Mouse as a Model for Chagas Disease: Does Mouse Represent a Good Model for Chagas Disease?

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Since the early years of its discovery, American trypanosomiase has been seen as a disease due to indirect damage via inflammation due to the immune response of a host against systemic tissue colonization by the *Trypanosoma cruzi* (Vianna 1911, Magarinos Torres 1928). The expression of this immune response has been studied over the century, but the mechanisms involving the pathogenesis of this disease are not well understood and remain a matter of debate (Kierszenbaum 1985, Hudson 1985).

Although there are many difficulties in correlating the chronic form of the disease in mice and humans, the mouse model has been the most studied. In addition, experimental American trypanosomiasis in mice has been proposed as a model for the study of autoimmune diseases. It has been emphasized that *T. cruzi* infections in mice offer an attractive means of investigation for the induction of autoimmunity and its consequences by (1) sharing of antigenic determinants (Acosta et al. 1985); (2) alteration of host cells surfaces by adsorption of *T. cruzi* released antigens (Muniz et al. 1970, Ribeiro dos Santos & Hudson 1980 a,b); (3) expressing parasite antigens on the surface of infected cells (Araujo 1985).

Moreover the parasite can invade either the primary (Savino et al. 1989, Gonçalves da Costa et al. 1991) or the secondary lymphoid organs (Brener & Chiari 1963, Gonçalves da Costa et al. 1984) transforming them into target organs since parasite antigens may transform the microenvironment and induce the destruction of transformed cells by cytotoxic lymphocytes. This process was described acting against sensitized neurons and muscle fibers (Kuhn & Mumane 1977), but it may occur systemically. Systemic infection occurs in immunocompromised hosts (Gonçalves da Costa et al. 1984) as well as in normal ones (Lenzi et al. 1996). The systemic and intense infection observed in mice has a correlation with severe clinical cases described in man by Chagas (1916), who was working mostly with children.

The balance of host/parasite relationship appears in some instances as the intensity of inflammatory infiltrate versus the parasite load. Experimental models allow the development of two polar expressions of this relationship: (1) absence of an inflammatory reaction in athymic nude mice; (2) an enhancement of myocarditis in infected mice where cyclophosphamide is given two days before infection (Gonçalves da Costa et al. 1984, Calabrese et al. 1996). An enhancement of myocarditis has been also observed in dogs after CY treatment (Andrade et al. 1987).

Immunomodulation can alter the flux of inflammatory cells to the site where the parasite or its antigens persist as well as the nature of the inflammatory type and subsets.

Contradictory results have been reported in different experimental studies upon superinfections. More severe lesions have been reported histopathologicaly in superinfections than in prime-infected mice (Fernandes et al. 1966), while other authors have shown very similar lesions in both groups in assays using genetically characterized *T. cruzi* clones (Lauria-Pires & Teixeira 1996).

Recently, it has been observed that mice that become chronically infected by a vaccination procedure using BCG associated with *T. cruzi* flagellar fraction antigens present a severe myosite after a superinfection with the same strain. This inflammatory infiltrate has shown a significant participation of eosinophils in comparison with lesions of prime-infected mice (manuscript in preparation). Little information has been brought out by the authors, but the occurrence of eosinophilia in the last stages of the acute period of infection was reported in patients by Emanuel Dias (1912). The role of eosinophils in antibody-dependent cellular cytotoxity (ADCC) has been reported in *in vitro*

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studies of cytotoxicity and by electron microscopy analysis of host cell-parasite interactions. Eosinophils are not the only cell type capable of displaying ADCC on *T. cruzi* infections, since lymphoid cells and neutrophils have also been implicated. It is important to stress that, in many experiments using CY where DTH to *T. cruzi* was enhanced, a rebound of circulating monocytes and PMN occurred and this affected the cellular infiltrate (Gonçalves da Costa & Calabrese 1996). The pathology induced by *T. cruzi* is mediated by T-cells (Gonçalves da Costa et al. 1984), which are of the $CD4^+$ phenotype, promoting a local DTH reaction, with a subsequent recruitment of circulating monocytes (Honteberyrie et al. 1987). Therefore some authors believe that both parasite and host share common determinants which are recognized by $CD4^+$DTH cells.

After tissue colonization and the establishment of lesions caused by a direct action of parasites occur, auto-reactive $CD4^+$T cells appear and reject syngenic heart grafts (Ribeiro dos Santos et al. 1992). Some authors, however, have demonstrated a predominance of $CD8^+$T cells in heart lesions, either in humans investigations (Higuch et al. 1997) or in experimental models (Tarleton 1990). It must be emphasized, nevertheless, that $CD8^+$T cells can also induce DTH and that only a small number of $CD4^+$ T DTH cells is necessary for a DTH induction. Those $CD4^+$ T cells attract a great number of effector cells (monocytes and macrophages or monocytes cells and PMN cells in function of the type of DTH) to the inflammatory site where specific parasite or parasite antigens remain. These effector cells arrive to the inflammatory site and promote the destruction of parasites and tissue structure through their hydrolytics products.

Lymphocytes bearing γδ TCR have been shown to play an important role in the early immune response to live intracellular microorganism and they seem to be correlated with an immunity to the parasites. It has also been shown that γδ T-cells may recognize non-peptide antigens that are not stimulatory for alpha-beta T-cells (Tanaka et al. 1994, Morita et al. 1995). In experimental Chagas disease, γδ T-cells appear expanded in the acute phase of murine infection by the CL strain of *T. cruzi.* It remains crucial, however, to explain the role of this cell population in the early response to a *T. cruzi* infection. Recently it has been shown that γδ T-cells may recognize non-peptide ligands that are not stimulatory for alpha-beta T-cells. Studies are being carried out in our laboratory to better understand the role of γδ T-cells in the murine acute phase of Chagas disease and for characterization of specific *T. cruzi* antigens involved in γδ cells activation. The expression of TCRs specific for similar phosphoantigens, either of endogenous or exogenous origin, induce a polyclonal γδ T-cells response, characterized by expressive crossreactvity against bacterial, protozoa or viral antigens. It has been demonstrated that in murine models gamma delta cells accumulate rapidly after infection with *Mycobacteria tuberculosis* (Tanaka et al. 1994), *Leishmania* (Uyemura et al. 1992), *T. cruzi* (Minoprio et al. 1989) and *Listeria monocytogenes* (Hiromatsu et al. 1992). Otherwise γδ cells can mediate specific CMI by recognizing invading pathogens directly (Tanaka et al. 1994).

γδ cells also appear as candidates for mediators of autoimmune diseases, since some correlations have been established with rheumatoid arthritis (Keystone et al. 1991), autoimmune thyroiditis (Roura-Mir et al. 1993) and autoimmune liver disease in which γδ cells cytotoxicity of hepatocytes was reported (Martins et al. 1996).

γδ T-cells can recognize damaged cells directly but some authors suggest that the presence of γδ T-cells in inflammatory lesions may be due to the ability of gamma-cells to control excessive tissue damage (Ferrick et al. 1996). They appear in the inflammatory infiltrate during the chronic human digestive form of Chagas disease (Rodrigo Corrêa de Oliveira, pers.comm.). Since a preferential localization of TCR γδ lymphocytes to epithelial surfaces has been described (Janeway et al. 1988), further investigations must be carried out to explain eventual differences between digestive and cardiac lesions in Chagas disease.

Differences between the experimental model and human autoimmune diseases have been established by the fact that in an experimental induced organ-specific autoimmune disorder it is possible to control the development of autoimmunity by deleting T-cells that are reactive to one well known initiating antigen (Critchfield et al. 1994); otherwise, an initiating target antigen has not yet been characterized in human T-cell mediated autoimmune diseases. Many of the difficulties normally found while comparing results between the murine model and human autoimmune disorders are beginning to disappear with the introduction of humanized mouse models – the HLA transgenic mice – for investigations of several diseases (Taneja & David 1998). These models bring the opportunity to test autoantigens for presentation by the HLA molecules. Regarding Chagas disease it is important to re-evaluate studies made during the chronic phase using the mouse model.

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