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Immunohistochemical characterization of infiltrating cells in human chronic chagasic myocarditis: comparison with myocardial rejection process

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Abstract. Cellular subpopulations that infiltrate the heart in human chronic chagasic myocarditis were defined immunohistochemically in endomyocardial biopsy (EMB) specimens. T cells formed 96.3% of the inflammatory infiltrate, predominantly CD8+ (cytotoxic/suppressor) T cells. The mean numbers of CD8+ and CD4+ (helper) T cells in the myocarditis were compared to those present in the myocardial rejection process. Mean numbers of CD8+ T cells were similar in both groups of EMB specimens while CD4+ T cell counts, CD4+/CD8+ ratios and CD4+ antigen expression were significantly lower in the chagasic group compared to the myocardial rejection group ($P < 0.002$). The persistent lower number and diminished expression of CD4+ T cells suggest an immunological imbalance in patients with chronic chagasic myocarditis. A possible participation of *Trypanosoma cruzi* parasites in the development of such immunological abnormalities is also discussed.

Key words: Chagas' disease – Myocarditis – Lymphocytes – Immunohistochemistry – Heart transplantation

Introduction

Chagas' disease is a widespread illness, endemic to some regions of South America, caused by infection with *Trypanosoma cruzi,* an intracellular parasite. In the acute stage of the disease, many organs are heavily parasitized, particularly the heart. Most patients recover without symptoms although in the long term some develop heart failure after an asymptomatic period. The inflammatory infiltrate plays an important role in the pathogenesis of heart failure in chronic chagasic disease (Barreto et al. 1986; Higuchi et al. 1987), but the exact mechanism responsible for the development of this inflammatory infiltrate is still a matter of dispute. Characterization of the cellular infiltrate in chronic chagasic myocarditis, as in other forms of myocarditis (Chow et al. 1989; Deguchi et al. 1987; Milei et al. 1990) is essential in the understanding of the immunopathogenesis, natural clinical outcome and for therapy.

The discrepancy between the severity of inflammatory infiltrate and the absence or scarcity of parasites in chronic chagasic myocarditis has suggested that immune mechanisms operate in the pathogenesis of this process (Torres 1941). Previous findings pointed to auto-immune mechanisms such as detection of common antigens between cardiac fibres and *T. cruzi* (Sadigurski et al. 1989), or the finding that lymphocytes from chronic chagasic rabbits reacted with non-infected allogeneic cardiac cells (Santos-Buch and Teixeira 1974).

The myocardial rejection in heart transplanted patients may also be considered to be an "auto-immune" myocarditis; the lymphocytes react against myocardial cells as non-self antigens. A comparison of the cellular infiltrate between both processes may give useful data about pathogenesis. The present work characterizes the inflammatory infiltrate in chronic chagasic myocarditis immunohistochemically and compares the mean numbers of CD4+ and CD8+ T cells in chronic chagasic myocarditis and in myocardial rejection from heart transplants of non-chagasic patients.

Materials and methods

This work was approved by the Ethics Committee of São Paulo Heart Institute. Myocardial T cell subsets were analysed in three groups of patients. In the group of chagasic patients, we firstly evaluated the percentage of T, NK and B cells presenting in the myocardium.

The first group contained chronic chagasic patients (group A) and this was divided into two subgroups. The first subgroup was designated A–I. T, natural killer (NK) and B cells were quantified in 13 right ventricular endomyocardial biopsy (EMB) specimens from chronic chagasic patients showing heart failure, a positive serological test for Chagas' disease and active lymphocytic myocarditis by EMB, according to previously described criteria (Higuchi et al. 1987). EMBs were performed to better evaluate the clinical condition of the patients and to assist in the choice of the most

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appropriate therapy. The characterization of T cell subsets was not performed in this group of biopsies.

A second group of EMBs from 22 patients with cardiac chronic Chagas' disease was studied to determine the number of CD4+ (helper) and CD8+ (cytotoxic/suppressor) T cells presenting in the myocardium. These constitute group A–II.

The second main group (B) comprised patients with myocardial rejection. CD4+ and CD8+ T cells were quantified in 22 right EMB specimens with histopathological diagnosis of acute myocardial rejection (working formulation's grades IB or IIIA; Billingham et al. 1990), from the last 22 non-chagasic patients submitted to heart transplantation in our institution. The basic disease of the patients was: dilated cardiomyopathy (11 cases), ischaemic heart disease (9 cases) or rheumatic disease (2 cases). The third group (C) was a control group (negative for myocarditis). CD4+ and CD8+ T cells were studied in myocardial fragments of right ventricle from 17 necropsy hearts less than 2 h post-mortem. These patients had died of non-inflammatory, myocardial disease at the São Paulo Heart Institute. The clinical diagnosis of these patients were primary pulmonary hypertension, acute aortic dissection or acute myocardial infarction. The fragments of hearts from patients showing acute myocardial infarct were taken from sites unaffected by the infarct. In spite of the minimal number of infiltrating cells, the proportion of CD4+ and CD8+ T cells normally presented in the myocardium was analysed.

Identification of lymphocyte subsets was performed using the avidin-biotin peroxidase complex technique in frozen sections of myocardial fragments according to previously described methods (Higuchi et al. 1991). The different subsets of lymphocytes were marked using specific monoclonal antibodies: CD4 (helper T cell) from Sao Paulo Medical School Laboratory of Immunology; CD8 (cytotoxic/suppressor T cells) and pan-B (B cells) from Dako-Patts, and NK cells from Pel-Freez Laboratory.

Positive cells for each marker were counted in all the fields for each EMB at a magnification of 400× and the mean number for high power field was obtained. The number of microscopic fields counted in each case varied from 8 to 25. The CD4+/CD8+ T cells ratio was also calculated.

The mean numbers of the different subsets of lymphocytes were compared by the Student's *t*-test; CD4+/CD8+ ratios were compared using the Mann-Whitney U test. Values of P lower than 0.05 were considered as statistically significant.

Results

The mean numbers of T, B and NK cells at 400× microscopic field and their proportions in chronic chagasic myocarditis (group A–I) were 13.0±5.4 (96.3%), 0.3±0.3 (2.2%) and 0.2±0.2 (1.5%) respectively.

The mean ages and mean numbers of CD4+ and CD8+ T cells of groups A–II (chronic chagasic myocarditis), B (myocardial rejection) and control group are shown in Table 1. The mean numbers of CD8+ in the chagasic group, myocardial rejection group and control group were 13.0, 10.6, and 0.6 respectively; the respective values for CD4+ were 4.5, 8.0 and 0.5 and for CD4/CD8 ratios were 0.3, 0.8 and 0.8.

There was no significant difference in the mean numbers of CD8+ T cells between groups A–II and B.

Table 1. Mean numbers of age, CD8+ and CD4+ T cells and CD4+/CD8+ ratios in the different groups of endomyocardial biopsies analysed (mean±SD)

Group	Age (years)	CD8+	CD4+	CD4+/CD8+
A Chagasic myocarditis	39±12	13.0±12.5	4.5±4.0	0.3
B Myocardial rejection	45± 9	10.6± 5.6	8.0±4.1	0.8
C Control group	48± 8	0.6± 0.5	0.5±0.3	0.8

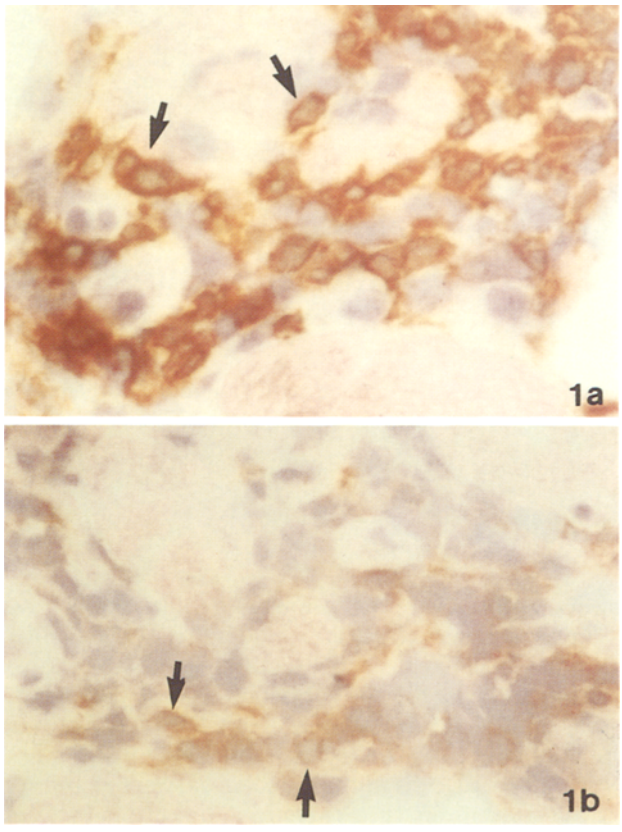

Fig. 1. Equivalent microscopical fields of myocardial frozen sections from a case of chronic chagasic myocarditis (group A–II) showing many lymphocytes immunoreactive for CD8+ T cell antigen *(arrows)* in **a** and lower numbers and with diminished expression of lymphocytes immunoreactive for CD4+ T cell antigen *(arrows)* in **b**. Avidin-biotin peroxidase method, ×400

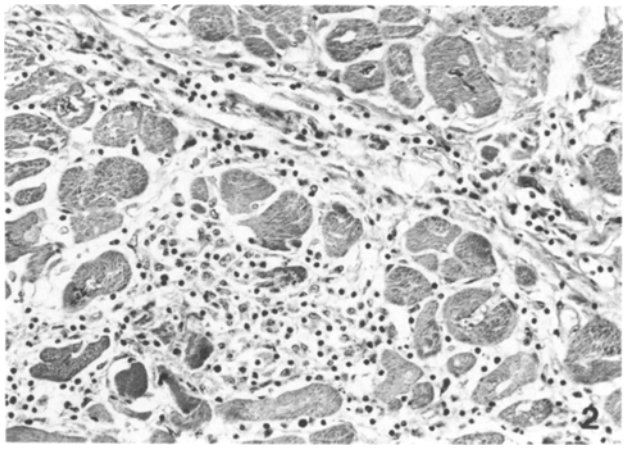

Fig. 2. Microscopic view of chronic chagasic myocarditis exhibiting lymphocytes encroaching upon myocardial fibres, thin interstitial fibrosis and moderate hypertrophy of the myocytes. Haematoxylin and eosin, ×250

The mean numbers of CD4+ T cells were significantly lower in the chagasic group compared to the myocardial rejection group ($P=0.002$). The expression of CD4+ T cells, that is the cell surface density of this antigen, was also markedly diminished in cases of chronic chagasic myocarditis than in rejection inflammatory infiltrate (Fig. 1a, b).

The CD4+/CD8+ ratios were significantly lower in the chagasic group when compared with the myocardial rejection group ($P<0.001$) and the negative control group ($P=0.008$) (Table 1).

Discussion

Santos-Buch and Teixeira (1974) demonstrated in vitro that lymphocytes from rabbits chronically infected with *T. cruzi* were able to lyse allogenic myocardial fibres but did not destroy allogenic renal cells. Teixeira et al. (1975) produced lymphocytic myocarditis in rabbits by injecting fractions of *T. cruzi*. These and other findings (Morris et al. 1990) have suggested that auto-immune mechanisms participate in the pathogenesis of chronic chagasic myocarditis (Fig. 2). Considering that the rejection phenomenon occurs by the same pathogenesis for auto-immune myocarditis (lymphocytes recognize myocardial cells as non-self antigens), both processes, chagasic and rejection myocarditis, may be comparable.

We have previously demonstrated (Higuchi et al. 1991), as have other authors (Pelletier et al. 1988; Rose et al. 1984), that myocardial rejection episodes are developed by both T cell subsets: CD8+ (cytotoxic/suppressor) and CD4+ (helper). No study has been carried out previously characterizing the inflammatory cells of chronic, human, chagasic myocarditis. We have demonstrated that T cells are the predominant subset of lymphocytes (96.3%) in this myocarditis, most of them being CD8+ T cells. When compared with the myocardial rejection process, we found similar numbers of CD8+ T cells, but significantly lower numbers of CD4+ T cells in chagasic myocarditis ($P=0.002$). The CD4+/CD8+ ratios were also lower in the chagasic myocarditis group compared with the myocardial rejection group ($P<0.001$) and the control group ($P=0.008$) while no difference was observed in the mean CD4+/CD8+ ratios between myocardial rejection and control groups. The intensity of CD4+ antigen staining was also diminished in the chagasic group. We considered this data a reliable and persistent finding compared with CD4+ T cells of rejection infiltrate taken into account that all EMB were processed in the same way. In the rejection process, CD4+ T cells were stained as strongly as CD8+ T cells.

Several experimental studies have shown that *T. cruzi* infected animals have altered immune responses, mainly during the acute phase (Harell-Bellan et al. 1985; Rowland and Kuhn 1978). The parasite induces a decrease in the expression of interleukin 2 receptors, and in the surface molecules of CD3+, CD4+ and CD8+ T cells (Kierszenbaum et al. 1989; Sztein et al. 1990) and this has been considered as a mechanism of evasion of the immune system attack by the parasite.

The persistence of immunodepression during the chronic phase of Chagas' disease is a controversial matter. Experimental studies (Reed et al. 1984, 1989) have shown a deficiency in helper T cell function in chronic Chagas' disease. Our findings of a lower number and decreased expression of CD4+ T cells in chronic chagasic myocarditis support the idea of selective immunodepression also in the late phase of human Chagas' disease. Would the parasites also be inducing immunosuppression in this phase of the disease? Recently, using anti-*T. cruzi* polyclonal antibody, we demonstrated parasite antigens related to the inflammatory infiltrate in myocardial sections from patients with chronic Chagas' disease (Higuchi et al. 1993), suggesting a direct role of the parasite in the pathogenesis of this myocarditis and also in phenotypic presentation of the lymphocytes. A vole for auto-immunity cannot be disregarded because the quantity of parasite antigens is minimal compared to the intensity of myocardial inflammation.

In conclusion, myocardial inflammatory infiltrates in chronic Chagas' disease are mainly composed of CD8+ T cells. Comparison with myocardial rejection process showed a lower number and a milder intensity of expression of CD4+ T cells in chagasic myocarditis. Persistent *T. cruzi* parasites in chronic phase of the disease may influence this particular inflammatory infiltrate.

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