

# Human Chronic Chagasic Cardiopathy: Participation of Parasite Antigens, Subsets of Lymphocytes, Cytokines and Microvascular Abnormalities

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*This article tries to demonstrate by new pathological findings (with the use of immunohistochemical technique and confocal laser microscopy) that chronic chagasic cardiomyopathy is a result of multiple factors involving myocarditis, immunodepression, severe fibrosis and microvessels dilatation and that all of these alterations are probably directly related with the presence of Trypanosoma cruzi parasites in the host associated with inadequate immunological response of the host.*

Key words: Chagas disease - myocarditis - *Trypanosoma cruzi* - immunodepression

In chronic Chagas disease, the severe myocardial fibrosis and disproportionate myocardial inflammation in view of the lack of *Trypanosoma cruzi* parasites, led to the proposals of theories of pathogenesis (Torres 1941, Muniz & Pena 1947, Cossio et al. 1974, Santos-Buch & Teixeira 1974) other than the parasite direction action. Many studies have demonstrated common antigens between *T. cruzi* and human myocardial fibers thus supporting theories of autoimmunity (Sadigursky et al. 1982, 1988, Levin et al. 1989, Cunha-Neto et al. 1995). According to this hypothesis, the process of myocarditis would perpetuate independently of the presence of the parasite which is rarely found associated with the inflammatory infiltrate (Acosta 1985, Ribeiro dos Santos et al. 1985). However, the autoimmune theory does not explain the multifocal nature of the myocarditis, with preference for certain specific regions of the heart such as the apical or the posterior left ventricular sites. Otherwise, frequent, positive xenodiagnosis during the chronic phase of Chagas disease and during episodes of reactivation in immunodepressed patients (by Aids, neoplasia or cardiac transplant) has shown that the parasite is present in the chronic phase and under active control of the immunological system of the host.

## THE PARASITE AND THE MYOCARDITIS

Knowledge of the exact role played by the parasite in the pathogenesis of chronic chagasic cardiopathy is of extreme importance to guide the therapeutic procedures. If the parasite is the principal cause of cardiac manifestations of the disease, the control of a possible autoimmune disease through immunodepressive drugs, may lead to the reactivation of the infectious agent.

The introduction of new techniques like immunohistochemistry (Higuchi et al. 1993) and PCR (Jones et al. 1993), has demonstrated a higher frequency of *T. cruzi* Ags and also a better association with myocardial inflammation.

Using an immunoperoxidase technique and anti-*T. cruzi* serum we found at least one positive section for *T. cruzi* Ags in seven of the eight hearts studied in chronic chagasic patients who died of heart failure (Higuchi 1993). The septum was the site at which *T. cruzi* Ags were most frequently encountered. In another series of 24 hearts, examining only a single section of the septum, 58% of the sections were positive and showed an association between the presence of *T. cruzi* Ags and a moderate or severe inflammatory infiltrate. There was no correlation between the quantity of Ags and the intensity of inflammatory infiltrate since very few *T. cruzi* Ags were associated with a severe or moderate inflammation, favoring the idea that the parasite Ags function as a trigger initiating the hypersensitive response against the myocardial fibers. On the other hand, cases with many pseudocysts of amastigotes frequently exhibited a week inflammatory infiltrate, suggesting that the dissemination of the parasites is associated with a deficient immunological response.

Thus, the pathogenesis of chronic myocarditis in Chagas disease is, in our view, directly related to the presence of the parasite, although additional mechanisms may be involved. As proposed by De Brito in 1962, *T. cruzi* may function as an adjuvant of myocarditis having the myocardial fibers as the main trigger or the myocarditis occurring as the result of a cross-reaction between common parasite and myocardial fiber Ags.

#### THE PARASITE AND THE IMMUNOSUPPRESSION

It is known from acute experimental studies (Tarleton 1988) and from human (Cunningham et al. 1980) infection that *T. cruzi*, like other parasitic infections, induces alterations in the immunological system of the host to circumvent host defense mechanisms before, during and after entry into the host cells. It has been demonstrated that *T. cruzi* decreases the expression of the lymphocyte surface molecules CD3+, CD4+ and CD8+ (Sztejn et al. 1990) which may favor its own survival. We have demonstrated in myocardial biopsy fragments that chronic chagasic myocarditis is constituted mainly by T cells (96%), predominantly the CD8+ T cell (Higuchi et al. 1993); these findings have been observed by others (Reis et al. 1993). The CD4+ T cells were present in lower numbers and were mildly stained compared to the CD8+ T cells. We later demonstrated that the number of CD8+ T cells increased in the presence of scarce or abundant *T. cruzi* antigens while the number of CD4+ T cells remained unchanged (Higuchi et al. 1997). These findings reinforce the hypothesis that *T. cruzi* Ags play a fundamental role in the development of chronic myocarditis, and that a certain degree of immunosuppression is present in this phase of the disease, thus maintaining parasite survival within the host. Administration of IL-2 (Reed et al. 1984) restores the immune response in experimental *T. cruzi* infection. *In situ* quantitative analysis of cytokines present in the myocardium from chronic chagasic patients by immunohistochemical techniques also revealed a severe, immune depressed helper T cell response: IL2+ and IL4+ cells were present in very low numbers of lymphocytes; however the number of IL4+ cells increases in cases with abundant pseudocysts of *T. cruzi* amastigotes, suggesting that this cytokine, as seen in other infectious diseases, is related to the dissemination of the parasite. On the other hand, IFN $\gamma$ + lymphocytes were present in higher numbers, mainly in the groups of negative cases or those with scarce *T. cruzi* Ags, suggesting that this cytokine is related to the control of the infection (Reis 1995). In contrast, experimental data in mice (Spinella et al. 1990) show that CD4+ T cells and

the Th2 line are responsible for the control of parasite infection and that both may be involved in the autoimmune response.

#### THE FIBROSIS AND THE MICROCIRCULATORY ALTERATIONS

Microvascular alteration have frequently been cited as possible mechanism in the pathogenesis of fibrotic lesions human chronic chagasic cardiopathy (Torres 1947). The alterations have been described as microspasms (Factor et al. 1985), microthrombi (Rossi et al. 1984), dysfunction of endothelial cells and increased platelet activity (Morris et al. 1990). In chronic human Chagas' cardiopathy we have also found recent and non occlusive, organized thrombi, and lymphocytic vasculitis, mainly in hearts exhibiting severe myocarditis, together with the presence of *T. cruzi* Ags. However the frequency of these findings are low to explain the severity of diffuse fibrosis and some lesions like the aneurism of left ventricle apex.

We studied the extracellular matrix and microcirculation in chronic Chagas disease and idiopathic dilated cardiomyopathy (Higuchi et al. 1999). Chronic Chagas' cardiopathy appears to be similar to other congestive cardiomyopathies as regards remodeling of the extracellular matrix. However, on analyzing thick myocardial sections, we observed that the alterations to the extracellular matrix are different in Chagas' cardiopathy and idiopathic, dilated cardiomyopathy. In the latter, the main alteration is rupture of the lateral connections with probable slippage of the myocardial fibers which are thin and stretched. In Chagas disease, the most important feature is a dense extracellular collagen accumulation enclosing each fiber or group of myocardial fibers. The microcirculation in the Chagas disease, different of the dilated cardiomyopathy hearts, showed enlarged diameter of the arterioles and capillaries which may explain part of the main fibrotic lesions in chagasic hearts: the aneurismatic fibrotic lesions observed at the apex and basal posterior wall of the left ventricle. These are probably ischemic lesions caused by low blood pressure perfusion in the watershed zone between two main coronary artery branches – the anterior descending and posterior descending arteries; and the right coronary and circumflex arteries, caused by the severe dilatation of the arterioles. Some ischemic lesions may occur as a consequence of "steal" phenomenon which occur in myocardium submitted to microvessels dilation; some areas may be underperfused like sinus node, AV node, and Hiss bundle. These structures have blood supply by the arteries whose trajectories are in the opposite side of the main branch and consequently may have ischemic lesions due to steal

phenomenon (Fig. 1). The cause of this dilatation would be periodic recirculation of the parasites, delivery of Nitric Oxide, development of inflammation and cytokines.

Summarizing, in our view, human chronic Chagas' cardiopathy is the result of a close interaction between the host and the parasite. Patients with a good immunological response may adequately circumvent the parasitic infection. Disturbance of the immunological response certainly plays a role in the chronic form of Chagas disease as noted by early investigators, and is responsible for the inadequate response by the host, leading to the persistence of the parasite and/or its products which are able to induce cross reactions between the myocardial fibers and the parasite. On the other hand, as in other parasitic infections, the microorganism is able to interfere with the immunological system of the host to protect itself and, at the same time, to facilitate its reproduction and propagation. The inflammatory response, which is probably recurrent, undergoing periods of more accentuated exacerbation, is most likely responsible for neuronal damage, microcirculatory dilatation, heart matrix deformities and consequent organ failure.

In our view, heart lesions in the chronic phase of Chagas disease are dependent on an exacerbated immunological response by the host against the parasite, causing injury to the myocardium. The indeterminate form of the disease is probably related to a more efficient immunoresponse against *T. cruzi*, fewer *T. cruzi* parasites, less myocardial

inflammation, less microvessels abnormalities and consequently fewer complications.

An scheme proposing the interactions of the main factors involved in chronic chagasic cardiopathy is provided (Fig. 2).

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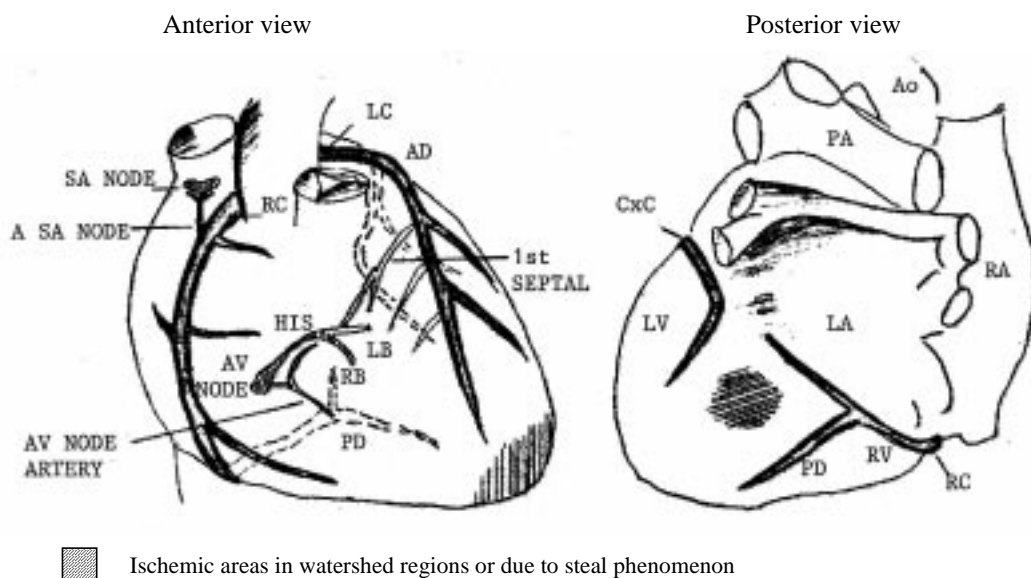


Fig. 1: schematic representation of ischemic lesions in chagasic hearts.

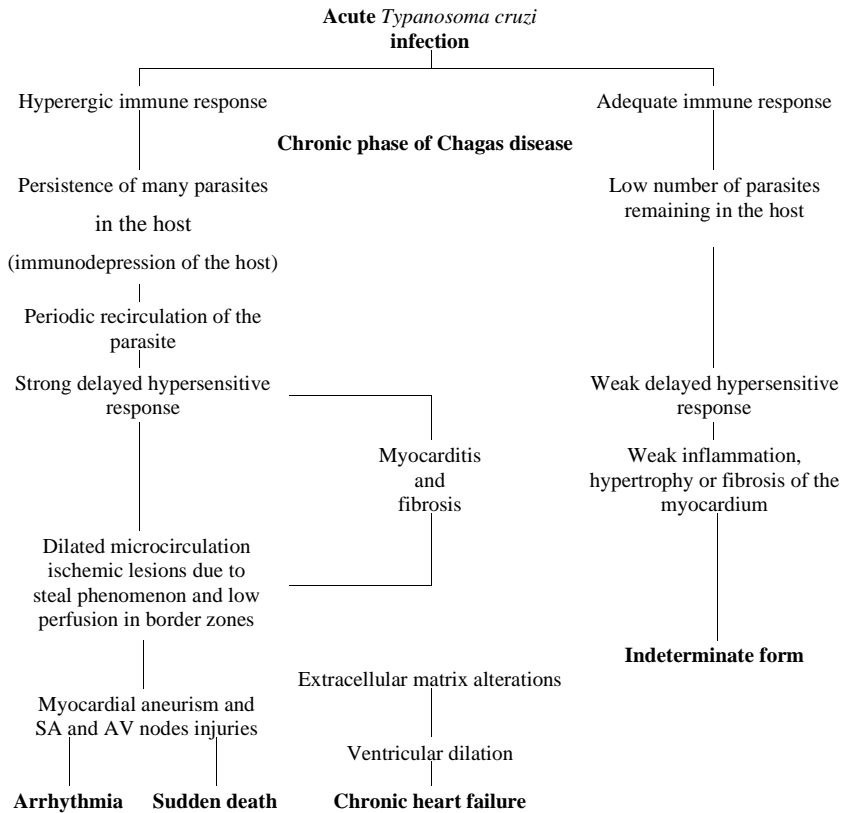


Fig. 2: representation of how the main pathogenetic factors are participating in the development of chronic Chagas' heart disease.

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