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Review

Pathophysiology of the heart in Chagas' disease: current status and new developments

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Abstract

In the present review we have summarized remarkable historical data on Chagas' disease studies putting special emphasis on histopathological findings and pathogenetic theories as well as recent discoveries based on the use of advanced modern technologies in pathology and immunology. A unified theory that links almost all of these findings is proposed. Chronic cardiac Chagas' disease represents the result of a close interaction between the host and the parasite, causing different clinical pictures: patients with an efficient immune response may adequately circumvent the parasitic infection and the individual will develop the indeterminate form. Deficient immune response of the host and/or a high initial parasitemia favor an immune imbalance that might lead to development of a permanent inadequate immunological response against the parasite. The inflammatory response, which is probably recurrent, undergoing periods of more accentuated exacerbation, is most likely responsible for progressive neuronal damage, microcirculatory alterations, heart matrix deformations and consequent organ failure.

Keywords: Chagas'disease; Fibrosis; Myocarditis; Cardiomyopathy; Histopathology

1. Introduction

Chagas' disease, caused by the hemoflagellate protozoan *Trypanosoma cruzi*, is a widespread disease that affects millions of people in South and Central America. The incidence of the disease varies from country to country, basically related to the implantation of national control programs to exterminate the domestic vector of *Trypanosoma cruzi*. Even though, according to the Word Health Organization, 16–18 million people are infected by the parasite in South America, and another 90 million are at risk of becoming infected. In Brazil, the prevalence is around 4%, corresponding to 6 million people [1]. Chronic chagasic cardiopathy (CCC) is the most devastating manifestation of Chagas' disease, affecting about a third of the infected people. Despite this obvious clinical importance the pathogenesis of CCC is still poorly understood. Chronic Chagas' disease is still an incurable disease, but only some of the infected patients exhibit late clinical manifestations. Most of the contaminated people remain asymptomatic (indeterminate form) and around 30% of them present cardiac and digestive complications, in a late phase of the disease. Patients presenting cardiopathy usually show progressive heart failure, cardiac arrhythmias or both. The pathogenesis of chronic Chagas' heart disease involves many interrelated factors that will be summarized in this review.

2. Outstanding historical facts

The first descriptions of the etiopathology and clinical characteristics of Chagas' disease were provided at the beginning of the last century by Chagas [2] and Vianna [3]. According to these authors, the acute phase of the disease is characterized by high numbers of *T. cruzi*

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parasites in the blood or lymphatic vessels, parasitism of almost all cell types, preferentially muscle fibers and inflammation. Carlos Chagas [4] pointed out differences between the acute and chronic forms of the disease, emphasizing the high degree of cardiac involvement with severe myocardial parasitism during the acute phase and low number of parasites associated with the myocarditis in the chronic form. Several early studies on Chagas' disease [5–7] have already emphasized the scarcity of parasites in histological sections during the chronic form as well as the presence of severe myocardial fibrosis.

In 1929 [8] and 1941 [9], Torres argued that chronic chagasic myocarditis was a result of an active and progressive myocarditis due to a continued action of the parasite associated with an 'allergic' state of the host. Some authors failed to demonstrate such an 'allergic' state by cutaneous tests [10,11] but Muniz and Penna in 1947 [12] provoked myocarditis and granulomas in the pleura by inoculating *T. cruzi* antigens in monkeys previously treated with endovenous injections of *T. cruzi* lysates. Mazza [13] and Mazza and Miyara [14] reported human cases of chronic Chagas' disease with allergic cutaneous manifestations while Mazza and Jorg [15] induced a Schwartzman phenomenon in dogs by injecting *T. cruzi* products. Andrade and Andrade [16] and Torres [9] postulated that chronic chagasic patients would react hyperergically to the presence of parasites.

The papers by Köberle and co-workers [17,18] had an outstanding influence on future strategies in Chagas research, by proposing an original and attractive idea: CCC should be considered as a neuronal cardiopathy that primarily affects the parasympathetic autonomous system. According to this hypothesis, megaesophagus, megacolon and cardiac dilation in Chagas' disease are consequences of the denervation of the parasympathetic autonomous system, whereas the myocardial inflammation should not be considered as an important element for cardiac failure [19–21]. Several authors have demonstrated [17,22–25] a diminished number of parasympathetic cardiac ganglion cells in chagasic hearts. Koberle [26] proposed that exacerbation of the sympathetic action would cause myocardial hypertrophy and dilation of the ventricles. In rats, high doses of catecholamines induced a dilated cardiopathy with an apical lesion [27]. A proposed *T. cruzi* neurotoxin has never been confirmed and a lack of correlation between parasympathetic ganglia lesions and cardiac alterations in chagasic patients has kept such theory in check [28–30]. Auto-immunity against neurons has also been suggested [31]. Recent studies did not identify neuronal depletion in the heart, only slight neuronal damage. The authors concluded that such neuronal lesions occur as an epiphenomenon in a heart presenting several pathological alterations, mainly inflammation and fibrosis [32,33]. Favoring such a point of view, degenerative lesions of neurons in CCC have been found to be dependent on the inflammatory infiltrate [30,34].

3. Knowledge obtained from endomyocardial biopsies

Endomyocardial biopsies from patients in different clinical stages of Chagas' disease have provided new insights on some important questions, in particular on the inflammatory process and the development of heart failure. An early autopsy study had suggested that inflammatory infiltrate was also present in patients presenting the indeterminate form of Chagas' disease [35]. However, more recent biopsy studies have outlined that patients with heart failure presented higher percentages of severe myocarditis, fibrosis and myocardial hypertrophy compared to patients in indeterminate and cardiac arrhythmic forms. According to these observations, CCC may be considered a progressive, fibrotic disease in which myocardial inflammation plays a fundamental role [36–38]. The microscopic characteristics of the myocardium in chronic chagasic hearts are shown in Fig. 1. Comparison of endomyocardial biopsies in acute and chronic phases demonstrated that patients in acute phase present 100% of myocarditis and 58% of *T. cruzi* antigens, whereas in patients in the chronic phase these values were reduced to 45% of myocarditis and 0% of *T. cruzi* antigens [39]. Another finding of this study was that the number of CD4+ T cells increased in parallel to the number of CD8+ T cells ($r=0.91$) in the acute phase but not in the chronic phase ($r=0.42$), suggesting an immunologic imbalance response in the late phase of the disease. In the chronic phase, patients with heart failure present a predominance of CD8+ T cells, with a CD4+/CD8+ T cell ratio of 0.8. The lack of parasite antigens in biopsy material from patients in the chronic phase is expected as, according with which we will describe latter, it seems necessary to examine several different sections of the heart to detect the parasite in this phase of the disease.

4. The parasite versus autoimmunity

The relative lack of parasites in the myocardium during the chronic phase originated many autoimmune theories, of both humoral and cellular origin. Cossio et al. [40] described a serum antibody in chagasic patients that reacted against myocardial and skeletal muscle, endocardium, vessels, and interstitium. This antibody was called EVI factor and was found in almost 100% of patients with CCC, whereas it was absent in normal individuals or in patients with other diseases [41]. However, immunoglobulins were not detected in myocardial biopsies from patients presenting CCC, indicating that the EVI factor probably does not participate directly in the genesis of myocarditis [42,43]. On the other hand, there is evidence for the participation of antibodies in some lesions. A necrotizing arteritis has been observed in humans and in experimental chagasic disease, probably related to the humoral response of the host to parasitic

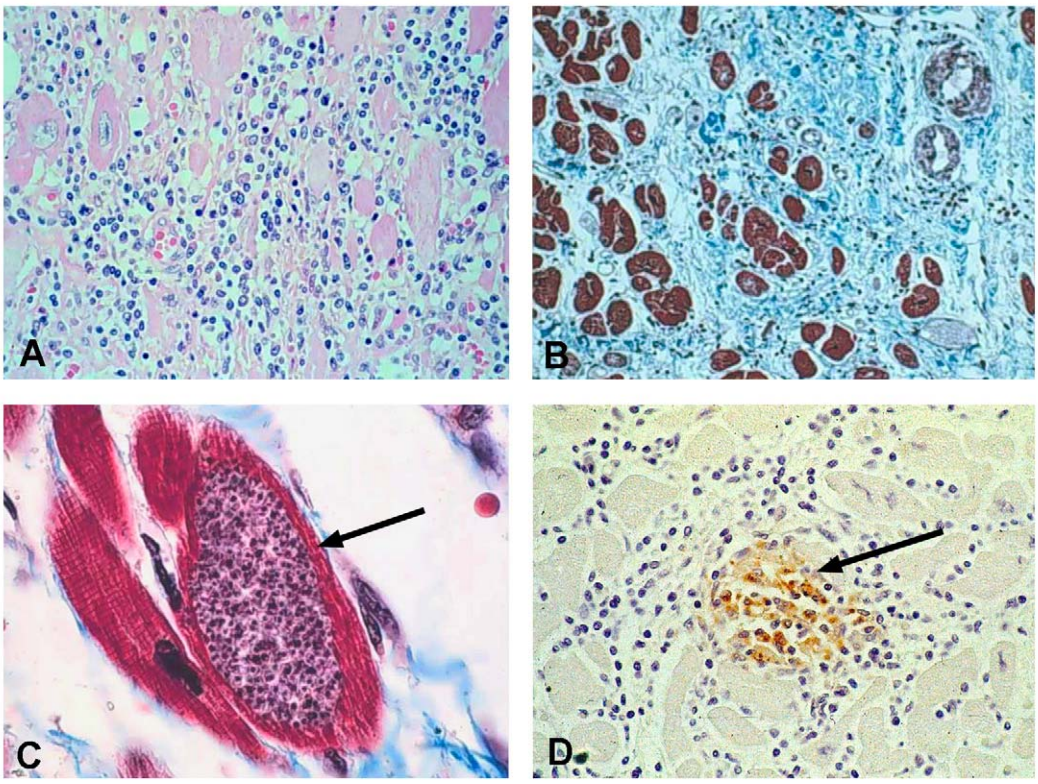

Fig. 1. Microscopic images demonstrating the main histopathological characteristics of chronic chagasic cardiopathy. (A) Severe myocarditis with many lymphocytes aggressing non-infected hypertrophic myocytes (H&E staining, objective magnification: 40×). (B) Diffuse myocardial fibrosis, involving each myocardial fiber and focal dense fibrosis associated with thickened arterioles, suggestive of ischemic injury (Masson trichrome staining, objective magnification: 20×). (C) A whole myocardial fiber containing a large pseudocyst of *T. cruzi* (arrow), that did not elicit inflammatory reaction (Masson trichrome staining, objective magnification: 100×). (D) Macrophages containing antigens of *T. cruzi* (arrow) in the middle of a granulomatous arrangement (immunoperoxidase technique, objective magnification: 40×).

antigens [44,45]. A growing number of reports have suggested a pathogenic role of antibodies present in the serum of chagasic patients that reacts against muscarinic and adrenergic receptors of cardiomyocytes [46,47]. The production of autoantibodies against β-adrenoreceptors and other receptors may interfere with cardiac and mechanical activities of myocardial cells [48–50].

Teixeira and co-workers [51–53] demonstrated that chronic *T. cruzi* infection induces the appearance of lymphocytes with specific cytotoxicity against myocardial fibers. Myocarditis was obtained by injecting several doses of subcellular antigens of *T. cruzi* in rabbits, suggesting cellular autoimmunity and delayed hypersensitivity to *T. cruzi* antigens. Common antigens between *T. cruzi* and human myocardial fibers, auto-reactive T cells specific to heart or nerve tissue antigens have been demonstrated in experimental animals and patients [54–59]. These autoimmune theories suggest that the myocarditis perpetuates independently of the presence of the parasite [60,61]. An interesting finding in favor of such a theory was obtained by Ribeiro-dos-Santos et al. [62] using a heterotopic heart transplant model. They transplanted syngeneic hearts of normal mice in ears of *T. cruzi* infected mice, resulting in rejection of the transplanted hearts by CD4+ T cells. However, a similar experiment performed by others did not result in graft rejection except when parasites could be found in the heart graft [63].

In our view, only autoimmunity is not sufficient to explain the multifocal nature of the myocarditis and the preferential location of fibrosis in certain regions such as the apical or the posterior left ventricular wall in CCC. Moreover, frequent positive xenodiagnosis during the chronic phase of Chagas' disease and during episodes of reactivation in immunocompromised patients (by AIDS, neoplasia or cardiac transplant) has provided evidence that the parasite is present under active control of the immunological system of the host in the chronic phase [64,65].

Knowledge of the exact role of the parasite in the pathogenesis of CCC appears extremely important to guide potential therapeutic strategies. If chronic chagasic 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 persistence of the parasite is the principal cause of the late cardiac manifestations of the disease, the control of a possible autoimmune disease through immunodepressive

drugs, may lead to reactivation of the infectious agent. In this hypothesis, the elimination of the parasite should be the primary focus of any therapeutic intervention.

The presence of *Trypanosoma cruzi* in the chronic phase of the disease has been already observed in early descriptions [3] and has been emphasized later by other authors [66,67]. Nevertheless, the number of parasites is disproportionately low in relation to the intensity of the myocarditis and whole myocardial fibers containing parasites do not elicit inflammation (Fig. 1C). Recent studies using modern techniques such as immunohistochemistry and polymerase chain reaction (PCR) have demonstrated higher frequencies of *T. cruzi* antigens (Ags) in CCC, providing evidence that the presence of *T. cruzi* Ags are indeed associated with myocardial inflammation (Fig. 1D). *T. cruzi* Ags were detected in 100% of hearts from chronic chagasic patients that died due to heart failure when several samples of the myocardium were analyzed [68,69]. In these studies, we observed no direct correlation between the amount of *T. cruzi* Ags and the intensity of the inflammatory infiltrate but a significant association between the presence of *T. cruzi* Ags and severe or moderate inflammation. Parasite Ags probably work as a trigger for the hypersensitive response against the myocardial fibers. Experimental studies in mice [70] and guinea pigs [71] have provided similar results. Jones et al. [72] have described a high incidence of *T. cruzi* DNA using the PCR technique in myocardial fragments exhibiting significant inflammation. *T. cruzi* antigens were also detected in myocardial biopsies [73].

Thus, in agreement with others [74], we believe that the pathogenesis of chronic myocarditis in Chagas' disease is directly related to the presence of the parasite, although additional immunological mechanisms are probably involved. De Brito [75] proposed that *T. cruzi* might function as an adjuvant for an immunological cross-reaction between common parasitic and myocardial fiber antigens, resulting in severe lymphocytic myocarditis.

5. Immunosuppression, cytokines, adhesion molecules and MHC antigens

Acute experimental studies [76] and reports in humans [77] have provided convincing evidence that *T. cruzi,* like other parasitic infective agents, induces alterations in the immunological system of the hosts to circumvent their defense mechanisms before, during and after entry into their cells. *T. cruzi* decreases the expression of the lymphocyte surface molecules CD3+, CD4+ and CD8+ [78] favoring its own survival. Studies on myocardial biopsy fragments from patients with chronic chagasic myocarditis have demonstrated that the inflammatory infiltrate was mainly composed of T cells, with a predominance of CD8+ T cells [79,80]. CD4+ T cells were present in lower numbers and were only mildly stained compared to CD8+ T cells. 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 [81]. 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 during this phase of the disease.

Administration of IL-2 [82] restores the immune response in experimental *T. cruzi* infection. In situ quantitative analysis of cytokines present in the myocardium of chronic chagasic patients by immunohistochemical techniques also revealed a severe, immune depressed helper T cell response: Very few lymphocytes stained positively for IL-2+ and IL4+; however the number of IL4+ cells increased in cases with abundant pseudocysts of *T. cruzi* amastigotes, suggesting that this cytokine, as 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 those groups of patients, in which *T. cruzi* Ags were absent or scarce, suggesting that this cytokine is related to the control of the infection [83]. It seems that Th2 response favors the permanence of the parasite in the myocardium in human chronic chagasic disease. In contrast, experimental data in mice [84] showed that CD4+ T cells and the TH2 subset are responsible for the control of parasitic infection and that both may be involved in the autoimmune response.

Macrophages are active cells for the control and killing of parasites by oxidative and non-oxidative mechanisms. On the other hand, macrophages may also serve as host cells that facilitate the replication and survival of pathogens. IL-10 is a cytokine that presents immunosuppressive functions by down-regulating the macrophage production of IFN-γ, NO and by decreasing expression of class II MHC antigens. Neutralization of IL-10 increases the resistance to infection with certain pathogens, including *T. cruzi*. Despite an early resistance, IL-10-deficient mice died within the third week of infection, whereas all control mice survived acute infection with a toxic-like syndrome, possibly mediated by systemic TNF-α overproduction [85,86]. This represents a highly interesting new field in research that deserves future studies. Some interesting gender differences have been observed in patients with CCC. Usually, women seem to present a better clinical outcome than men [87]. In an autopsy study of chagasic patients that died due to severe heart failure, we found a significant difference between men and women regarding the composition of the myocardial inflammatory cells [88].

Adhesion molecules are very important for the development of inflammation. Although the normal myocardium presents ICAM-1 on endothelial cells, we detected an up-regulation of this molecule and induction of VCAM-1 expression on capillaries and venules in patients with CCC. Besides the endothelium, some cases also presented induction of ICAM-1 expression on the sarcolemma of

myocytes [89]. Adhesion molecules seem to be over-expressed not only in the myocardium, but also in the blood of patients with Chagas' disease. Laucella et al. [90] described reduced serum levels of some adhesion molecules after specific chemotherapy with benznidazole in children with the indeterminate phase of Chagas' disease, suggesting that measurement of these molecules could be a valuable tool to evaluate the parasitological clearance.

MHC antigens are also up-regulated in the myocardium of patients with CCC. We detected an up-regulation of class I MHC in the sarcolemma of myocytes (Fig. 2C), and there is also evidence for an over-expression of class II MHC in endothelial cells of patients with CCC [89,91]. However, it is important to realize that up-regulation of adhesion molecules and MHC antigens in CCC appear to be related to the presence of myocarditis. We did not detect over-expression of these molecules in the myocardium of patients with idiopathic dilated cardiomyopathy [89] that did not present myocardial inflammation (Fig. 2D). Since inflammatory cytokines have been demonstrated in inflammatory foci in hearts of patients with chagasic cardiomyopathy, they are probably responsible for the up-regulation of adhesion molecules and MHC antigens [92]. However, over-expression of adhesion molecules on the endothelium is probably very important to perpetuate inflammation and the up-regulation of class I MHC antigens on myocytes could represent a target for CD8+ T lymphocyte adhesion, promoting direct cytotoxicity.

6. Heart failure and natriuretic peptides

Atrial and brain natriuretic peptides (ANP and BNP, respectively) are elevated in the blood and ventricular tissues in several cardiac diseases evolving with cardiac hypertrophy and heart failure. The main stimulus for a ventricular production of these peptides is elevation of the wall stress of the ventricular chambers. In these settings, the ventricular production of ANP and BNP can be increased dramatically, approaching the levels in the atria, where these peptides are normally produced.

With regard to Chagas' disease, there is evidence for elevated blood levels and ventricular up-regulation of ANP and BNP in both acute experimental and chronic human chagasic cardiomyopathy [93–95]. It is interesting to note that myocarditis itself does not appear to influence the expression of ANP in ventricular myocytes. In CCC, the expression of ANP was restricted to the subendocardial region, where wall stress is higher, and the peptide was not found around inflammatory foci located away from the subendocardial region [94]. As in other cardiac diseases, the measurement of BNP in the blood of patients with Chagas' disease is considered to be a very sensitive and specific method to detect asymptomatic heart dysfunction [95]. Since the detection of BNP in the blood is easy to perform, this can lead to reevaluation of the concept of the indeterminate phase of the disease.

In summary, ANP and BNP are elevated in the blood and ventricular tissues of patients with chagasic cardiomyopathy and ventricular dysfunction. However, the increased synthesis of the peptides seems not to be directly related to inflammation, but is probably due to the altered hemodynamic conditions and the elevation of the wall stress in the cardiac chambers.

7. Dilated chagasic cardiopathy versus idiopathic dilated cardiomyopathy

In this section, idiopathic dilated cardiomyopathy (IDC) is defined as a poorly functioning dilated heart without evidence of coronary artery disease, valve disease, congenital heart malformation or uncompensated hypertensive heart disease. CCC is frequently used as a model of a dilated cardiomyopathy with a defined etiology, in the concept that both probably have a similar structural disarrangement that leads to ventricular dilatation. Experimental works with *T. cruzi* have been performed with the aim to achieve a better understanding of dilated cardiomyopathy and its treatment [96–98]. However, we like to point out some important histopathological differences that have been observed between IDC and CCC that alert for different therapeutic approaches. In contrast to CCC, IDC is usually characterized by absent or only mild myocarditis and less fibrosis that does not surround each myocardial fiber [39]. A network of fibrillar collagen enveloping the myocardial fibers and tethering each other is important for the maintenance of normal shape and efficient contraction of the heart [99,100]. In IDC, attenuation and rupture of extracellular matrix connections occur that might favor the slippage of those 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, which probably prevents their normal distension and contraction (Fig. 2A,B). The lateral connections are preserved within the groups of cardiac fibers, which usually are severely hypertrophic [101]. The presence of parasitic antigens may induce fibrogenesis.

Myocardial lymphocytes and macrophages may favor the development of fibrosis by production of cytokines and growth factors [83,102]. Regarding some growth factors, we have observed a strong correlation between numbers of PDGF-A+ and PDGF-B+ cells. In contrast, there was a lack of correlation between PDGF-A and TGF-β1; PDGF-A and GM-CSF; and between PDGF-B and TGF-β1. GM-CSF and TGF-β1, which are considered important elements for the immune response against *T. cruzi* parasites, were present in very scarce amounts [103,104]. In contrast, IL-15 seems to be involved in survival and proliferation of CD8+ T cells and IL-2 [105,106]. Recent

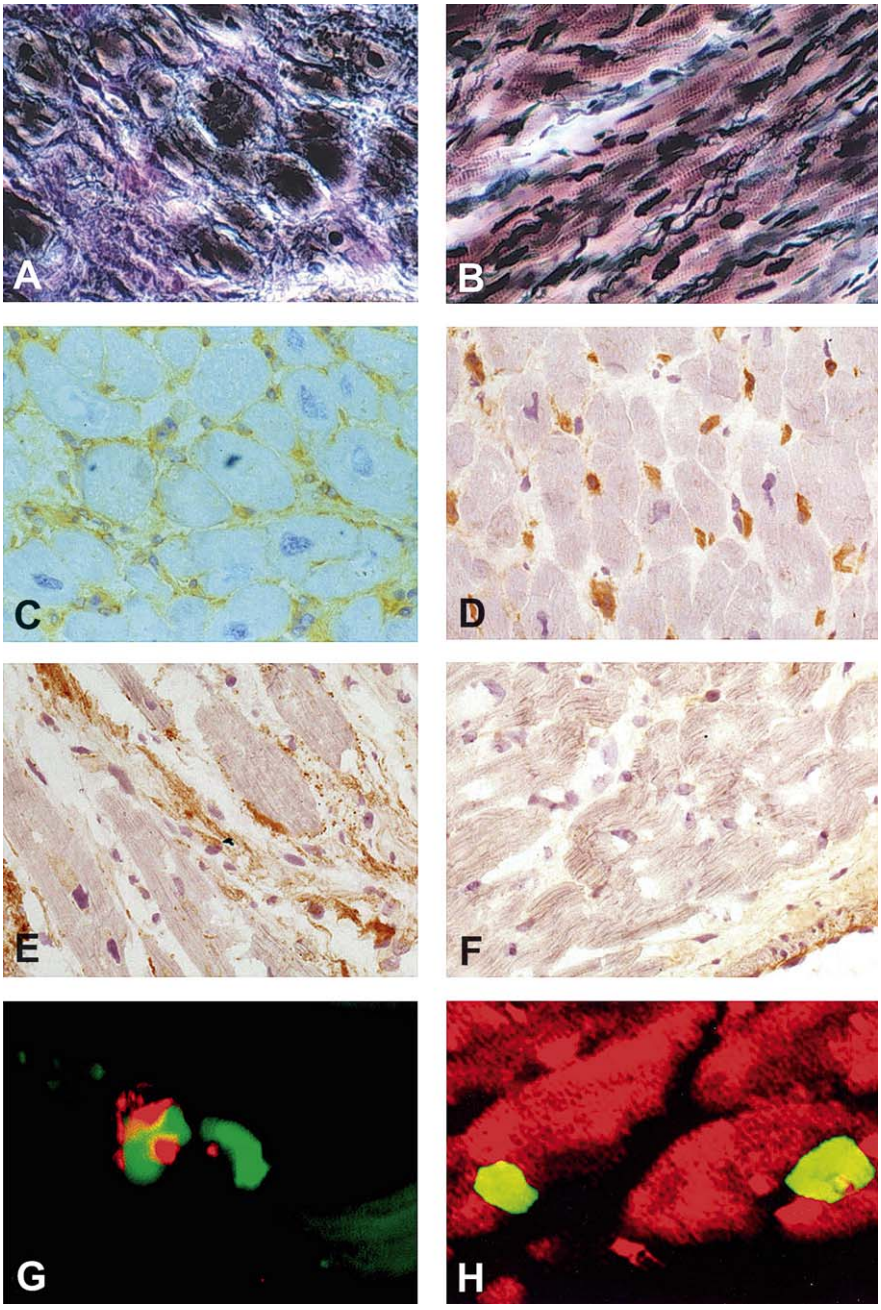

Fig. 2. Microscopic features comparing chagasic cardiopathy (left side) and idiopathic dilated cardiomyopathy (right side). (A) Staining of the extracellular matrix in chagasic myocardium by the Del Rio Hortega technique, emphasizing increased collagen fibers surrounding the myocytes (struts, coins and weaves) (objective magnification: 16×). (B) Dilated cardiomyopathy presents attenuated and ruptured collagen fibers (objective magnification: 40×). (C) Class I HLA antigens are present on the membranes of myocytes in chagasic hearts. (D) Dilated cardiomyopathy presents class I HLA antigens on endothelial cells of capillaries, whereas no staining of myocytes becomes obvious (immunoperoxidase technique, objective magnification: 40×). (E) Membrane attack complex of the complement (C5b-9) is expressed on the membranes of myocytes in chagasic myocardium. (F) Dilated cardiomyopathy shows positivity only in the subendothelial layer of arterioles and venules, as seen in the right lower corner (immunoperoxidase technique, objective magnification: 40×). (G) TUNEL-staining in chagasic myocardium is restricted to apoptotic interstitial cells (green fluorescence). At least one of the apoptotic interstitial cells is double-stained with the human macrophage marker CD68 (red fluorescence) (objective magnification: 40×). (H) In contrast, many TUNEL-stained apoptotic cardiomyocytes (yellow fluorescence) are present in dilated cardiomyopathy. Red fluorescence indicates muscular elements as stained with Phalloidin (objective magnification: 40×).

studies have indicated that IL-15 may also play an important role in chronic chagasic myocarditis. Addition of IL-15 induced growth of CD8+ T cell cultures, obtained from chagasic endomyocardial biopsies [107] and IL-15+ T cells were found to be abundant in chronic chagasic myocardial sections [108].

It has been shown that endothelial cells infected with *T. cruzi* display higher platelet adherence and aggregation [97]. The neuraminidase produced by the parasite removes sialic acid components from the endothelial surface and may favor linkage with thrombin. Large amounts of C3 and C5b-9 products were present on the surface of *T. cruzi* amastigotes and could explain the resistance of amastigotes to destruction by complement action, as well as the presence of trypomastigote decay-accelerating factor [109]. These products may also favor thrombosis [110]. In chronic human chagasic disease, the mechanism of release of growth factors has not been completely clarified and a possible role of sub-lytical amounts of MAC (C5b-9—membrane attack complex of the complement cascade) inducing such release was studied, comparing IDC and CCC. C5b-9 was detected on the sarcolemma of myocytes in hearts with CCC but not in hearts from patients with IDC (Fig. 2E,F). These findings suggest that C5b-9 may be involved in the pathogenesis of the fibrosis that surrounds each myocardial fiber in chagasic specimens [111]. This type of fibrosis has also been demonstrated by confocal microscopy [101] and scanning electron microscopy [112]. The presence of the parasite in the extracellular matrix may induce fibrogenesis although the pathogenesis is not very well understood [113]. On the other hand, *T. cruzi* has collagenolytic and proteolytic properties [114], which may contribute to the destruction of the extracellular matrix, favoring cardiac remodeling and heart failure.

Cardiomyocyte apoptosis as well as proliferation have been described in congestive heart failure, but their clinical relevance remains unclear. Interestingly, we have observed different amounts of myocardial cell apoptosis between IDC and chagasic hearts. In a recent study, apoptosis of myocardial fibers correlated with bad long-term outcome

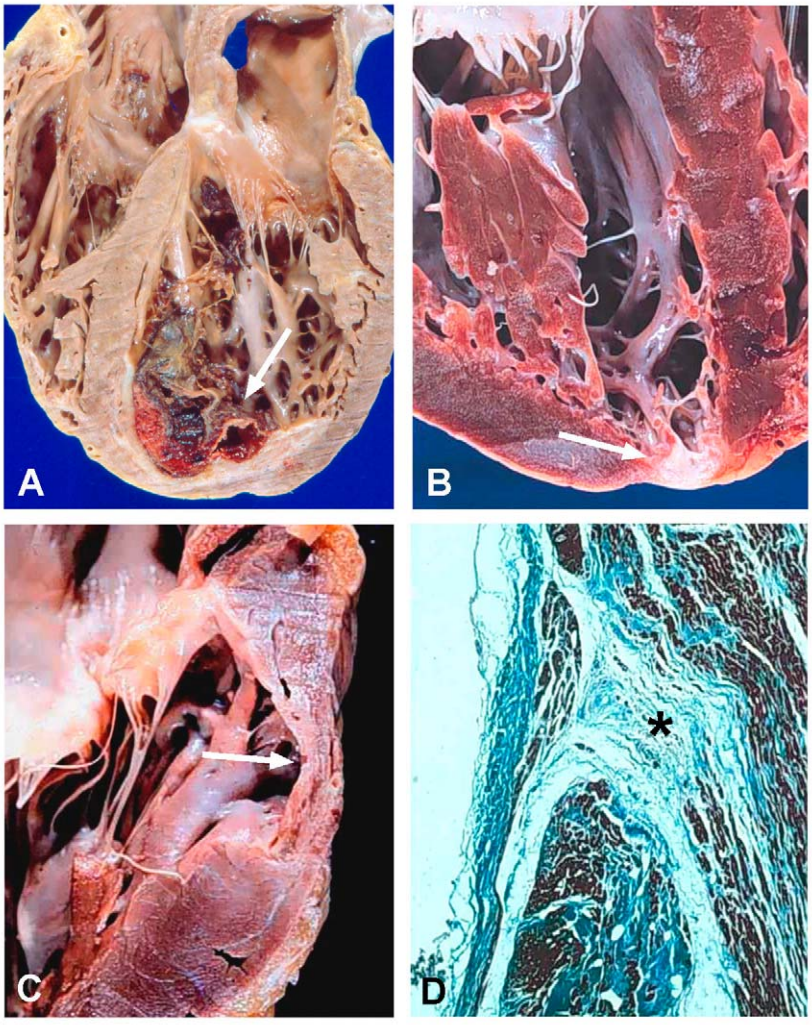

Fig. 3. Macroscopic and microscopic chagasic features, emphasizing the segmental myocardial fibrosis. (A) A chronic chagasic heart exhibits severe dilation of left and right ventricles plus thinning, fibrosis and thrombosis of the left ventricular apex (arrow). (B) A moderately dilated and hypertrophic left ventricle showing segmental fibrosis on the apex (arrow), considered to be a pathognomonic lesion of Chagas heart disease. (C) Fibrotic thinning of the myocardium adjacent to the mitral valve at the posterior wall of the left ventricle (arrow). (D) Microscopic view of the bifurcation of the His bundle showing segmental fibrosis (asterisk) at the origin of the right bundle branch (Masson trichrome, objective magnification: 2.5×).

after a surgical treatment of IDC [115]. In an ongoing study, we are analyzing whether apoptosis of myocardial cells occurs in chagasic patients with severe heart failure. Apoptosis was detected by a fluorescent TUNEL assay (Roche) and by caspase-3 immunohistochemistry in myocardial fragments from chagasic hearts. By both methods, apoptotic cells were detected in high numbers in all fragments examined. However, all TUNEL- or caspase-3 positive cells were interstitial cells and no apoptotic cardiomyocyte at all could be detected [116]. The majority of the TUNEL- and caspase-3 stained interstitial cells showed morphological characteristics of inflammatory mononuclear cells. A double-labeling approach indicated that a great percentage of apoptotic interstitial cells stained positively for the human macrophage marker CD68 (Fig. 2G) and also demonstrated that apoptosis of CD4 T-cells seems not to be an important event in human chronic Chagas' disease. Negative TUNEL-staining of cardiomyocytes was also obtained by Rossi et al. [117], leading to the conclusion that the absence of apoptosis in cardiomyocytes suggests that necrosis rather than apoptosis should be considered as the mechanism of myocardial cell loss in CCC. In an in vitro study, the transialidase produced by *T. cruzi* inhibited the apoptosis of Schwann cells [118]. The role of *T. cruzi* in such phenomenon needs further investigation. In contrast, experimental *T. cruzi* infection was accompanied by the occurrence of severe apoptosis of lymphocytes [119]. Furthermore, in vitro, CD4+ T cells apoptosis exacerbated parasite replication in co-cultured macrophage infected with *T. cruzi* [120]. The mechanisms of such a replication have been suggested to be related to prostaglandins E2/TGF-β in macrophages and it was shown that cyclooxygenase inhibitors almost completely ablate it [121].

8. Microcirculation, fibrosis and ischemic lesions in chagasic hearts

Severe myocardial fibrosis associated with microvascular alterations has been described long time ago [16,122]. Recent experimental studies on acute Chagas' disease have demonstrated microvascular alterations characterized by microspasms [123], microthrombi [124], dysfunction of endothelial cells and increased platelet activity [125,126]. All of these phenomena may play an important role in the further development of myocytolysis and fibrosis. In our previous work analyzing the interstitial alteration versus microvascular status in chagasic and non-chagasic dilated hearts, a severe microvascular dilatation was only observed in chagasic hearts [101]. We hypothesize that the lack of arteriolar contraction might be due to the presence of vasodilator substances induced by the inflammation and/or by the parasite. This may cause impaired myocardial irrigation in distal areas of the coronary branches. Such a low blood pressure perfusion should be present mainly at the watershed zones between two main coronary artery

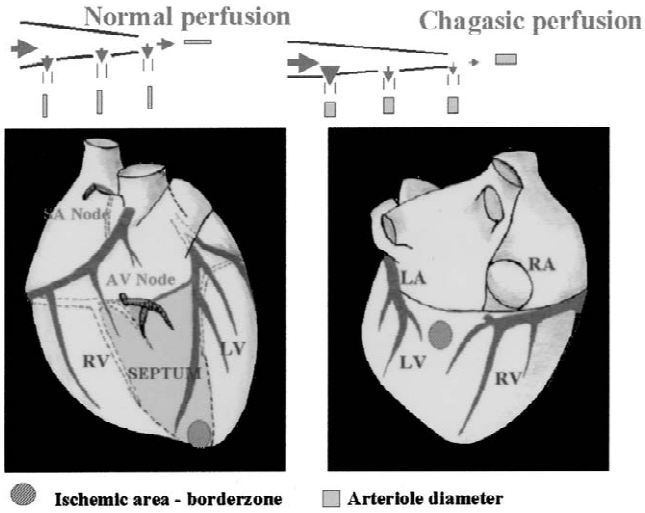

Fig. 4. Schematic representation of cardiac blood perfusion in a chagasic heart: Autopsy hearts from chagasic patients with heart failure present a typical microvascular dilatation that may cause impaired blood perfusion in borderzones. This scheme would explain focal fibrotic lesions in the following heart regions: (1) between anterior descending and posterior descending branches causing apex aneurism; (2) between circumflex and distal right coronary arteries causing posterior wall fibrotic lesion; and (3) between first septal perforating artery and atrioventricular nodal artery originating from dominant coronary artery at the crux cordis leading to segmental fibrosis of the His bundle and its branches (LA, left atrium; RA, right atrium; RV, right ventricle; LV, left ventricle; SA, sinoatrial node; AV, atrioventricular node).

branches. The resulting ischemia would explain fibrotic lesions at the following loci: (1) the apex (Fig. 3A,B), a watershed zone between the anterior descending and the posterior descending arteries; (2) the posterior lateral wall of the left ventricle (Fig. 3C), a borderzone between the right coronary and circumflex arteries, and (3) fibrotic segmental lesions in the conduction system [127,128] (Fig. 3D) which is doubled irrigated. In addition, the epicardial coronary artery cross-sectional luminal areas are diminished in left ventricular wall dysfunctional segments where they frequently exhibit the appearance of fibrotic thinning of the myocardium that sometimes was substituted by adipose tissue [112,129]. These regions are generally related to the origin of sustained ventricular tachychardia in chronic chagasic patients and they exhibit a similar histopathological pattern as a healed myocardial infarction. Similar as in ventricular sustained tachycardia of myocardial infarction scars, the radiofrequency by catheter ablation may block such malignant arrhythmia [130]. Fig. 4 demonstrates schematically such possible ischemic focal lesions.

9. Conclusions

In conclusion, in our view chronic cardiac Chagas' disease represents the result of a close interaction between

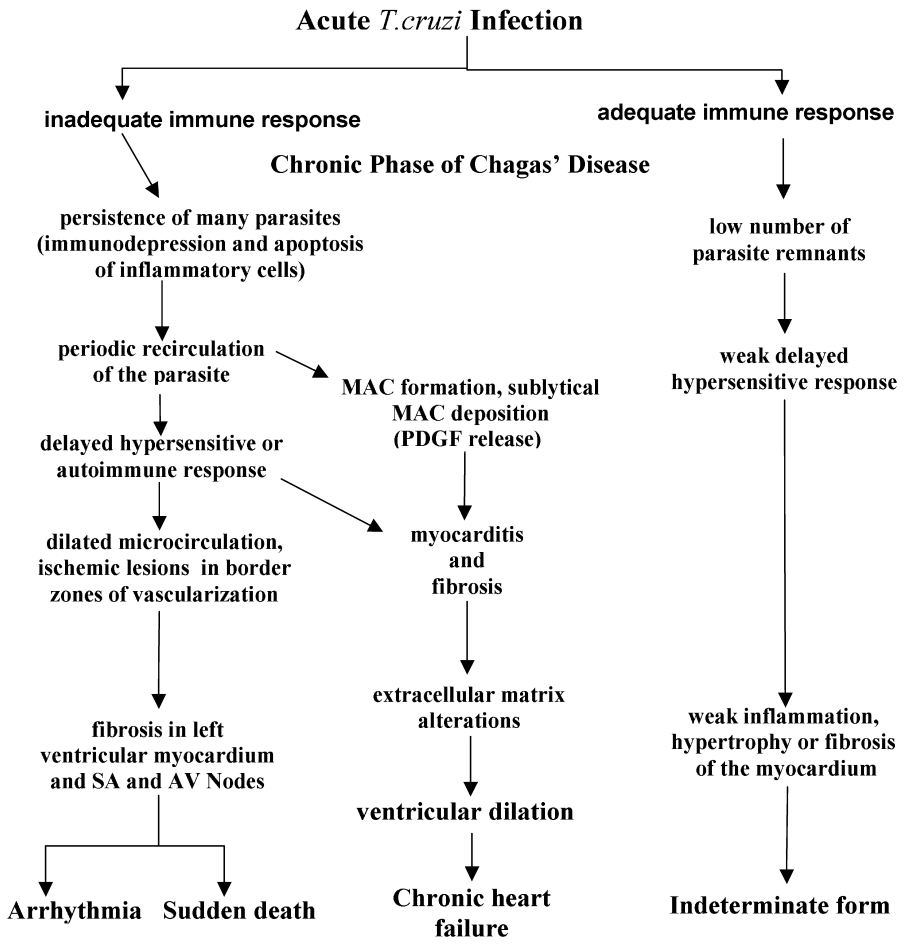

Fig. 5. Hypothetical scheme of the main factors involved in chronic chagasic cardiopathy and their hypothetical interactions that might lead to different cardiac lesions (MAC, membrane attack complex of the complement cascade).

the host and the parasite, causing different clinical pictures. A scheme of the main factors involved in chronic chagasic cardiopathy and their hypothetical interactions that might lead to different cardiac lesions is provided in Fig. 5. Patients with a good immune response may adequately circumvent the parasitic infection and the individual will develop the indeterminate form. Deficient immune response of the host and/or a high initial parasitemia favor immune imbalance that might lead to development of a permanent inadequate immunological response against the parasite. The inflammatory response, which is probably recurrent, undergoing periods of more accentuated exacerbation, is most likely responsible for progressive neuronal damage, microcirculatory alterations, heart matrix deformations and consequent organ failure.

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