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IMMUNOREGULATORY MECHANISMS AND CHAGAS' DISEASE

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During the course of experimental Chagas' disease, several immune disorders occur. In the acute phase, T and B cell polyclonal activation is associated to immunosuppression. At the chronic stage, T cells – of the TH2 subset – participate to the pathology characteristic of Chagas' disease. Data obtained after infection of BALB/Xid mice suggest that polyclonal activation may be dependent on B1 (CD5) cell-activation. Moreover, these mice fail to develop the pathological features of the chronic infection. Control of lymphokine secretion might play a key role in the clinical status of Chagas' disease.

Key words: Trypanosoma cruzi – B cell polyclonal activation – immunosuppression – TH1 and TH2 subsets – B1 (CD5 B) cells – immunopathology

Chagas' disease, due to the protozoan *Trypanosoma cruzi* is the major cause of sudden death in Latin America. The World Health Organization estimates to 15-18 million people suffering from this disease. Among them, 20 to 30% will develop the chronic Chagas heart disease or megavisceres. Because of the absence or scarcity of parasites during the chronic stage of the infection and of the features of histopathological lesions, hypothesis of autoimmunity has been argued. Pathology is represented by mononuclear inflammatory infiltrates with necrotic foci leading to the destruction of myocardial or nerve fibers. The consequence is heart failure and sudden death. Until now, the fine mechanisms of the cardiomyopathy or nerve damage are not clearly elucidated.

The murine experimental model has represented, this last decade, a useful tool to better understand the immunological status of the infected host. During acute experimental infection, all the compartments of the immune system are disturbed as illustrated by T and B cell activation in lymphoid organs, macrophage activation, cytokines production, thymic atrophy, elevated autoantibody production (Harel-Bellan et al., 1983; Minoprio et al., 1986a; Russo et al., 1989; Spinella et al., 1989; Leite de Moraes et al., 1991). The role of this high degree of disturbance in the following clinical state of the chronic disease is beginning to be investigated.

The early immune picture of the infection is represented by a tolerance-like state in relation to the parasite. Non specific and autoreactive reactivities emerged with a low response against the parasite itself (Minoprrio et al., 1987). However, this breakdown of tolerance is not complete and few weeks after infection, a significative response against the parasite is developing, that is turned to account serological diagnosis. Several parameters of immunosuppression have been described as lack of response to exogenous antigens injected at the time of infection, IL2 suppression, mitogen unresponsiveness, suppression of specific cytotoxic activity (Cunningham et al., 1978; Ramos et al., 1978; Harel-Bellan et al., 1983; Chromanski & Kuhn, 1985; Plata, 1985). Moreover, polyclonal activation may contribute to the development of nonspecific B cell responses compared to parasite specific ones.

The participation of different subsets of lymphoid cells has been extensively demonstrated by *in vivo* treatment of animals at the time of and following infection. Indeed, treatment with anti-CD4, anti-CD8 and or anti-Ia monoclonal antibodies resulted in higher parasitemia and susceptibility to the infection (Minoprio et al., 1987; Russo et al., 1988; Spinella et al., 1989; Tarleton, 1990). Moreover, treatment with anti-CD4 or anti-Ia abolishes the B cell polyclonal activation. *In vivo* treatment with cytokines

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also resulted in controlling the infection and abolishing the immunosuppression (Chromanski & Kuhn, 1985; Reed, 1988). However, no observation of the chronic stage of the infection was observed in any of this reports. According to the anti-CD4 and anti-Ia treatments, the reason was the death of the animals due to the high parasitemia before the onset of the chronic stage.

Recent data in our laboratory have shown that infected BALB/c mice sharing the *Xid* mutation (affecting the B cell compartment of the immune system) are able to control the parasitemia and do not show the pathological features of the chronic infection, i.e., the characteristic inflammatory infiltrates in heart and muscles (Minoprio et al., 1991). These results allowed us to suggest a role for B1 (CD5 B) cells in the triggering of the pathology of chronic *T. cruzi* infection. Indeed, the *Xid* mutation affects mainly the B1 (CD5 B) cell compartment that is developmentally blocked. As recently described, the B1 cells may produce IL10 that in turn regulates negatively the proliferation of TH1 cells (O'Garra et al., 1990). It is probable that, in *T. cruzi* normal infected mice, the production of IL10 after activation of B1 cells contribute to a shift to TH2 cells with secretion of B cell factors like IL5 and IL6 contributing to the polyclonal B cell activation. The negative control of IL10 upon TH1 stimulation could explain the low levels of IL2 and IFN-γ produced in mice acutely infected with *T. cruzi*. In contrast, since B1 cells are lacking in BALB/Xid mice, these mice might control the *T. cruzi* infection stimulating TH1 cells with secretion of IL2 and IFN-γ, lymphokines known to have a protective role against the acute stage of *T. cruzi* infection (Chromanski & Kuhn, 1985; Reed, 1988).

In the context of chronic infection, we asked for the participation of T cells in the development of mononuclear inflammatory infiltrates. Previous work of Laguens and col. had shown that mononuclear cells from chronically infected mice were able to transfer pathological lesions in naive recipients (Laguens et al., 1981). Moreover, analysis of T lymphocyte populations into the inflammatory infiltrates had shown that T cells represent about 5% of the total mononuclear cells present in the inflammatory infiltrates (Ben Younes-Chennoufi et al., 1988), the majority of them being CD4+. Upon transfer into naive recipients, it was clearly shown that pathology may be mediated by CD4+ but not by CD8+ T cells, in absence of parasites (Hontebeyrie-Joskowicz et al., 1987). T cell lines derived from chronically infected mice have been obtained; they are of the CD4 subset and reproduce, when transferred to naive recipients, a pathology similar to that described in chronically infected mice. These lines are possibly autoreactive because they are able to transfer a local delayed type hypersensitivity reaction in presence of parasite extract or of mouse nerve extract. The role of autoreactive CD4 T cells in the destruction of syngeneic hearts has been demonstrated by Ribeiro Dos Santos and collaborators (This issue, and Ribeiro Dos Santos et al., 1992).

With respect to the helper activity of the T cell lines derived from chronically infected mice, they display a strong activation of syngeneic B cells in a polyclonal way as illustrated by the high levels of different immunoglobulin isotypes secreted. Indeed, *in vivo* injection of the T cell line G-05 induces polyclonal B cell activation measured by the number of immunoglobulin isotype-secretory cells (Spinella et al., 1990). The isotypic pattern in recipient mice is quite similar to that observed in chronic infected mice (Minoprio et al., 1986a), with a predominance of IgG2 isotypes. The lymphokine secretion of this CD4 cell line indicates that it belongs to the TH2 subset with production of IL4, IL5, Il6 and IL3. These lymphokines present in the culture supernatant of the G-05 T cell line participate to the B cell polyclonal activation *in vivo*. In preliminary experiments (Leite de Moraes, unpublished results), significant levels of IL6 are observed in the serum of acute infected mice. This observation argues for the activation of TH2 from the early stage of the infection although IL6 may be produced not only by T cells but also by macrophages.

In summary, the role of CD4 T cells in the immunopathology of the experimental Chagas disease is demonstrated. Very likely, the TH2 subset is involved in the chronic pathology performing (i) recruitment of monocytes in the tissues to constitute inflammatory infiltrates, (ii) polyclonal B cell activation with secretion of high levels of autoantibodies. The triggering of TH2 rather than TH1 in mice infected with *T. cruzi* remains to be elucidated. Activation of B1 cells may be one of the potential candidate for this regulation.

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