IS THE THYMUS A TARGET ORGAN IN CHAGAS' DISEASE?

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1. Introduction

The involvement of T cells in the course of a variety of infectious diseases has been demonstrated by several lines of evidence. Particularly concerning Chagas' disease, it has been recently shown that CD4 - bearing T lymphocytes are responsible for the anti-cardiac muscle autoreactivity occuring in the chronic phase of the affection (Hontebeyrie-Joskowicz, 1991; Ribeiro dos Santos *et al.*, 1992), where CD8 + cells are involved in protective mechanisms (Tarleton 1991).

In this respect, although peripheral T lymphocytes are largely recognized as playing a key role in the immunopathology of infectious diseases, much less data are available concerning putative alterations of intra-thymic T cells. In the present work, we shall review a number of findings recently come out, concerning the thymus in the murine model of some Chagas' disease. Nonetheless, before going into the specific data obtained on this subject, it is worthwhile to briefly discuss some general features regarding thymic lymphocytes and microenvironmental components in normal conditions.

2. Intra-thymic T cell differenciation : general comments

The thymus gland is a central lymphoid organ, in which bone marrow-derived T cell precursors, undergo a complex process of maturation, eventually leading to the migration of positively selected thymocytes to the T-dependent areas of peripheral lymphoid organs. This differentiation process, that involves a positive selection of some thymocytes and negative selection of many others (the latter accompanied by massive apoptosis), allows T lymphocytes to distinguish self from non-self proteins, representing the vast majority of the so-called T cell repertoire. Along with intra-thymicT cell differentiation, lymphocyt es modulate the expression of a number of membrane proteins. For instance, the molecule named CDI, typical of immature cortical thymocytes is lost along with differentiation. Conversely, CD4 and CD8 proteins, absent in the most immature thymocytes, are simultaneously expressed in the majority of cortical (still rather immature) thymocytes, being then further modulated, so that the mature medullary subsets are either CD4 or CD8 single positive cells. Lastly, the CD3/Ti complex, in the context of which, the antigen recognition will take place, is expressed in low densities in cortical lymphocytes whereas their density increases when cells further differentiate acquiring the medullary phenotype (see review Boyd and Hugo, 1991).

It should be pointed out that key events of intra-thymic T cell differentiation are driven by influence of the thymic microenvironment. This later corresponds to a tridimentional network composed of distinct cell types, such as epithelial cells, macrophages and dendritic cells, 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), interleukins 1, and 6 (Le *et al.*, 1988a) and granulocyte-macrophage colony stimulator factor (Le *et al*, 1988b), and b) cell-to-cell contacts, including those occurying through classical adhesion molecules (Nonoyama *et al.*, 1988) and, most importantly, the paramount interactíons with differentiating thymocytes that are mediated by the major histocompatibility complex products, highly expressed on thymic epithelial cell membranes (see review van Ewijk, 1991). Thus, MHC c1ass I proteins interact with the CD8 molecule whereas MHC class II binds to the CD4 complex. These interactions are determinant in defining the positive versus negative selection of thymocytes bearing the distinct T cell receptor rearrangements.

Although thymic epithelial cells (TEC) can be generally characterized by the presence of cytokeratin-containing intermediate filaments and desmosomes, the epithelial reticulum is a heterogeneous tissue in which morphological and antigenic differences are detected, that can be evidenced by means of anti-TEC monoclonal antibodies (MAb), able to define cells in different regions of the thymic lobules (van Ewijk, 1991), and by Mab specific for distinct cytokeratins (Savino & Dardenne, 1988a, 1988b). Interestingly, the use of a panel of antibodies, against either thymic fragments or cytokeratins, can be considered as relevant tools in the study of thymic pathology.

Besides the above discussed phenotypically - defined TEC heterogeneity, a particular TEC-containing structure has been isolated, namely the so-called thymic nurse cell complexes (TNC). These are lymphoepithelial multicellular structures formed by one TEC harbouring 20-200 rather immature thymocytes (Wekerle & Ketelson, 1980).

3. The thymic lymphoid compartment in Chagas'disease

One of the features occuring in acute *T. cruzi* infection is a thymic atrophy defined by loss of thymus weight and cellularity, whose severity correlated with the parasite dose applied.

The decrease in cell numbers essentially corresponded to a cortical thymocyte depletion, so that in infected animals, the cortical region of the thymic lobules virtually disappears. In keeping with this, a consistent decrease in the percentage of CD4/CD8 double positive cells (normally located in the cortex) is observed. In parallel with this, there is an augmentation in the percentage values of double negative as well as CD4 and CD8 single positive thymocytes. Moreover, the proportion of thymic lymphocytes expressing high densities of the CD3 complex was enhanced along acute infection (Leite de Moraes *et al.*, 1991).

One might argue that, such thymocyte alterations occuring in acutely-infected animals are stress-associated, since similar findings could be induced by injection of glucocorticoid hormones. In fact, high levels of circulating corticosterone can be detected in acute *T. cruzi* infection. Nonetheless, adrenalectomy did not alter the thymic atrophy and thymocyte subset changes seen in the murine acute Chagas' disease (Leite de Moraes *et al.*, 1991). Thus, low numhers of cycling cells are detected in *S. mansoni*-infected animals (Silva Barbosa *et al.*, 1992).

Regarding chronic infection, less data is so far available. Yet, we have shown that in *T. cruzi* chronically-infected mice, the thymus weight, cellularity and CD4/CD8-defined subsets, progressively returns to values similar to those of age-matched control animals (Leite de Moraes *et al.*, 1992). However, as further discussed below, an important aspect yet to be

determined concerns whether the intrathymically generated T cell repertoire is changed or not after infection.

4. The thymic microenvironment in acute *T. cruzi* infection

In addition to the thymocyte changes summarized above, *T. cruzi* acutely infected mice exhibited changes in the thymic microenvironment. For example, epithelial 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 expression of cytokeratin 8 and 18, restricted to cortical TEC in normal conditions, was also found as medullary clusters or isolated cells (Savino *et al.*, 1989). Interestingly, similar changes were seen in animals developing experimental schistosomiasis (Silva Barbosa *et al.*, 1992).

More recently, we noticed that the total numbers of thymic nurse cells was decreased in acute *T. cruzi* infection (manuscript in preparation).

Besides the phenotypic changes in epithelial cells located in different areas of the thymic lobules, alterations in the expression of functional molecules could already be evidenced in experimental acute Chagas' disease. For example, we evidenced a transient decrease in thymulin serum levels (Savino *et al.*, 1989). Moreover the Ia-bearing cellular network was rather denser as compared to control non-infected mice. In this respect, and taking into account the importance of intrathymic MHC expression for normal thymocyte differentiation, it is possible that abnormal MHC distribution in the thymus from infected subjects affects the physiological generation of the T cell repertoire.

Lastly, we should discuss the modulation of extracellular matrix (ECM) components of the thymic microenvironment in acute *T. cruzi* infection, 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 (see review, Savino and Lannes-Vieira, 1991). In acutely-infected animals, we evidenced a progressive increase in intra-lobular ECM expression, that actually preceeded thymocyte depletion (Savino *et al.*, 1989).

It is important to note that, microenvironmental alterations occuring in acutely infected mice, are paralleled by an intra-thymic invasion of the parasite, that can infect both epithelial and non-epithelial dendritic cells (Savino *et al.*, 1989; Gonçalves da Costa *et al.*, 1991). These findings place the possibility that, at least some of the changes above discussed may be related to the presence of parasite antigens within the thymus.

5. Anti-thymic cell autoantibodies in Chagas'disease

Polyclonal B and T cell activation, together with anti-self reactivity, appears to be a common finding in murine Chagas' disease, including examples of target epitopes not appearing in the parasite (Minoprio *et al.*, 1986a, 1986b, 1980), and epitopes shared by molecules of the host and the infectious agent (Petry *et al.*, 1988).

As regards anti-TEC autoreactivity, we noticed that *T. cruzi* acutely infected mice develop circulating anti-TEC antibodies (Savino et al., 1989). Interestingly, during the chronic phase of both human and murine Chagas' disease, anti-thymocyte antibodies with cytotoxic properties were detected in the sera (Savino *et al.*, 1990). In this particular case, anti-thymocyte antibodies cross-reacted with parasite antigens. Latter findings could be interpreted as an example of molecu-

lar mimicry between parasite-derived proteins and molecules of the normal thymic cells. It should be mentioned that consequence(s) of this particular process in terms of the host's immune response represent(s) a remain(s) to be determined.

6. Conclusions and perspectives

The data above reviewed provide a strong evidence that both the lymphoid and microenvironmental compartments of the thymus are significantly affected as a consequence of *T. cruzi* infection. It appears that changes in the thymocyte subsets, 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 infection. On the other hand, it is also apparent that much more results should come out before we can conceive more precisely whether common or specific mechanisms are involved in generating such thymic abnormalities.

Finally, a relevant question to be further addressed concerns the putative influences of these alterations in modulating the host's immune system. In this respect, the analysis of T cell receptor gene rearrangements whithin the thymus and in the peripheral lymphoid organs, following infection, may bring valuable information regarding a possible plasticity of the T cell repertoire.

Acknowledgment

This work was partially supported with grants from CNPq (Brazil), INSERM (France) and WHO/TDR/World Bank (Geneva).

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