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Pathogenesis of Chronic Chagasic Myocarditis

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1. Introduction

Chronic chagasic cardiomyopathy (CCC) is the most serious manifestation of the chronic form of Chagas' disease and constitutes the most common type of chronic myocarditis in the world [1-5]. Chagas' disease, a chronic illness caused by the flagellate parasite *Trypanosoma cruzi* (*T. cruzi*), was first described in 1909 by the Brazilian physician Carlos Chagas [6]. The insect vectors of the disease are present throughout most of South and Central America, and their zone of distribution extends across the southern United States [7]. It was estimated by year 2000, that in endemic areas 40 million people were considered to be at risk of infection, being 20 million already infected. Every year near 200,000 new cases are expected to happen, and 21,000 deaths per year occur [8].

Although always considered to be confined to Latin America, due to migratory movements from endemic countries to Europe and North America, Chagas' disease is being detected more frequently in developed countries. Europe is estimated to have from 24,001 to 38,708 (lower or upper limit of estimate, respectively) immigrants with *T. cruzi* infection [1]. In the United States 6 autochthonous cases, five transfusion related cases and five transplant associated cases have been reported, but migratory movements still remain the main source of Chagas' disease. It has been estimated that around 89,221 to 693,302 infected Latin Americans migrated to the United States in the period 1981 to 2005 [3].

Two phases of the disease can be distinguished: (1) acute phase, with transiently high concentration of parasites in tissue and blood, nonspecific symptoms, and a 5% myocarditis incidence, lasting 4 – 8 weeks; and (2) chronic phase, lasting lifelong. Chronic phase can be presented as indeterminate form, characterized by lack of symptoms and normal ECG and normal radiographic examination of the chest, esophagus and colon. Approximately 60 – 70% of patients remain in this form for the rest of their lives. Only 20 - 40% of infected individuals,

10 - 30 years after the original acute infection, will develop cardiac, digestive or mixed form of the disease, characterized by the appearance of megaviscera (dilated cardiomyopathy, megaesophagus and/or megacolon). It poses a substantial public health burden due to high morbidity and mortality [3, 7, 9].

CCC is manifested by a chronic, diffuse, progressive fibrosing myocarditis that involves not only the working myocardium but also the atrioventricular (AV) conduction system, autonomic nervous system and microcirculation [10 - 12]. This leads to cardiomegaly, cardiac failure, arrhythmias, thromboembolism, and death [11]. Colon and esophagus are also commonly affected by Chagas' disease, being megacolon with constipation and megaesophagus with achalasia also features of the disease [7].

2. Pathogenesis of Chagas' myocarditis

Milei et al. proposed a combined theory that could explain the pathogenic mechanism in chronic chagasic myocarditis [2, 13] that has been previously reviewed by us [14]. This hypothesis is based on three ingredients: the parasite, host immune system and fibrosis. These ingredients are proposed as being the primary causative agents of damage on myocardial tissue, conduction system, autonomic ganglia and nerves and microvasculature.

2.1. First ingredient: The parasite

The role of *T. cruzi* in the chronic phase has been previously underestimated due to the fact that its presence was believed to be scarce and unrelated to the inflammatory infiltrate present at this stage. Nowadays, the involvement of the parasite in the chronic phase has been well documented. Using dissimilar methods, different authors demonstrated either the persistence of *T. cruzi* or parasite antigens in mice [15], the parasite DNA sequence amplified by the polymerase chain reaction (PCR) [16, 17], *T. cruzi* antigens from inflammatory lesions in human chagasic cardiomyopathy [18], or the immunohistochemical finding of the parasite in endomyocardial biopsies with PCR confirmation [19]. This would suggest a direct role for the parasite in the perpetuation of myocardial inflammation. In other words, the antigen stimulation would persist throughout the chronic stage, even though the parasites are not morphologically detectable by light microscopy [20].

The role of parasitemia is more controversial. High parasitemia correlated with severity of disease in one report [21], but showed no association in another [22]. Interestingly, it has been observed that immunosuppression reactivates rather than ameliorates the disease, as seen in patients receiving immunosuppressive therapy to prevent transplant rejection and in AIDS patients. Accordingly, many experimental models where strains of genetically manipulated mice lacking various immune functions showed increased susceptibility to develop the disease [23].

2.1.1. Life cycle of *Trypanosoma cruzi* (Figure 1)

When a reduviid bug feeds from an infected mammal, it takes up circulating trypomastigotes, which reach then the bug's gut. There, they differentiate to amastigotes, which proliferate and start to differentiate into epimastigotes. In this process, when amastigote is still sphere-shaped but has developed its flagellum, some authors call this stage spheromastigotes. Then, it elongates its cell body and flagellum, taking the classical epimastigote shape. At this stage, the parasite undergoes metacyclogenesis, differentiating in metacyclic trypomastigotes, the infective form for mammals. When the bug feeds again, it excretes trypomastigotes with feces, which in turn reach blood torrent through bug's wound. Trypomastigotes can infect a wide variety of host cells, within them it differentiate into amastigotes and proliferate. Then, they can differentiate into trypomastigotes again, reach circulation and infect new cells. If an uninfected bug feeds from the animal in the moment of parasitemia, cycle starts again [24].

2.1.2. Genetic variability of *Trypanosoma cruzi* and its relation to its pathogenesis

The genetics of *T. cruzi* caught the attention of researchers in late 80' and early 90'. First studies on variability were performed analyzing electrophoretic variants on cellular enzymes. The

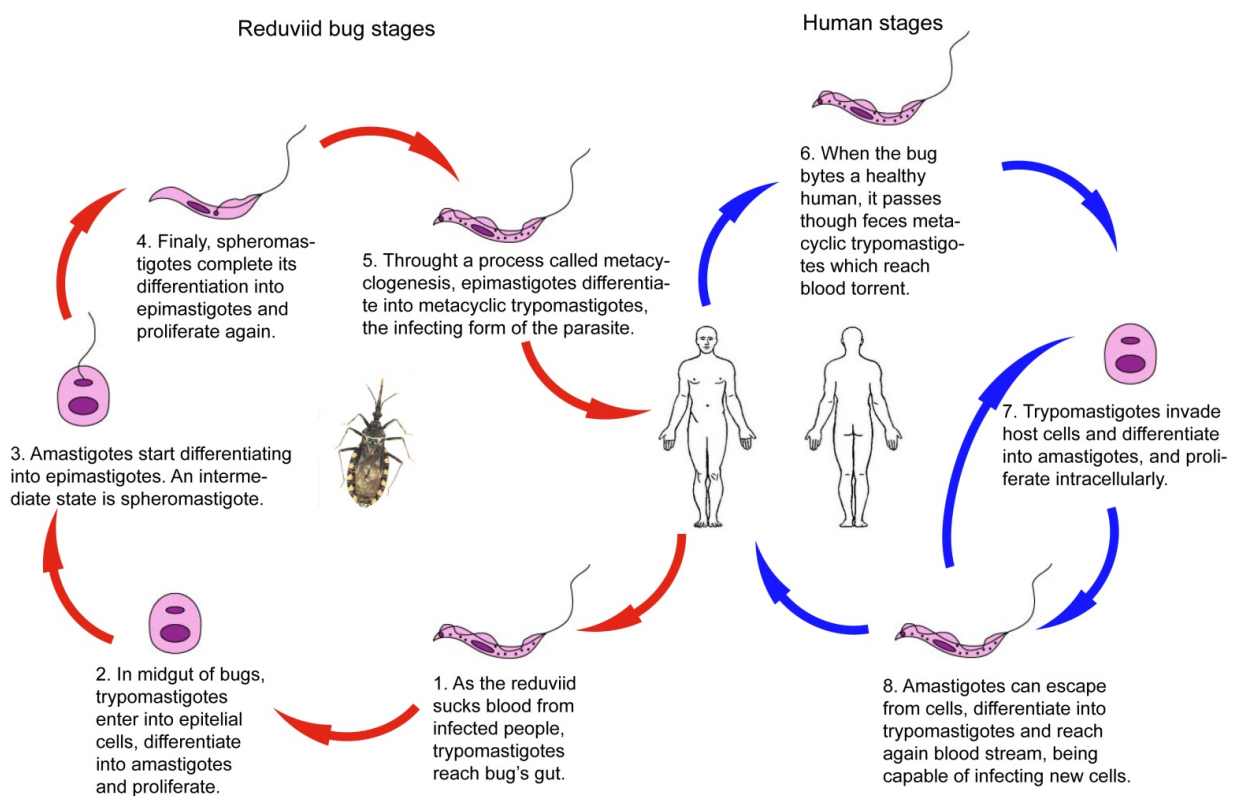


Figure 1. Life cycle of *Trypanosoma cruzi*

groups resulting were called zymodemes and were named Z1, Z2, Z3. Only Z2 was associated with domestic transmission cycle.

The development of PCR based techniques allowed the study of new variant regions and the characterization of multiple variants of a great number of genes. All these variants showed significant correlation with each other, suggesting the existence of two subtypes of *T. Cruzi* based on these data [25]. Moreover, *T. cruzi II* which is clearly linked to human pathology, being *T. cruzi I* mainly related to infection of wild sylvatic mammals. Even, applying LSSP-PCR to the study of the variable region of kinetoplast minicircle from *T. cruzi* provided evidence of a differential tissue distribution of genetically diverse *T. cruzi* populations in chronic Chagas' disease, suggesting that the genetic variability of the parasite is one of the determining factors of the clinical form of the disease [26].

2.1.3. Cell host invasion and intracellular survival by *Trypanosoma cruzi*

Once *T. cruzi* reaches blood torrent, it invades a great variety of cells in the host. When parasiting non phagocytic cells, *T. cruzi* uses some surface glycoproteins to attach to cell: gp82, gp30 and gp35/50. All three glycoproteins are known to induce calcium mobilization from intracellular reservoirs. Gp82 is linked to the phospholipase C (PLC) and inositol 1,4,5 – triphosphate (IP3). Gp 35/50 is associated to increasing intracellular levels of cyclic AMP. On the other side, cruzipain, a protein known to be secreted by *T. cruzi*, acts on kininogen and produces bradykinin, which binds to its receptor, further increasing intracellular calcium. Increased intracellular calcium produces modifications in cytoskeleton that lead to parasite endocytosis [27].

In the parasitoforous vacuole, mainly by the action of gp85/TS a glycoprotein with trans-sialidase action, and TcTox, a protease, the parasite degrades the membrane of the vacuole, escapes from it and proliferates within the cell [28].

2.1.4. Molecular mimicry

The induction of autoimmunity by similarities between *T. cruzi* and host epitopes has been long proposed as a mechanism that leads to tissue damage in the chronic phase of the disease. Both humoral and cellular autoimmune responses have been described, but we will discuss them in more detail in the section of immune system. The real importance of molecular mimicry in the pathogenesis of chagasic myocarditis is still a matter of debate [29].

Although it seems that in some cases this mechanism triggers autoimmunity, in many others, autoimmunity seems to be an epiphenomenon of cellular destruction, with exposition of intracellular epitopes not normally exposed to the immune system. This, in turn may activate autoreactive lymphocytes leading to the appearance of autoantibodies that are not the cause of damage, rather a consequence [29].

The most important cross reacting epitopes of *T. cruzi* and the correspondent epitopes in humans are listed in table 1, as well as the kind of immune response they elicit.

2.2. Second ingredient: Host immune system

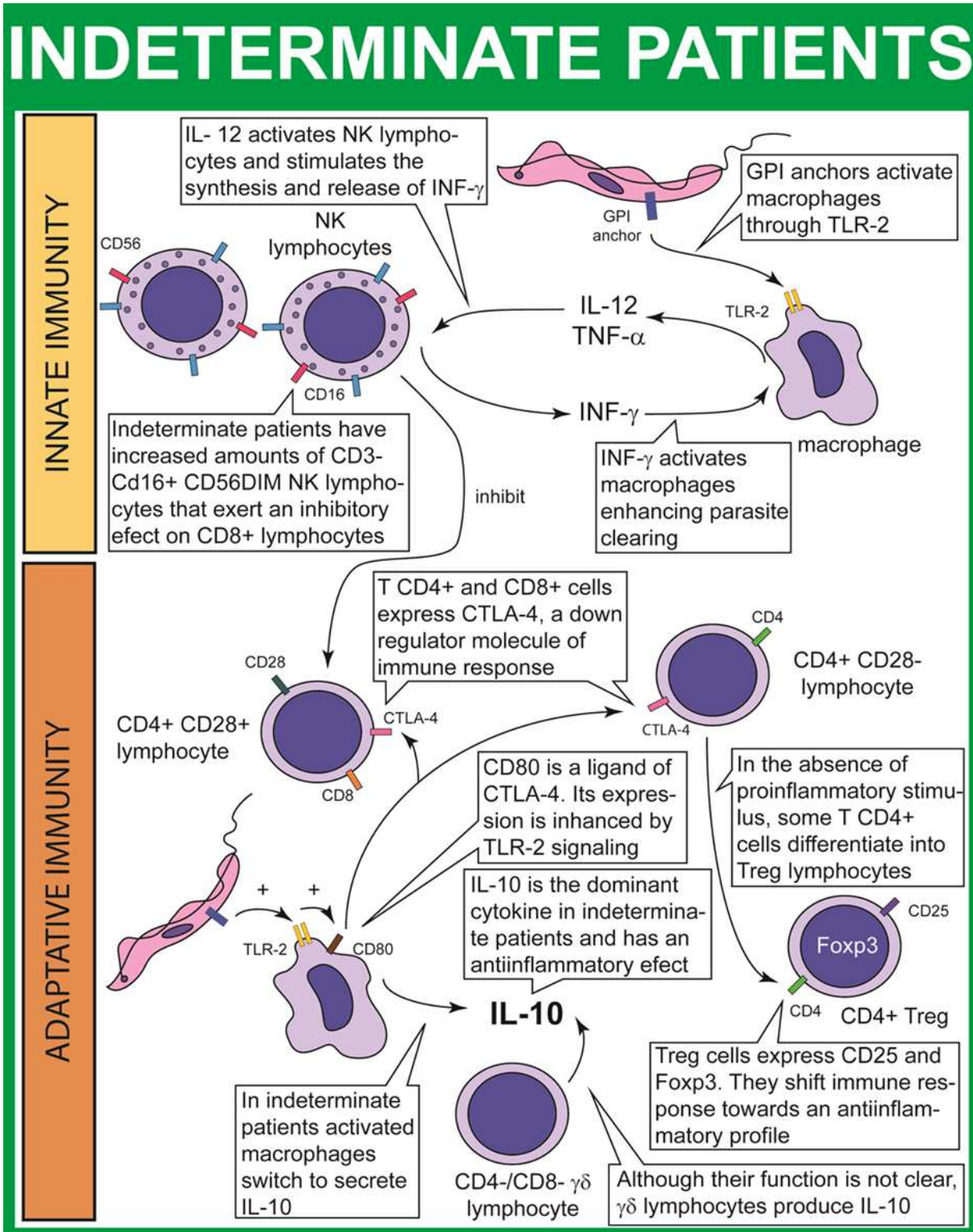
When the three ingredients theory was first proposed [2, 13], second ingredients were mainly T lymphocytes and macrophages. In the subsequent years some evidence grew about the participation of humoral immune system through autoantibodies in the pathogenesis. As a consequence, the whole immune system of the host is now considered as the second ingredient.

As described earlier, mononuclear cells persist in the chronic stage of the disease, contributing to the inflammation through its products of secretion or through its own cytotoxicity (suppressor T cells) and cytolytic action (macrophages) [13]. As previously stated, molecular mimicry may be the main explanation of autoimmunity, triggering both cellular and humoral autoreactivity [29]. Figure 2 summarizes the most important immune events in CCC pathogenesis.

Parasite antigen	Human Antigen	Immune reaction
B13	Cardiac myosin heavy chain	Autoantibodies Autoreactive T cells
R13 (ribosomal protein)	Ribosomal protein β_1 -adrenergic receptor M_2 -muscarinic receptor 38-kDa heart antigen	Autoantibodies
Ribosomal protein PO	β_1 -adrenergic receptor	Autoantibodies
FL-160	47-kDa neuron protein	Autoantibodies
Shed acute-phase antigen (SAPA)	Cha antigen	Autoreactive T cells
TENU2845/36 kDa	Cha antigen	Autoantibodies
Calcireticulin	Calcireticulin	Autoantibodies Autoreactive T cells
Galactosyl-cerebrosides	Galactosyl-cerebrosides	Autoantibodies
Unknown	Neurons, liver, kidney, testis	Autoantibodies
Sulphated glycolipids	Neurons	Autoantibodies
150-kDa protein	Smooth and striated muscle	Autoantibodies
Cruzipain	Cardiac myosin heavy chain M_2 -muscarinic receptor	Autoantibodies
Microsomal fraction	Heart and skeletal muscle	Autoantibodies
Cytoskeleton	95-kDa myosin tail	Autoantibodies
SRA	Skeletal muscle Ca^{2+} dependent SRA	Autoantibodies
MAP	MAP (brain)	Autoantibodies
Soluble extract	Myelin basic protein	Autoantibodies Autoreactive T cells
55-kDa membrane protein	28-kDa Lymphocyte membrane protein	Autoantibodies

Table 1. Examples of cross-reacting epitopes [12, 29]

A



B

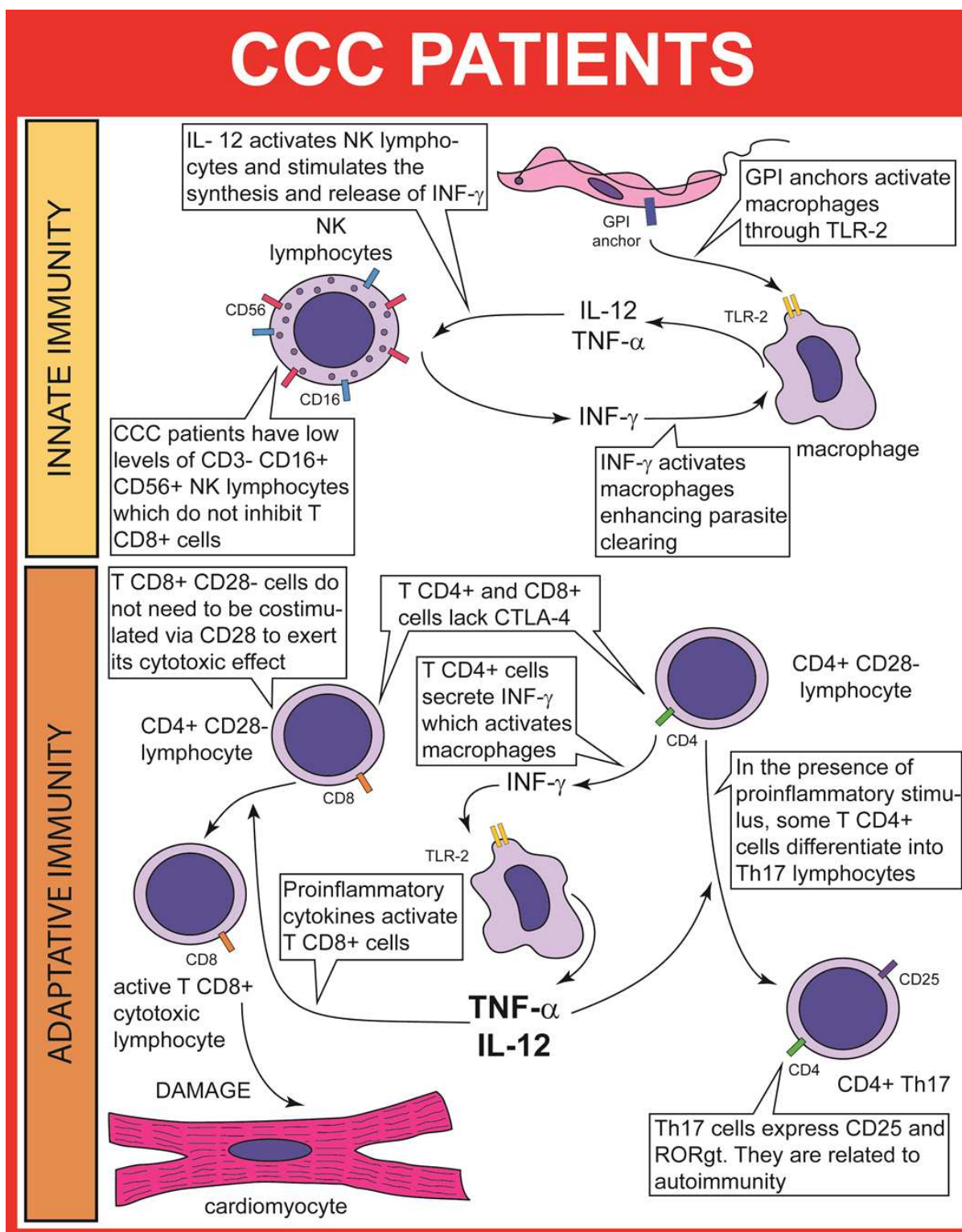


Figure 2. A. The immune pathogenesis of Chagas disease in indeterminate patients. The presence on numerous down regulating mechanisms shift the response towards an anti-inflammatory profile. B. The immune pathogenesis of Chagas disease in CCC patients. Cells evolve towards a proinflammatory profile, with development of autoimmunity.

2.2.1. Innate immunity

In recent years innate immunity came to the attention of researchers of Chagas' disease pathogenesis. The role of NK cells has been particularly studied in early and late indeterminate phases of the disease and in CCC patients. In early indeterminate patients, compared to non infected people, increased values of pre-natural killer (NK)-cells (CD3⁺ CD16⁺ CD56⁻), and higher values of proinflammatory monocytes (CD14⁺ CD16⁺ HLA-DR⁺⁺) were found. The higher values of activated B lymphocytes (CD19⁺ CD23⁺) contrasted with impaired T cell activation, indicated by lower values of CD4⁺ CD38⁺ and CD4⁺ HLA-DR⁺ lymphocytes, a lower frequency of CD8⁺ CD38⁺ and CD8⁺ HLA-DR⁺ cells; a decreased frequency of CD4⁺ CD25^{HIGH} regulatory T cells was also observed. All these data suggest a rather proinflammatory profile [30]. This profile may be useful to limit parasitemia and confine infection to tissues. In fact, it has been demonstrated that NK cells are important in defence against the spread of parasitic infection [31], and are an important source of INF- γ , a key cytokine to activate macrophages and help with parasite clearance [32].

In late indeterminate phase, CD3⁺CD16⁺CD56⁺ and CD3⁺CD16⁺CD56^{DIM} NK cells are increased but are in normal range in CCC patients, suggesting a protective role for them [33]. NK cells showing CD56^{DIM} may play a role in the down modulation of cytotoxic deleterious T CD8⁺ response reported in CCC patients [34].

Monocytes display different cytokine profile. In indeterminate patients they produce more IL-10 [35] while in CCC patients they produce more TNF- α [36], leading to a proinflammatory profile that could be responsible for chronic myocarditis. Conversely *in vitro* experiments culturing monocytes from indeterminate and CCC patients showed a predominant production of INF- γ in the former and IL-10 in the later [37]. Also, monocytes of indeterminate patients showed downregulation of Fc- γ R, TLR and CR1 molecules, related to an impaired phagocytic capacity [38].

Toll-like receptors (TLR) are also implied in the response to acute infection with *T. cruzi*. TLR-2 has been shown to recognize GPI surface molecules from the parasite. *In vitro* and *in vivo* studies have demonstrated that macrophages stimulated with GPIs through TLR-2/CD14 receptors produce NO, TNF- α and IL-12 [39]. Toll-like receptor 4 (TLR4)-deficiency genotype D299G/T399I occurred more frequently in asymptomatic (14.8%) than CCC patients. TLR1-I602S, TLR2-R753Q, TLR6-S249P, and MAL/TIRAP-S180L did not associate with CD or CCC. These findings indicate that curbed TLR4 activation might be beneficial in preventing CCC [40].

A key role of complement in infection control has been clearly established. The complement activating molecules C1q, C3, mannan-binding lectin and ficolins bound to all strains analysed; however, C3b and C4b deposition assays revealed that *T. cruzi* activates mainly the lectin and alternative complement pathways in non-immune human serum [41]. Mannose-binding lectin (MBL) initiates complement on *Trypanosoma cruzi* through the MBL-associated serine protease 2 (MASP2). MASP2 polymorphisms, specially g.1961795C, p.371D diplotype (short CD), occurred at a higher frequency among symptomatic patients, compared with the indeterminate group, highlighting the importance of complement in the pathogenesis of CCC [42].

2.2.2. Cellular adaptative immunity

The role of immune cells in the pathogenesis of Chagas' heart disease has been de dominant hypothesis for many years. The paucity of parasite cells in the inflamed myocardium and the presence throughout the evolution of the disease of macrophages and lymphocytes in patched infiltrates lead to this hypotesis. As early as in 1929, Magariños Torres, observing those infiltrates postulated an "allergic" mechanism for CCC. Further, Mazza and Jörg followed this thought and supported the "allergic" theory [13].

The study of circulating lymphocytes in peripheral blood of chagasic patients showed an increase in the percentages and actual numbers of double-positive cells of the phenotype CD3+/HLA-DR+, as well as decrease in the percentage of CD45RA+/CD4+ and CD45RA+/CD8+ T cells, indicating greater numbers of activated T cells circulating. Consistent parallel increases were seen also in the B lymphocyte subset which stained double-positive for CD19/CD5 [43]. These results were similar for both indeterminate and CCC patients. Moreover, T cells from chagasic patients do not express the co-stimulatory molecule CD28 [44] but express high levels of HLA-DR molecules [45]. Some interesting differences were demonstrated between indeterminate and CCC patients. CD28⁻ T cells in indeterminate patients showed expression of CTLA-4, which recognizes the same ligands as CD28, but instead of inducing cell activation it causes down modulation of T cells. On the contrary, T cells in CCC patients do not up-regulate CTLA-4 [46].

Monocytes from indeterminate patients, when infected in vitro with *T. cruzi*, express low levels of HLA-DR and high levels of CD80, a ligand for CTLA-4 [47]. The interaction of these monocytes with CTLA-4⁺ T cells leads to the expression of IL-10, a cytokine known to down-modulate inflammatory responses [35]. This is not observed in CCC patients. CD28⁻ T cells, not expressing CTLA-4, express TNF- α and INF- γ [44].

In the same direction, CD4⁻CD8⁻ $\gamma\delta$ T cells are found to be increased in indeterminate patients compared with CCC ones. These cells are also linked to the production of IL-10 and a down modulatory effect on inflammation [48].

Cells infiltrating myocardium have also been studied. As demonstrated with immunostaining of endomyocardial biopsies by our group, leukocytes infiltrating myocardium in Chagas' disease were approximately 50% macrophages, and 50% lymphocytes, mainly T lymphocytes [49]. Further immunohistochemical characterization of these cells with CD45R for lymphocytes, CD20 and lambda and kappa light chains for B lymphocytes, CD45R0 for T lymphocytes and CD68 for macrophages, confirmed these findings [2].

Autoreactive T cells have caught the attention of many investigators. In experimental models, CD4⁺ T cells from infected mice showed a proliferative response to the exposition to human cardiac myosin heavy chain and to *T. cruzi* B13 protein. They also arrested the beating of fetal heart cells and, more importantly, induced myocarditis in immunized mice and promoted rejection of transplanted normal hearts in the absence of *T. cruzi* [50]. Also, it has been described that T cells infiltrating the myocardium of chagasic patients cross react with human cardiac myosin heavy chain and to *T. cruzi* B13 protein and express high levels of INF- γ and low levels of IL-4, switching to a Th1 profile [51].

A second group of autoreactive T cells have been characterized, that react to Cha antigen in human heart. Cha antigen is a protein in human myocardium of unknown function that is recognized sera from chagasic patients. When anti-Cha T cells are transferred to non infected mice, they cause myocarditis and stimulate anti-Cha autoantibodies production [52].

In recent years, a newly described T cell, named Treg, has come to attention in relation to Chagas' disease pathogenesis. These cells are characterized by the expression of CD4 and CD25. Treg cells are increased in indeterminate patients compared to CCC, which correlates negatively with levels of activated CD8⁺ [33]. In a recent review on the role of these cells on the pathogenesis of CCC it is highlighted that indeterminate patients have a higher frequency of Treg cells, suggesting that an expansion of those cells could be beneficial, possibly by limiting strong cytotoxic activity and tissue damage. Indeterminate patients also show an activated status of Treg cells based on low expression of CD62L and high expression of CD40L, CD69, and CD54 by cells from all chagasic patients after *T. cruzi* antigenic stimulation. Moreover, there was an increase in the frequency of the population of Foxp3⁺ CD25^{High}CD4⁺ cells that was also IL-10⁺ in the IND group, whereas in the cardiac (CARD) group, there was an increase in the percentage of Foxp3⁺ CD25^{High} CD4⁺ cells that expressed CTLA-4 [53].

An additional mechanism is the bystander activation. This is the activation of autoreactive lymphocytes by antigen presenting cells in a proinflammatory environment [54]. This kind of autoreactive T cells activation has been described in Chagas' disease [55].

2.2.3. Humoral adaptative immunity

The importance of humoral immunity in controlling *T. cruzi* acute infection has been clearly established. Mice lacking B lymphocytes rapidly succumb to infection [56]. But the fact that attracted most attention from researchers is the production of a wide variety of autoantibodies.

The first autoantibody to be described was one that reacted to endocardium, blood vessels and interstitium of skeletal muscle (EVI) [57], but was the same group of investigators who recognized the heterophil nature of the antibody and realised that had no pathogenic role [58].

Another autoantibody, studied by our group, was anti-laminin antibody [59, 60]. These antibodies were shown to react against *T. cruzi* amastigotes and trypomastigotes and human laminin [61] and deposition of this antibody in marked thickened basement membranes of myocytes, endothelial cells, and vascular smooth muscle cells was shown by us with light microscopy, electron microscopy and immunohistochemical techniques in endomyocardial biopsies of chagasic patients [62] but then we found that only 50% of patients had the antibody on their sera and no correlation with disease severity could be established [59].

Anti-myosin antibodies are postulated by some authors to be generated through molecular mimicry with two *T. cruzi* antigens: B13 protein [63] and cruzipain [64, 65]. Although cruzipain antibodies mainly react to skeletal muscle myosin, they can cause conduction disturbances when transferred to uninfected mice and, when transferred to pregnant animals, they caused conduction disturbances in pups [65]. On the other hand, immunosuppressed mice did not mount any humoral response when immunized with myosin but still develop myocarditis [66].

This fact made some authors doubt on the molecular mimicry hypothesis and rather consider antibodies to myosin a consequence of myocyte damage [67].

Antibodies that react with muscarinic receptors are also being intensely studied. In early 1990's IgG from chagasic patients was observed to bind to muscarinic M2 receptors and activate them [68]. These anti-muscarinic antibodies were found to increase intracellular cGMP and decrease cAMP [69] and were positively related to the presence of dysautonomia [70]. These antibodies also causes accumulation of inositol phosphate and nitric oxide synthase stimulation, with a negative inotropic effect on myocardium [71]. As mentioned before, anti-muscarinic autoantibodies are positively related to the presence of dysautonomia [70], the presence of achalasia in chagasic patients [72], sinus node dysfunction [73], but are not related with the degree of myocardial dysfunction [73, 74], nor with the presence of brain lesions [75]. In fact patients with cardiomyopathy and left ventricular dysfunction but without autonomic dysfunction show low levels of anti-muscarinic antibodies [76].

Autoantibody	Hypothetic pathogenic role	Reference
Anti-Cerebroside	Probably related to neurological symptoms	[77]
Anti-Gal	Apparently protective	[78]
Anti-Brain Microtubules	Unknown	[79]
Anti-Ribosome	Unknown	[80, 81]
Anti- UsnRNPs	Unknwnown	[82]
Anti-Sulfatides	May cause myocarditis and induce arrhythmias	[83]
Anti-Galectin-1	Increased in CCC patients	[84]
Anti-Cha R3	Specific of CCC	[85]
Anti-Desmoglein-1	Related to Penphigus foliaceum	[86]
Anticardiolipin	Unknown	[87]
Anti- TrkA, TrkB and TrkC	Prevents apoptosis of neurons and helps cellular invasion	[88]
Anti-MBP	Related to gastrointestinal form	[89]

Table 2. Less studied autoantibodies in Chagas' disease

Antibodies against β_1 -adrenergic receptors are also intensely studied. Described in early 1980's [90] these antibodies increased cAMP in mouse atrial fibers, increasing the release of PGE_2 and TXB_2 causing diminished contractility [91]. Increased cAMP activates PKA and then increases the intracellular calcium concentration. This causes in turn inhibition of the Na^+/K^+ -ATPase and stimulates Ca^{2+} -ATPase activity leading to intracellular depletion of K^+ and further increase in Ca^{2+} . These alteration alter contractility and electric impulse generation and conduction [92]. Antiadrenergic autoantibodies titers could not be related to the severity of left ventricular dysfunction [74] and patients with overt cardiomyopathy but without autonomic dysfunction show low leves of these antibodies [76]. Antibodies against β_2 -adrenergic receptors have also been described but are mainly related to megacolon [93].

Antibodies against atrio-ventricular (AV) node and sinus auricular node tissues have been studied as markers of chronic cardiopathy condition. When compared in chronic chagasic

cardiopathy patients, non-chagasic cardiopathy patients, indeterminate chagasic subjects, healthy blood donors as controls, they more frequently found in chronic chagasic cardiopathy, but not enough to be good markers for chagasic cardiopathy group. Besides, no clear association with complex rhythm or conduction aberrations was found [94].

Many other autoantibodies have been described (table 2) but are not so widely studied and their role in pathogenesis of chagasic myocarditis is not clear.

2.2.4. Genetic factors

Human Leucocyte Antigen (HLA) have show some relation to de development of CCC. HLA-B40 and Cw3 combination was protective for CCC [95], as resulted DRB1*14, DQB1*0303 [96], HLA-DQB1*06 [97] and HLA-A68 [98]. On the other hand, HLA-C*03 [99], DRB1*1503 [100], DRB1*01, DRB1*08, DQB1*0501 [96] and HLA-DR16 alleles [98] were positively related to the development of CCC.

A number of other genes related to immune system have been studied in order to determine their relation to a predisposition to develop CCC. In table 3 we list those positively related to the appearance of CCC [101].

Gene	Polymorphism
CCL2/MCPI	- 2518
CCR5	+ 53029
TNF- α	- 308G/A, -238G/A, -1031T/C
LT- α	+ 80A/C, + 252A/G
BAT-1	- 22C/G, - 348C/T
NF-kB	- 62, - 262
IL-1 β	- 31, + 3954, + 5810
IL-1RN	+11100T/C
IL-4	-509C/T
IL-10	- 1082G/A
IL-12 β	+ 1188A/C
INF- γ	+874T/A
MAL/TRIAP	S180L
MCP-1	-2518a/G
MIF	-174G/C
TGF- β 1	+10T/C

Table 3. Genetic polymorphisms related to CCC. Adapted from [101, 102].

2.2.5. The cytokines and chemokines

Although proinflammatory cytokines seem to be necessary for controlling parasitemia during acute phase of the disease [101], CCC patients display a rather proinflammatory cytokine while indeterminate patients display a down modulator one. CCC patients have increased levels of

TNF- α and CCL2 than indeterminate patients [103, 104]. Infiltrating macrophages from CCC patients express INF- γ , TNF- α and IL-6 but show low levels of IL-2, IL-4 and IL-10 [105-107]. Also CCR5, CXCR3 and CCR7 and their ligands are increased in hearts of CCC patients, as well as monocytes expressing CXCR3, CCR5, CXCL9 and CCL5 [101]. It has been shown that INF- γ and CCL2 induce myocytes to secrete arial natriuretic factor and cause hyperthrophy [108], and IL-18 and CCR7 ligands, which are increased in CCC, cause cardiomyocyte hyperthrophy and fibrosis [109-111]. Cultures of peripheral blood mononuclear cells from patients with moderate and severe cardiomyopathy produced high levels of TNF- α , IFN- γ and low levels of IL-10, when compared to mild cardiomyopathy or cardiomyopathy-free patients. Flow cytometry analysis showed higher CD4+IL-17+ cells in peripheral blood mononuclear cells cultured from patients without or with mild cardiomyopathy, in comparison to patients with moderate or severe cardiomyopathy, reflecting a relative protective effect of IL-10 and IL-17 compared with INF- γ and TNF- α [112]. In another experiment in which CD8+ in culture were stimulated with trypanosomal antigens, those cells from patients with CCC produced larger amounts of INF- γ and TNF- α than those obtained from indeterminate patients [113].

2.3. The third ingredient: Fibrosis

Fibrosis is one of the most striking characteristics of CCC. In our experience with endomyocardial biopsies, fibrosis had replaced between 8,2 and 49% of contractile myocardium, with only one patient having less than 10% [49]. In our experience with autopsies of hearts, fibrosis was more extensive in conduction system than in contracting myocardium [2]. The deposition of laminin in extracellular and basement membranes has been implicated in the pathogenesis of inflammatory process, as laminin is able to bind proinflammatory cytokines [114]. The inflammatory infiltrate in CCC is related to the production of cytokines such as INF- γ , TNF- α , IL-18, CCL2 and CCL21, that may have modulator actions on fibrotic process [101].

3. Pathophysiological consequences of myocarditis

With the perpetuation of inflammation, necrosis and scarring fibrosis, damage to all histological components of myocardium occurs. Damage to contracting myocardial fibers determines contractile failure as well as electrophysiological disturbances. Conduction system, nervous autonomic system and microvasculature are also damaged and as a consequence they cause further damage to contractile myocardium and produce electrical instability.

3.1. Dysautonomia

As early as 1922 Carlos Chagas noted that the chronotropic response to atropine was altered in chagasic patients [115], but it was not until late 1950's that Köberle published his works showing impressive neuronal depopulation in microscopic sections obtained from the intercaval atrial strip in chagasic patients using a standardized technique of cardiac intramural neuronal counting developed by himself [116, 117]. These findings led to the "neurogenic hypothesis" [118], which explained all megas in Chagas' disease as a consequence of neuronal depletion.

Although many other authors claimed to have confirmed this finding [119, 120], other authors called to attention about the criteria used to diagnose neuronal depletion because of the great variability in the number of neurons in autonomic ganglia [121] and they also remark that the only right criterion to establish neuronal depletion is the presence of proliferation of satellite cells, with the formation of Terplan's nodules, a characteristic lesion described as proliferating satellite cells which replace degenerating neurons, forming nodular structures. These lesions, once considered pathognomonic, can be found in other cardiomyopathies [121]. The same author could not confirm the loss of neurons or denervation in CCC [122]. Finally, it was demonstrated that, using Terplan's nodules as diagnostic criterion, CCC patients with heart failure has more neuronal depletion than patients with dilated cardiomyopathy of other causes [120]. In our experience the neuroganglionic involvement was variable in autopsies of chagasic hearts [11].

According to neurogenic hypothesis [118], early and irreversible damage to the parasympathetic system during acute phase of the disease causes a catecholaminergic cardiomyopathy, but this point of view has been debated and evidence is contradictory. Functional test performed in CCC patients demonstrated impaired parasympathetic heart rate regulation: metaraminol, phenylephrine and atropine intravenous injections, facial immersion, Valsalva maneuver, head-up and head-down tilt tests, respiratory sinus arrhythmia, hand grip, graded dynamic exercise, and spectral analysis of Holter recordings [123-130], but a careful analysis of these data showed that many patients had normal autonomic function and most patients had heart failure, that could explain autonomic dysfunction per se [131]. But the study of indeterminate patients has shown conflicting results. While some authors could demonstrate impaired autonomic function [132, 133] others could demonstrate that autonomic function was normal in patients without myocardial damage and that abnormalities in autonomic dysfunction was proportional to heart dysfunction, leading these authors to propose that these abnormalities arise as a compensating mechanism for the progressive left ventricular dilatation [134, 135]. These findings led to a new "neurogenic theory", which considers autonomic dysfunction as secondary to ventricular dilatation and hemodynamic alterations, but once installed, acts synergistically with parasitism and inflammation to cause further myocardial damage [136].

3.2. Microvascular damage

Microcirculation abnormalities have been demonstrated in experimental models as well as in clinical practice [137]. Many investigators have found abnormal myocardial perfusion using isonitrite-99m-technetium [138] and thallium-201 [139, 140] scintigraphy in chagasic patients with normal epicardial coronary arteries. Furthermore, the progression of left ventricular systolic dysfunction is associated with both the presence of reversible perfusion defects and the increase in perfusion defects at rest [141, 142]. Anatomopathological studies in humans also provided evidence of microvascular damage in CCC. In late 1950's first reports showing collapse of arterioles and intimal proliferation [143] caught the attention of investigators. Also, microthrombi have been described [144]. In endomyocardial biopsies we also found thickening of capillary basement membranes [49].

Additional evidence of microvascular damage was obtained from experimental models. Vascular constriction, microaneurysm formation, dilatation and proliferation of microvessels has been demonstrated [145-148].

Many factors have been advocated in the genesis of these lesions. First, the parasite itself. It was shown that *T. cruzi* produces a neuraminidase that removes sialic acid from de surface of endothelial cells. This results in thrombin binding and platelet aggregation [149]. *T. cruzi* also produces tromboxane A₂ (TXA₂), specially during amastigote state [150], also favouring platelet aggregation and vascular spasm. Direct parasitism of endothelial cells by *T. cruzi* has also been demonstrated, and this causes the activation of the NF-κB pathway increasing the expression af adhesion molecules [151], and secreting proinflammatory cytokines [152] and iNOS [153].

Endothelin-1 (ET-1) is another proposed pathogenic element. Elevated levels of mRNA for preproendothelin-1, endothelin converting enzyme and endothelin-1 were observed in the infected myocardium [154], and elevated levels of ET-1 have been found in CCC patients [155]. Mitogen-activated protein kinases and the transcription factor activator-protein-1 regulate the expression of endothelin-1, and both are shown to be increased in myocardium, interstitial cells and vascular and endocardial endothelial cells [156]. Besides, treatment with phosphoramidon, an inhibitor of endothelin converting enzyme, decreases heart size and severity of pathology in an experimental model of Chagas' disease [157]. Moreover, the use of bosentan, a dual endothelin A (ETA) receptor and endothelin B (ETB) receptor was accompanied by a significant increase in parasitemia and tissue parasitism or inflammation and reduced the infection-associated increase in NO_x serum concentration, suggesting that ETA and ETB may play a role in the control of *T. cruzi* infection probably by interfering in NO production [158].

Inflammation also produces dysfunction of endothelial cells. Macrophages secrete TXA₂ and platelet activating factor (PAF) that act on endothelium causing vasoconstriction [159]. Endothelial cells infected *in vitro* with *T. cruzi* lose their antithrombotic properties in response to interleukin 1 β (IL-1β) [160, 161].

It is remarkable that, although the data presented, endothelial function seems to be normal in CCC patients without heart failure, as measured by increases in blood flow in response to acetylcholine and sodium nitroprusside [162]. A normal endothelial function has also been found using pulse plethysmography in 40 asymptomatic patients with Chagas' disease compared with healthy controls, although a prothrombotic and proinflammatory state has been noted in Chagas' disease patients [163].

4. A combined theory that could explain the pathogenic mechanism in chronic chagasic myocarditis

With the perpetuation of inflammation, necrosis and scarring fibrosis, damage to all histological components of myocardium occurs. Damage to contracting myocardial fibers determines contractile failure as well as electrophysiological disturbances. Conduction system, nervous

autonomic system and microvasculature are also damaged and as a consequence they cause further damage to contractile myocardium and produce electrical instability. Figure 3 illustrates with a flow chart the interactive network of different elements in the pathogenesis of CCC.

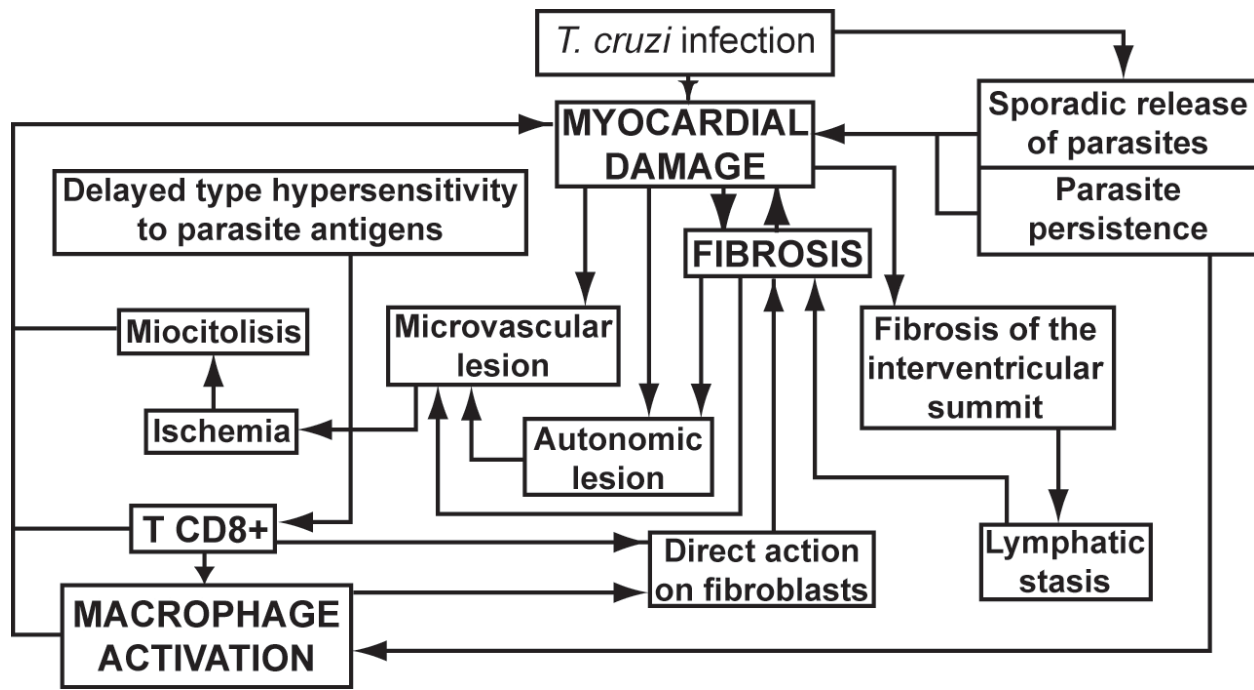


Figure 3. Schematic representation of the integrated theory of multiple factors that determine myocardial damage in CCC.

5. Conclusions

As shown across the sections of this chapter, the numerous hypothesis about pathogenic pathways of CCC have supporting data and pitfalls. All hypothesis finally interact with each other, giving us the idea that none of these theories explains the development of CCC by itself. Rather, it seems more feasible that all of these conform a network of damaging elements, and that all elements cause and/or enhances each other. The triggering element is obviously the interaction between parasite and host's immune system. Cell parasitism, the inflammatory process and consequent necrosis and fibrosis cause damage to contracting myocardium, autonomic system, conduction system and microcirculation. Autonomic damage causes impaired regulation of microvasculature and further alterations in blood flow. Ischemia causes more myocardial damage. Necrosis exposes intracellular epitopes and causes autoantibodies production, with more necrosis, fibrosis and so on. It seems that, if adequate down modulator immune mechanisms work properly, this vicious circle stops and patients do not develop cardiomyopathy, rather they remain in an indeterminate form lifelong.

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