atrophy is essentially due to a thymocyte depletion leading to an important reduction in the cortical region of thymic lobules. Actually, in some cases, the cortex virtually disappears. Besides (and possibly partially secondary to) this thymocyte death and resorption, the microenvironmental tridimentional network undergoes a densification process, as can seen by the shrinkage of the thymic epithelial reticulum.

## 4. STRUCTURAL CHANGES IN THE THYMIC EPITHELIUM

As briefly stated above, it is possible that some of the structural changes occuring in the thymic microenvironment along with the development of an infection process, are secondary to the mechanical rearrangements following thymocyte death. Nonetheless, there is some evidence suggesting the existence of intrinsec and rather particular changes in the TEC network. For example, ultrastructural studies of the thymus in experimental murine rabies virus infection revealed specific TEC alterations corresponding to an increase in the cytoplasmic vacuoles and appearance of cysts bordered by ciliated epithelial cells (Savino et al., 1987). Moreover, we recently evidenced that *in vivo*, not only macrophages but also thymic epithelial cells could be infected by *Trypanosoma cruzi* (Gonçalves da Costa et al., 1990).

Concerning AIDS thymus, severe changes in the thymic epithelium were noted by us and others. Instead of the normal TEC network, thymuses from HIV infected patients beared round or spindle-shaped cells forming large epithelial (keratin-positive) clusters in which the typical slender cytoplasmic processes were not seen. Moreover, histological signs of TEC injury eventually leading to focal epithelial necrosis were reported (Seemayer et al., 1984; Savino et al., 1985).

In addition to these structural data, some monoclonal antibody-defined thymic epithelial cell subsets were found to be altered *in vivo* in individuals infected with either parasites or viruses. We showed that in mice undergoing acute Chagas' disease, cells recognized by the MAb ER-TR.5 (normally restricted to the thymic medulla) could be detected in both inner and subcapsullary cortex. Conversely the TEC subset defined by the expression of cytokeratin 8 and 18, and that is cortex-restricted in normal conditions, was also found as medullary clusters or isolated cells (Savino et al., 1989). Interestingly, similar changes were recently seen in animals developing experimental schistosomiasis (Silva Barbosa et al., 1990).

The studies carried out on AIDS thymuses (Savino et al., 1985) also revealed changes in TEC subsets, as demonstrated by the decreased in the numbers of cells defined by the MAb anti-p19 and TE-4, that define in the normal thymus the medullary/subcapsullary TEC subset (Haynes et al., 1983, 1984).

## 5. THYMIC HORMONE PRODUCTION AND HLA-DR/Ia EXPRESSION

Besides the morphological changes in the profile and subsets of the thymic epithelial cell network, alterations in the expression of functional molecules could already be evidenced in some models of infectious diseases in which they have been investigated. Thus, the serum levels of one chemically-defined thymic hormone namely thymulin, were found to be decreased in AIDS patients (Dardenne et al., 1983). These findings were further confirmed when we detected a decreased immunohistochemical labelling of thymulin in thymus frozen sections from AIDS subjects (Savino et al., 1985).

More recently, we studied thymulin production in mice acutely infected with *T. cruzi*. In contrast to what was found in acquired immunedeficiency syndrome, only a minor decrease of thymulin was detected in parasite infected animals, even in late infection stages (Savino et al., 1989).

As regards the expression of class II MHC gene products, we showed that, contrasting to the normal positive cellular framework, HLA-DR expression in AIDS thymuses was decreased (or even absent) in some epithelial regions, where only dendritic non-epithelial (keratin-negative) cells were labeled (Savino et al., 1985).

This pattern was however not detected in mouse models of viral of parasitic diseases we have already analysed. In *T. cruzi* acutely infected animals, the la-bearing cellular network was rather denser as compared to control non-infected mice (Savino et al., 1989). Similarly, in rabies virus infected mice, the la-positive framework remained strongly labeled (Savino et al., 1987).

## 6. *IN VITRO* INFECTION OF CELLS OF THE THYMIC MICROENVIRONMENT

A series of recent studies now strongly suggest that cultures of thymic epithelial cells can be used as a further tool to better analyse the relationships between infectious agents and the thymic microenvironment. We showed that a murine TEC line as well as primary cultures of thymic phagocytic cells can be infected by *T. cruzi* (Savino et al., 1989), and that even in conditions of relatively low infectivity (5%) TEC cultures exhibited a slight, yet consistent, decrease in thymulin production (Leite de Moraes, 1989). Moreover, preliminary data suggest that cytokeratin expression may be altered in infected TEC growing *in vitro* (unpublished).

Concerning viral infection, it was demonstrated that primary cultures of human TEC could be infected by measles virus and by cytomegalovirus, resulting in distinct specific cytopathic effects (Numasaki et al., 1989a). Particularly for measles virus, not only virus particles were detected within cultures TEC, but they yielded the formation of syncitia and virus replication was evidenced (Numasaki et al., 1989b). Moreover, these authors showed that measles virus infected TEC exhibited phenotypic changes revealed with a variety of MAb that specify distinct TEC markers. This same research group recently succeeded in infecting cultured human TEC with HIV-1. Infected cultures revealed cellular disarrangements, giant cell formation and eventual cytolysis (Numasaki et al., 1989c).

## 7. ANTI-THYMIC EPITHELIAL CELL AUTOANTIBODIES IN INFECTIOUS DISEASES: AN EXAMPLE OF MOLECULAR MIMICRY?

Anti-self reactivity, involving both B and T cell autoimmune responses, appears to be a common finding in infectious diseases, as those evidenced for *T. cruzi* infection (Minoprio et al., 1986a, b). As regards anti-TEC autoreactivity, we noticed that *T. cruzi* acutely infected mice develop circulating anti-TEC antibodies (Savino et al., 1989). In the same vein, we and others demonstrated the presence of immunoglobulins and complement components bound to epithelial cells of AIDS thymus (Savino et al., 1985; Pekovic et al., 1987). More recently, Ig-binding sites were also evidenced in thymuses from *Schistosoma mansoni* infected animals (Silva Barbosa et al., 1990).

One interesting question raised from these data refers to the triggering for clonal expansion. As recently revealed by Minoprio et al. (1988), most of the MAb obtained by fusioning myeloma cells with splenocytes from *T. cruzi*-infected mice do not recognize parasite epitopes.

Nonetheless, in other examples the epitope recognized is shared by molecules of the host and the infecious agent. Thus, *T. cruzi* and astrocytes bear common MAb-defined epitopes in a ganglioside (Petri et al., 1988). Specifically concerning the thymic epithelium, it was showed that a MAb directed against the p.19 protein of the HTLV-1 (human T cell leukemia virus type 1) also recognized a cytoplasmic epitope of the normal human thymic epithelium (Haynes et al., 1983). More recently, similar findings were reported in terms of epitopes shared by the thymic epithelium and distinct components of HIV, including thymic hormones (Naylor et al., 1987; Wu et al., 1988: Parravicini et al., 1988). Particularly in respect to thymosin $\alpha$-1, an aminoacid homology with the HIV peptide T was demonstrated (Nguyen & Scheving, 1987). In addition to these findings, we recently observed that sera from rabbits immunized with a saline extract derived from adult *S. mansoni*, were able to decorate the epithelial network when apllied on thymus frozen sections (manuscript in preparation).

This later series of data drives us to the hypothesis that the so-called *molecular mimicry* between viral – or parasite – derived proteins and molecules of the normal thymic epithelium,

may be a rather common phenomenon. This might explain the frequency of anti-TEC autoreactivity detected in distinct infectious diseases. In any case, it should be mentioned that other consequences of the this particular molecular mimicry in terms of the host's immune response represent a completely open avenue for investigation.

## 8. INCREASE IN THYMIC EXTRACELLULAR MATRIX ALONG WITH ACUTE INFECTIONS

The last aspect to be discussed concerns the modulation of the extracellular matrix (ECM) component of the thymic microenvironment, and its parallelism with thymocyte death. In the last few years we cumulated evidence showing that the expression of basement membrane proteins, namely type IV collagen, laminin and fibronectin, is dramatically increased in atrophic thymuses. Thus, in a variety of experimental and human infections resulting in severe thymocyte depletion, an important intralolular ECM-containing network was consistently observed, as in murine rabies (Savino et al., 1987), acute experimental Chagas' disease (Savino et al., 1989), murine schistosomiasis (Silva Barbosa et al., 1990) as well as congenital human measles or cytomegalovirus infections (Fonseca & Savino, unpublished). Interestingly, this phenomenon was also evidenced after injecting mice with a single dose of hydrocortisone, known to promote thymocyte death (Lannes Vieira et al., 1990). Kinetic studies in both hydrocortisone and *T. cruzi in vivo* models revealed that such increase in ECM production actually preceeds thymocyte depletion. These data together with our preliminary findings suggesting that fibronectin appears to enhance thymocyte death *in vitro*, raised the hypothesis that the increase in thymic extracellular matrix occuring as a general feature in acute infectious diseases, may be somewhat related to thymocyte death.

## 9. CONCLUSIONS AND PERSPECTIVES

The bulk of data above reviewed represents in our opinion a strong evidence that the microenvironmental compartment of the thymus can be pleiotropically affected as a consequence of infection. It appears that changes in the thymic epithelial cell network pattern, together with an increase in thymic extracellular matrix production, might be considered as general features in individuals undergoing acute infections.

On the other hand it is also apparent that much more results should come out on this subject so that we can conceive more precisely which are the similarities and differences (in respect to the thymic microenvironment) that can be evidenced in distinct viral or parasitic diseases.

Finally, an important question to be further addressed concerns on the putative influences of these thymic microenvironmental alterations in terms of the pathophysiology of the disease.

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