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INVITED REVIEW
CHRONIC CHAGASIC CARDIOPATHY: THE PRODUCT OF A TURBULENT HOST-PARASITE RELATIONSHIP.

Maria de Lourdes HIGUCHI(1)

SUMMARY

The pathogenesis of chronic chagasic cardiopathy is still a debated matter. In this review, the main theories raised about it since the first description of the disease in 1909 by Carlos Chagas, are considered. The scarcity of *T.cruzi* parasites into the myocardium and the apparent lack of correlation between their presence and the occurrence of myocardial inflammatory infiltrate, have originated many theories indicating that chronic Chagas' cardiopathy is an autoimmune disease. Recently however, papers using immunohistochemical technique or PCR have demonstrated a strong association between moderate or severe myocarditis and presence of *T.cruzi* Ags, indicating a direct participation of the parasite in the genesis of chronic chagasic myocarditis. Different patterns of cytokine production seem to have important role in the outcome of the disease. Participation of the microcirculatory alterations and fibrosis as well as the relationship with the parasite are also emphasized. Finally, the author suggests that the indeterminate form of the disease occurs when the host immunological response against the parasite is more efficient while the chronic cardiopathy occurs in patients with hyperergic and inefficient immune response.

KEYWORDS: Chronic chagasic cardiopathy; Review.

INTRODUCTION

Chagas' disease, or American trypanosomiasis is endemic throughout Latin America. According to WHO, currently around 15 million people are infected with the disease which remains an important public health problem in this part of the world[22]. At present, chronic Chagas' disease is still incurable and only some of the infected patients exhibit late clinical manifestations. Why most remain assymptomatic during life, dying of other causes (indeterminate form) while the rest present a complicated outcome, frequently leading to death, is an unsolved question. The pathogenesis of chronic Chagas' heart disease is apparently very complex, involving many interrelated factors, as we will come to see in this review.

HISTORICAL ASPECTS

The etiopathology and clinical characteristics of the disease were first described by CHAGAS[13] and VIANNA[92] at the beginning of this century. The acute phase of Chagas' disease is characterized by the proliferation of *T. cruzi* parasites and their dissemination by the blood or lymphatic vessels throughout the organism, affecting nearly almost all cell types, although preferentially muscle fibers, associated with severe inflammation. In 1916, CHAGAS[14] described certain differences between the acute and chronic forms of the disease, emphasizing the high degree of cardiac involvement and myocardial parasitism, mainly in the acute form of the disease. CHAGAS pointed out that the parasites were also responsible for the myocarditis in the chronic form and that, since they are fewer in number than in the acute phase, they could not be detected in histological sections. He also emphasized that fibrosis was an important feature of the chronic form and probably responsible for cardiac arrhythmia. Different morphological and clinical aspects have been better described by more recent authors[9,46,53].

In chronic Chagas' disease, the severe myocardial fibrosis and disproportionate myocardial inflammation in view of the lack of *T. cruzi* parasites, led to the proposals of other theories of pathogenesis. In 1929[88] and also 1941[89] TORRES argued that chronic chagasic myocarditis was based on an "allergic" mechanism and postulated that the inflammatory process was an active, progressive myocarditis resulting from the continued action of

(1) Departamento de Patologia, Instituto do Coração, Av. Dr. Enéas de Carvalho Aguiar 44, 05403-000 São Paulo, SP, Brasil.

the parasite associated with an "allergic" state of the host. Further research, however, failed to demonstrate such an "allergic" state by cutaneous tests[59, 65]. However, MUNIZ & PENNA in 1947[60] believed they had provided experimental evidence for such a hyperergic mechanism through the demonstration of myocarditis and granulomas in the pleura after the direct inoculation of *T.cruzi* antigens in monkeys previously treated with endovenous injections of *T. cruzi* lysates. MAZZA[52] and MAZZA & MIYARA[55] reported human cases of chronic Chagas' disease with allergic cutaneous manifestations which they termed "esquizotripanides" while MAZZA & JORG induced a Schwartzman phenomenon in dogs by injecting *T. cruzi* products[54]. ANDRADE & ANDRADE[5] and TORRES[17] postulated that the myocardium of chronic chagasic patients would react hyperergically to the presence of parasites since they found very few parasite pseudocysts, disproportionate to the inflammatory process. Reinforcing this idea, DE BRITO in 1962[20] produced an allergic myocarditis by injecting homogenate of the myocardium associated with *T. cruzi* fragments as an adjuvant. He postulated that chronic chagasic myocarditis was a hyperergic reaction against myocardial fibers activated by *T. cruzi* antigens which functioned as an adjuvant.

This line of research, studying the mechanisms by which the parasite induced chronic myocarditis, suffered a marked change with KÖBERLE's series of works[41,42] which presented an original and attractive idea: that chronic, chagasic cardiopathy was a neuronal cardiopathy, as myocardial inflammation was not always present and it did not explain the severe hypertrophy present in almost all cases. This idea was based on the theory current at the time concerning the pathogenesis of megaesophagus and megacolon in Chagas' disease where denervation of the parasympathetic autonomous system is the basic mechanism explaining the dilation of these organs[35,43,44]. According to KÖBERLE, a diminished number of parasympathetic cardiac ganglion cells (as demonstrated by many authors[7,45, 64]) with exacerbation of the sympathetic action would explain the myocardial hypertrophy and dilation of the ventricles. Fibrosis would result from hypoxic lesions due to coronary underperfusion. The number of neurons in the atrial myocardium of chagasic patients is diminished compared to normal hearts[41]. ORIA & RAMOS had previously found significant lesions of the autonomic nervous system in the hearts of chagasic patients and correlated these lesions with electrocardiographic alterations found in the patients[64]. KÖBERLE did not believe that the parasites played a significant role in the development of chronic myocarditis. Neuronal lesions were initially explained by the action of a neurotoxin derived from the parasites, mainly during the acute phase of the disease. This theory caused great impact at the time, leading to quite a number of investigations on the subject. OLIVEIRA[62] considered chagasic heart disease to be a catecholaminogenic cardiopathy as he had induced myocardial hypertrophy with an apical lesion by the administration of high doses of catecholamines in rats. Other studies were performed which favored the theory of cardiac denervation in the pathogenesis of the disease[37, 63]. However, the *T. cruzi* neurotoxin has never been confirmed and many studies demonstrated the absence of correlation between lesions of the parasympathetic ganglia and cardiac alterations in chagasic patients[3,48,51,81]. RIBEIRO DOS SANTOS described an anti-neuron antibody in experimental Chagas' disease, and suggested that auto-immunity against neurons would be the mechanism of neuronal destruction, occurring mainly during the acute phase, but also in the chronic phase of the disease[70]. Until now, however, the real meaning of neuronal depletion in the cardiac ganglia is still a matter of debate; recent studies have not identified neuronal depletion in the heart, only slight neural damage which the authors conclude had occurred as an epiphenomenon of many changes affecting the chronic chagasic heart, mainly inflammation and fibrosis [19,74]. Favoring this argument, previous works have demonstrated that degenerative lesions of neurons in Chagas' cardiopathy seemed to be dependent on the inflammatory infiltrate[6,51] which disappeared when anti-inflammatory drugs were administrated[6].

RECENT FINDINGS

New techniques involving molecular biology and the use of endomyocardial biopsy have examined new aspects of the pathogenesis of chronic chagasic cardiopathy. Pathological studies in necropsies of patients suffering sudden or accidental death had suggested that hearts of patients in the indeterminate phase of chronic Chagas' disease exhibit similar but less intense histological alterations when compared to hearts from chagasic patients with heart failure[49,50]. The analysis of endomyocardial biopsies from patients in different clinical stages of the disease (indeterminate, cardiac without heart failure, and cardiac with heart failure) demonstrated that the inflammation is more frequent and more severe in the group with heart failure. Severe fibrosis and myocardial fiber hypertrophy were seen only in the groups with cardiac alterations. These findings suggest that chronic chagasic cardiopathy is a progressive, fibrotic disease, and that the myocardial inflammation plays a fundamental role in development of cardiac failure[10,12,30], also probably causing neuronal injury. The pathogenesis of the inflammatory infiltrate, therefore, is the main doubt to be clarified.

Autoimmunity, both humoral and cellular were suggested first. An immune response against *T. cruzi* antigen/s would induce cross immunoreactions against the myocardial structures, resulting in autoimmune myocarditis. This line of research began with COSSIO et al.[15] who described an antibody present only in chagasic patients. Using indirect immunofluorescence in sections of myocardium and skeletal muscle, these investigators observed that the antibody, termed the EVI factor, reacted against the endocardium, vessels and interstitium. It was present in almost 100% of patients with chronic chagasic cardiopathy, in 40% of assymptomatic chagasic patients and absent from normal individuals or patients with other diseases. The presence of immunoglobulins in the myocardial biopsy from a cardiac chagasic patient provided evidence of a pathogenetic role for the EVI factor in chagasic cardiopathy[16]. In contrast to another study[56], however, the immunofluorescence findings in myocardial biopsies from chronic chagasic cardiac patients, did not demonstrate immunoglobulins, fibrinogen or complement (C3) in frozen sections, indicating that, if the EVI factor is present, it probably does not participate directly in the genesis of myocarditis[32]. The same authors who described the EVI factor were unable to reproduce

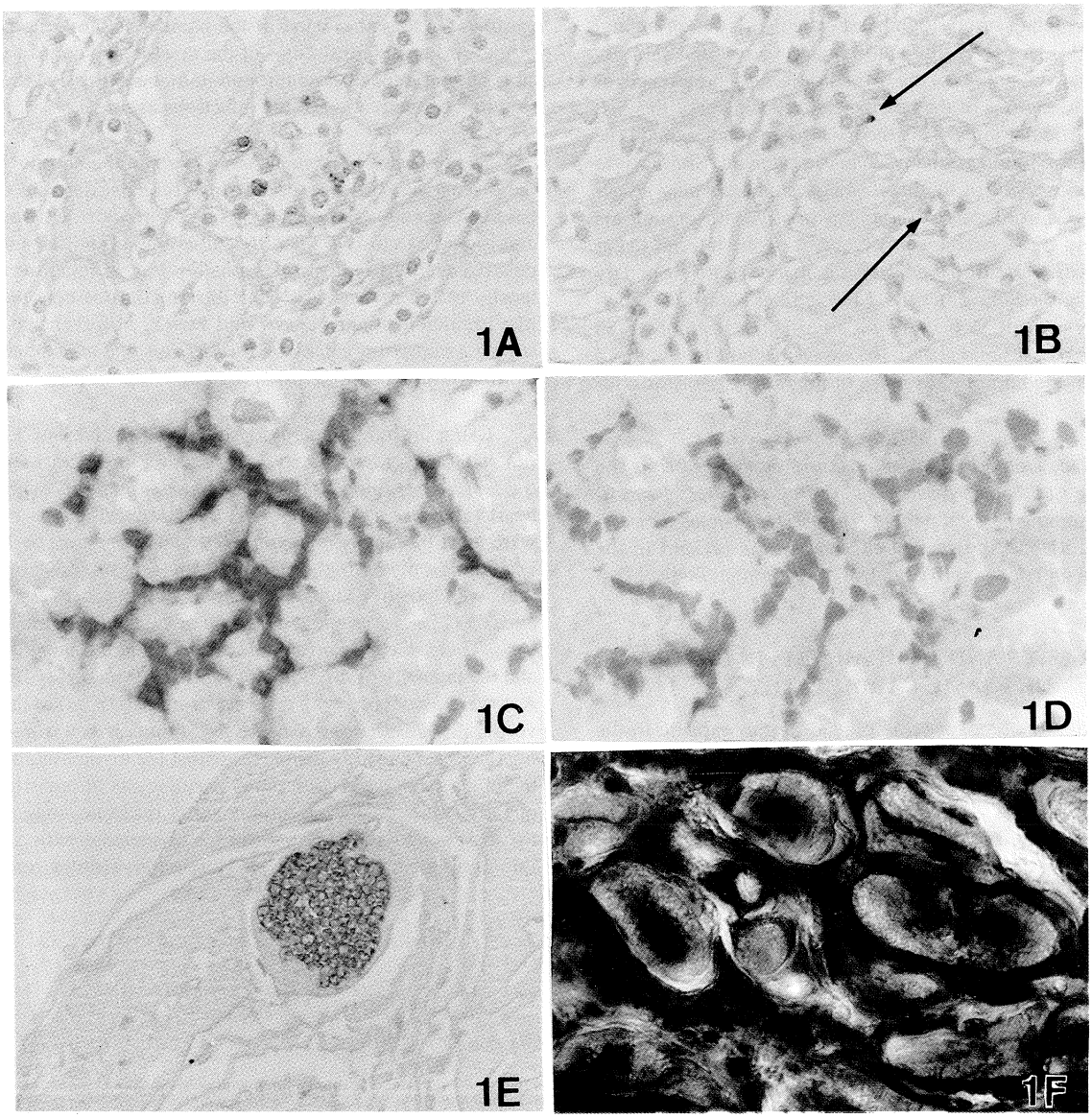

Fig. 1 – Microscopic views of human chronic chagasic myocarditis. A) Anti-*T.cruzi* immunoperoxidase stained slide exhibiting many amastigotes or fragmented *T.cruzi* Ags within a severe inflammatory infiltrate (400x). B) Anti-*T.cruzi* immunoperoxidase procedure showing two positive structures (arrows) in severe myocardial inflammation (630x).C) Anti-CD8+ immunoperoxidase procedure exhibiting numerous, dark brownish lymphocytes surrounding myocardial fibers. D) Anti-CD4+ immunoperoxidase technique demonstrating scarce and mildly stained lymphocytes. E) An intramyocytic pseudocyst of amastigotes stained by anti-*T.cruzi* serum, not eliciting an inflammatory reaction (400x) F) Del Rio Hortega technique demonstrating severe, dense collagen surrounding each myocardial fiber (400x).

their previous results, concluding that the factor probably has no pathogenetic role[40]. Examining cellular autoimmunity, several studies by TEIXEIRA et al.[79,85,86] experimentally demonstrated that chronic *T. cruzi* infection may induce the appearance of lymphocytes showing cytotoxic activity against myocardial fibers, but not against allogeneic non-cardiac cells. These authors were able to produce chagasic myocarditis by injecting several doses of subcellular antigens of *T. cruzi* in rabbits. These experiments strongly suggested mechanisms of cellular autoimmunity and delayed hypersensitivity to *T. cruzi* antigens in the pathogenesis of Chagas' disease.

Many studies have demonstrated common antigens between *T. cruzi* and human myocardial fibers thus supporting theories of autoimmunity [17,47,77,78]. 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[1,71] 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. This theory also does not explain the digestive lesions found in many chagasic patients. Similarly to the situation observed in the heart, megaesophagus may be accompanied by chronic myositis, occasionally with granuloma formation, and the secondary involvement of the intermuscular neuronal plexuses. Neuronal depletion is marked with secondary proliferation of the Schwann cells. Muscle fiber parasitism, however, is rarely present. A necrotizing arteritis has been observed in a few human cases and also in the experimental disease, probably related to the humoral response of the host to parasitic antigen/s[2,21,71].

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 MYOCARDITIS IN CHRONIC CHAGASIC CARDIOPATHY

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 and assist in the development of vaccines as defended by some authors. If the myocarditis is an autoimmune process independent of the presence of the parasite, administration of a vaccine may also induce myocarditis. On the other hand, if the parasite is the principal cause of the 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 presence of *Trypanosoma cruzi* in the chronic phase of the disease has been observed since the first descriptions[91] and has been recently emphasized by other authors[4,87]. However, even employing exhaustive histological examination, the number of positive sections is disproportionately low in relation to the intensity of the myocarditis. The introduction of new techniques like immunohistochemistry[29] and PCR[38], however, 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 7 of the 8 hearts studied in chronic chagasic patients who died of heart failure[29]. 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 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 hypersensitivy response against the myocardial fibers. On the other hand, cases with many pseudocysts of amastigotes frequently exhibited a weak inflammatory infiltrate, suggesting that the dissemination of the parasites is associated with a deficient immunological response. Experimentally, similar results have

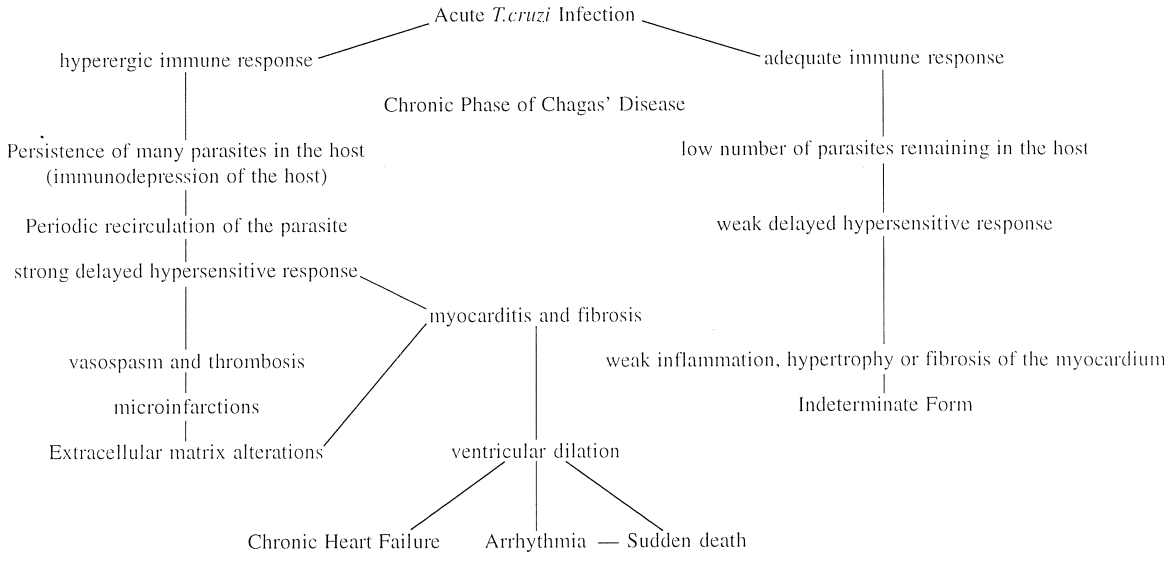

Fig. 2 – Schematic representation: the main pathogenetic factors involved in chronic Chagas' heart disease

been found in mice[95] and guinea pigs[26]. Reinforcing the significance of the parasite in the development of chronic myocarditis, JONES et al.[38] demonstrated a higher incidence of *T. cruzi* DNA by the PCR technique in myocardial fragments exhibiting significant inflammation. Presence of *T. cruzi* antigens was also detected in "in vivo" myocardial tissue.[11]

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 parasite and myocardial fiber antigens[20]. In accordance with this idea, amino acid sequences very similar to those in myosin, were recently demonstrated in *T.cruzi*,[17]. This finding may also explain the tropism of the parasite for the muscle fibers where it establishes a definite, chronic foothold since the similarity of the amino acid sequence may facilitate the incorporation and multiplication of the the parasite.

THE PARASITE AND IMMUNOSUPPRESSION IN CHRONIC CHAGAS' DISEASE

It is known from acute experimental studies[28,66,84] and from human[18,92] 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[27]. It has been demonstrated that *T. cruzi* decreases the expression of the lymphocyte surface molecules CD3+, CD4+ and CD8+[82] 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[31]; these findings have been observed by others[56,68]. The CD4+ T cells were present in lower numbers and were mildly stained compared to the CD8+ T cells. In this study we compared chagasic myocarditis with the myocardial rejection process. Considering that Chagas' myocarditis is an autoimmune process in which lymphocytes attack the myocardial fibers of the host, it would be expected that the lymphocyte populations are similar to those in myocardial rejection where the myocardial fibers are seen as non-self cells by the lymphocytes. However, the number of CD4+ T cells and the intensity of their staining by immunohistochemical techniques were diminished in Chagas' myocarditis compared to the myocardial rejection process[92]. 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[33]. 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[67] 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 severely, 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, IFNγ+ 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;[69] In contrast, experimental data in mice[72,80] 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 PARASITE, FIBROSIS AND MICROCIRCULATION

The classic report of TORRES[89] and later works by other authors[5,39] have emphasized fibrosis and the microvascular alterations in the chronic cardiac form of Chagas' disease. Recent experimental works on acute Chagas' disease have demonstrated microvascular alterations manifested as microspasms[23], microthrombi[75], dysfunction of endothelial cells and increased platelet activity[58,76] all of which may play an important role in the further development of myocytolysis and fibrosis. 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* antigen/s[34]. These phenomena seem to be directly related to the presence of the parasite. It has been shown that endothelial cells infected with *T.cruzi* display higher platelet adherence and aggregation[83]. The neuraminidase produced by the parasite may explain this complication since it removes sialic acid components from the endothelial surface, favoring linkage with thrombin. *T.cruzi* amastigotes have been demonstrated to accumulate large amounts of C3 by products and C5b-9 on their surface although not inserted into the lipid layer, explaining why the amastigotes are resistant to destruction by complement action. These complement by products may also favor thrombosis[36]. This alteration would also promote the formation of cardiac mural thrombi, and consequently, thromboembolic phenomena. Additionally, when the parasite is present in the extracellular matrix, it may induce fibrogenesis[94]. On the other hand, *T. cruzi* has collagenolytic and proteolytic properties[24] which may destroy the extracellular matrix, leading to cardiac remodeling and heart failure. A complex network of fibrillar collagen enveloping each myocardial fiber and tethering one to the other plays a major role in the maintenance of normal shape and efficient contraction of the heart[73,93]. Chronic Chagas' cardiopathy appears to be similar to other congestive cardiomyopathies as regards remodeling of the extracellular matrix [25,58]. However, on analyzing thick myocardial sections, we observed that the alterations of 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, probably preventing their normal distension and contraction. The lateral connections are preserved within the groups of cardiac fibers which usually are severely hypertrophic (HIGUCHI et al., unpublished data).

Summing up, it appears that in Chagas' disease there is a close interaction between the host and the parasite. Genetic factors are also probably important in the outcome of the infection. 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, when antigenic, 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 alterations, heart matrix deformities and consequent organ failure.

In our view, heart lesions in the chronic phase of Chagas' disease are dependent on an exaggerated 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*, less myocardial inflammation and consequently fewer complications such as fibrosis, thrombosis and necrosis.

An attempt to schematize the main factors involved in chronic chagasic cardiopathy (figure 2) is provide below.

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RESUMO

Cardiopatia crônica chagásica: o produto de uma interação parasita-hospedeiro turbulenta

A patogênese da cardiopatia crônica chagásica ainda é assunto controverso. Na presente revisão as principais teorias patogenéticas propostas, desde a descrição da doença por Carlos Chagas em 1909, são abordadas. A escassez de parasitas do *T.cruzi* no miocárdio e a aparente falta de correlação entre sua presença e a ocorrência de infiltrado inflamatório no miocárdio originaram várias teorias de autoimunidade na patogênese da cardiopatia crônica chagásica. Entretanto, trabalhos recentes têm demonstratado a presença de Ags do *T.cruzi* em associação com infiltrado inflamatório chagásico através de técnicas de imunoperoxidase e de PCR, sugerindo fortemente uma participação direta do parasita na gênese dessa miocardite. Diferentes padrões de produção de citocinas parecem desempenhar importante papel na evolução da doença. Ressalta-se também a possível participação do parasita em lesões da microcirculação e fibrose. Finalmente, o autor sugere que a forma indeterminada ocorre em pacientes cuja resposta imunológica contra o *T.cruzi* é mais efficente, enquanto que pacientes com cardiopatia crônica são aqueles com resposta contra o parasita ineficiente e hiperérgica.

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