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Chapter 7

Chagas Cardiomyopathy: Role of Sustained Host-Parasite Interaction in Systemic Inflammatory Burden

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Abstract

The economic and social burden associated with Chagas disease morbidity and mortality is regrettably large in Latin America causing more deaths than does any other parasitic disease. Inflammatory dilated cardiomyopathy is, by far, the most important clinical consequence of *Trypanosoma cruzi* infection. The insidious persistence of this parasite determines chronic myocarditis progression. The clinical outcome is multifactorial and depends on the particular parasite strain and virulence factors, the infective load and route of infection, the parasite ability to by-pass the protective immune response, the intensity and type of immune response during the acute infective phase, and the host genetic background. From the immunological viewpoint, host control of *T. cruzi* has been shown to depend on both humoral and cell-mediated adaptive responses and from the innate immune system. In this review, we discuss the most relevant literature conveying information on the relevance of identifying a subset of systemic inflammatory molecules as potential markers of cardiovascular risk morbidity and mortality in patients with Chagas disease. Concurrently, a direct role for the parasite in the perpetuation of myocardial inflammation is substantiated. Ultimately, host-parasite interactions determine the course of the ongoing systemic inflammation and the perpetuation of myocardial inflammation in genetically predisposed patients.

Keywords: Chagas disease, myocarditis, cardiomyopathy, inflammation, immune system, cardiovascular risk

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1. Introduction

Chagas disease is a parasitic disease caused by the *Trypanosoma cruzi* that affects over 12 million people in Central and South America, causing more deaths than any other disease of its kind. Large migrations of infected people from the endemic areas are usually observed mainly in the United States of America and Europe. The most frequent cardiac complications of chronic Chagas disease are left ventricular dilation and dysfunction, aneurysm, congestive heart failure, thromboembolism, ventricular arrhythmias, and sudden cardiac death. Chagas disease diagnosis is based on serology, namely immunopositivity for immunoglobulin G antibodies to *T. cruzi.*

Inflammatory dilated cardiomyopathy is, by far, the most important clinical consequence of *T. cruzi* infection. The chronic chagasic cardiomyopathy (CCC) is roughly progressive, and its treatment does not differ from that of any other non-chagasic cardiomyopathy in the absence of strong evidence. Clinical symptoms usually include dyspnea, palpitations, precordial pain, syncope and eventually, sudden death.

Epidemiological data show high mortality and morbidity resulting from the cardiovascular disease in chagasic patients. However, there are no hints to suspect cardiovascular risk in the silent period of the disease (asymptomatic form). Noticeably, inflammatory factors are upraised during the silent period of the Chagas disease. Like atherogenesis, immune-inflammatory-mediated effector mechanisms commanded by Th1/Th17 cells are involved in the pathophysiology of Chagas disease, having a similar histological hallmark which includes Th1/Th17 cells, macrophages, and a characteristic cytokine profile.

Host control of the *T. cruzi* appears to depend on both humoral and cell-mediated adaptive responses, and on the innate immune system as well [1]. The cytokines strongly activate multiple functions relevant to cardiovascular homeostasis. According to the literature on the subject, there is robust evidence of a systemic upraised level of inflammatory mediators in patients with Chagas disease suggesting that the interplay between the parasite aggressiveness and the host immune response might have a key role in the perpetuation of myocardial inflammation.

The role of the parasitemia is more controversial associated with immunosuppression, disease reactivation, and disease severity. Due to the arousal of a strong and specific immune response against the parasite, nearly two-thirds infected people become protected and may stay in an indeterminate stage of the infection characterized by low parasitemia level for 10 or even up to 40 years after the prime infection. The other one-third infected people, however, develop symptoms, entering the symptomatic chronic stage of infection typically characterized by cardiomyopathy.

The antigenic stimulation though persists all over the chronic stage. Then, the clinical outcome depends on multiple factors like parasite persistence, the particular *T. cruzi* strain, the infective load and virulence factors, the route of infection and sidestepping the host immune response by the parasite, the strength of the immune response at any time, and of course, genetic predisposition [2]. In fact, the sustained parasite-host immunity interactions induce systemic inflammatory mediators' upregulation and fibrosis, both crucially involved in myocardial tissue damage and the resulting disturbances in the cardiac conduction system, mainly affecting the autonomic ganglia, nerves, and the microvasculature.

Unlike the study of the classical risk factors, research studies on how inflammatory status affects the development and determines the progression of cardiomyopathy have not yet identified or clinically validated relevant biomarkers [3]. Interestingly, secluded evidence suggests that the inflammatory status might be associated with increased morbidity and mortality. Deepening our understanding of the pathophysiology of Chagas disease, it will make way to identifying new molecular targets for the design of CCC prophylactic vaccines and therapeutic drugs.

2. Cardiomyopathy genes

Dilated cardiomyopathies (DCM) are characteristically defined by the presence of left ventricular dilatation, and contractile dysfunction [4]. Genetic mutations that involve genes encoding the cytoskeleton sarcomere, nuclear envelope proteins and others account for up to 35% of the total cases. Hypertrophic cardiomyopathies (HCM) and dilated (DCM) cardiomyopathies are heart muscle diseases related to genes variants encoding sarcomere proteins [5]. Among these proteins, the most common are the β-myosin heavy chain (MYH7) [6], the cardiac myosin-binding protein C (MYPBC3) while the myosin light chain (MYL3) and the regulatory myosin light chain (MYL2) are rare [7]. Certain variants in sarcomere genes also cause DCM, albeit less frequently. Of note, variant location does not absolutely predict whether it will trigger HCM or DCM.

Heart failure associated with cardiomyopathies is often caused by mutations in sarcomeric genes, resulting in contractile dysfunction and cellular damage. This may stimulate the production of a robust proinflammatory response. Intriguingly, flow cytometry analysis revealed a significant increase in total macrophages and classically activated proinflammatory (M1) macrophages in DCM hearts as compared with normal hearts. Serum cytokine analysis in dilated cardiomyopathy hearts showed a striking increase in interleukin IL-6 in rodents. Furthermore, RNA-seq analysis revealed the upregulation of inflammatory pathways in DCM hearts. Altogether, these data indicate a robust proinflammatory response in DCM hearts, likely in response to cellular damage triggered by an MYBPC3 mutation and the resultant contractile dysfunction [8]. In addition, other genes have been implicated in DCM, particularly the monocyte chemoattractant protein-1 gene polymorphism [9].

The epigenetic factors that contribute to myocarditis include consumption of alcohol or drugs, exposure to toxins, and metabolic and endocrine disturbances. The typically presenting symptoms are related to congestive heart failure, and can also include circulatory collapse, arrhythmias, and thromboembolic events [10].

3. Anatomopathological findings of Chagas cardiomyopathy

Chagas disease is typified in the WHO classification within the group of specific diseases of the myocardium. Alternatively, the denomination of cardioneuropathy has been proposed to express the frequent and severe importance of the autonomic affectation and the consequent dysautonomia associated with the functional and clinical alterations. The observed

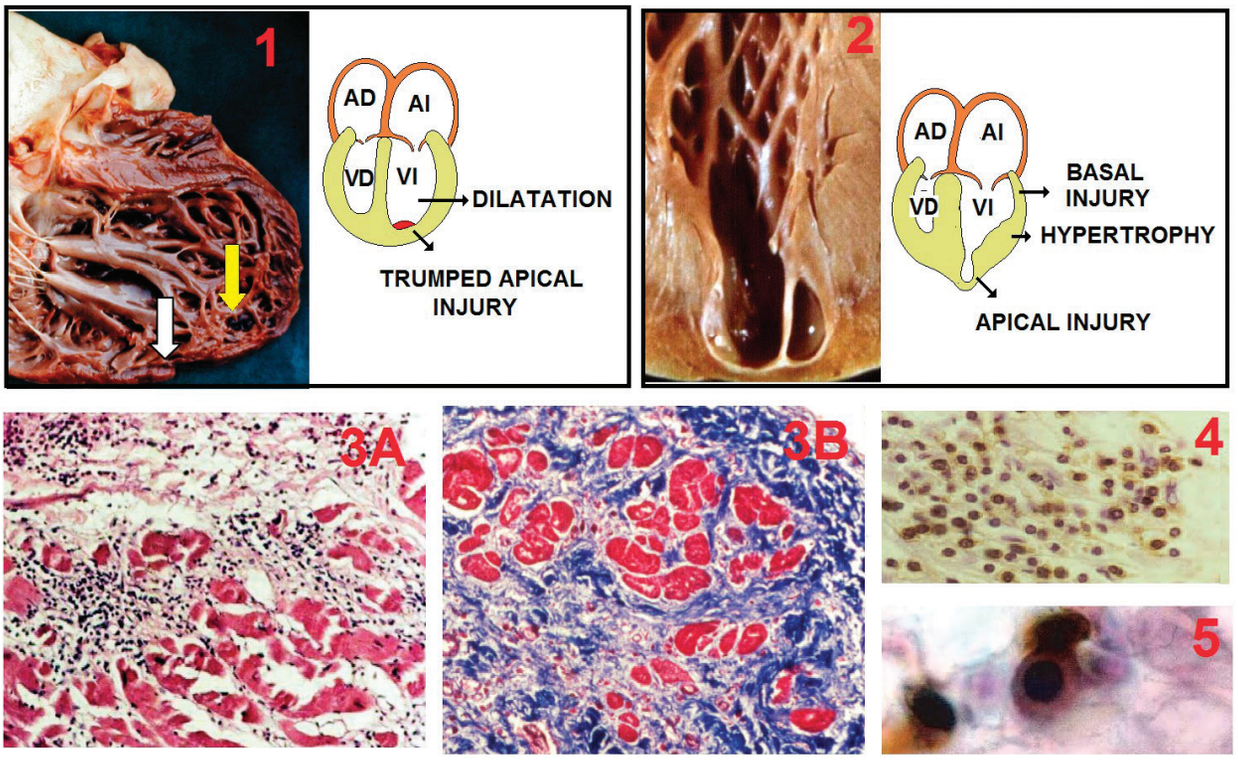

Figure 1. Anatomopathological findings. Figure courtesy of Dr. José Milei. Panel 1, left. High-grade heart dilatation. Thining of the apical wall of the left ventricle (white arrow) and cavitary thrombus (yellow arrow). Panel 1, right. Schematic representation. Panel 2, left. Characteristic apical aneurysm. Panel 2, right. Schematic representation. Panels 3–5. Histological features. Panels 3A and 3B. Microscopically, myocardial lesions consisted of a chronic inflammatory process with fibrotic scars and extensive mononuclear infiltrates. Panel 4. Immunostaining for T lymphocyte. Positive cells express CD45R0 antigen (brown); specialized myocardial cells have almost disappeared. Extensive mononuclear infiltrate, the majority of them being T lymphocytes. X20. Panel 5. Double immunostaining for the simultaneous demonstration of T lymphocytes (CD45RO) and macrophages (CD68). T lymphocytes (brown) in close contact with a macrophage (pinky cytoplasm). X1000.

macroscopic alterations are: (a) cardiomegaly (more than 500 g weight) and (b) characteristic apical lesion associated with localized parietal thinning, rarely presenting as posterior basal or parietal, or an apex aneurysm. Some authors propose two characteristic morphological types of chronic chagasic cardiopathy: "type I" or concentric, is characterized by a predominance of left ventricular hypertrophy with little dilatation or none, and circumscribed closed apical lesion and "type II" or eccentric, characterized by a wide dilatation and opening of the apical zone and frequently associated with thrombosis. Pathological evolution can progress from the concentric to eccentric type (**Figure 1**).

4. Clinical expression of the acute phase of Chagas disease

The Chagas disease presents two phases [10]. The initial acute phase, lasting for about 2 months after the infection and characterized by high parasitemia and parasite invasion to the tissues, is asymptomatic or shows mild unspecific symptoms. In this phase, symptomatic patients usually develop characteristic skin lesions or unilateral purplish swelling of the lids,

usually known as the Romana's sign, and can present fever, headache, difficulty in breathing, lymphadenitis, vague myalgia, and abdominal or chest pain. In this phase too, immunosuppression, and the decreased inflammatory response result in increased parasite load in locally infected tissues upon generalized tissue invasion [11].

5. Clinical expression of the chronic phase of Chagas disease

To date, regardless the chronic phase be asymptomatic or symptomatic with cardiological alterations, the prognosis on the eventual development of heart disease in a given patient is not feasible.

5.1. Clinical asymptomatic expression of the chronic phase

The reason most patients with Chagas do not develop heart disease is uncertain. The first studies suggested that asymptomatic patients without clinical and cardiological alterations and the general population had a similar cardiovascular risk, but over time, epidemiological data suggest a higher risk than presumed.

Collectively, the studies highlight the importance of studying the early inflammatory parameters indicative of early cardiovascular damage, evaluating potential implication on morbidity and mortality, and prognostic and therapeutic relevance.

5.2. Clinical symptomatic expression of the chronic phase

The clinical expression of the chronic phase usually manifests as alterations in the cardiac conduction system, mainly arrhythmogenesis and dysrhythmias derived from the fibrotic and atrophic lesions compromising the AV nodule, and the His bundle and branches, and autonomic dysfunction as well. Dysautonomia may affect blood pressure and cardiac frequency, eventually leading to orthostatic hypotension, syncope, and heart failure. Myocardial alterations are associated with chronic inflammation, fibrous hypertrophy, fibrosis, myocytolysis, tissue depletion of neurons, and vascular damage contributing to sudden death or heart failure. Some clinical features may result from upstream molecular mimicry and cardioactive autoantibodies production. Patients with chagasic cardiomyopathy produce anti-ß1 and -ß2 adrenergic, and anti-M2 cholinergic autoantibodies in the heart. These autoantibodies, originally directed against the parasite, would indistinctly recognize similar antigenic determinants in the host, a phenomena known as mimicry.

6. Factors that define blood inflammatory outcome in Chagas disease

The sustained systemic inflammatory burden may result from many host-parasite interactions, whereby the interplay of the natural and adaptive immune host response with the parasite will result in a varying degree of tissue damage, host aggression, and clinical outcome.

Then, the perpetuation of the Chagas disease inflammatory phenotype [10] is critical in understanding the complexity of the clinical outcome given that immunological homeostasis in chronically infected hosts could be spoiled by both parasite and host immune molecules and cells [10].

6.1. Factors that promote parasite evasion and persistence

The presence of the parasite [11] or its products like DNA, and other constitutive molecules in blood, myocardium, and the autonomic tissues lead to sustained immunological stimulation and *T. cruzi* evasive strategies. Today, the DNA amplification by polymerase chain reaction and other sensitive assays allow detecting parasites or their components in chronic symptomatic patients.

6.1.1. Parasitemia and sustained antigen stimulation

There is scarce evidence linking Chagas seropositivity with cardiovascular events in asymptomatic patients [9]. The perpetuation of *T. cruzi* antigens in cardiac tissue and the immune-mediated dysautonomia might be implicated in the cardiovascular pathogenesis [12] being responsible for the asymptomatic-to-the *cardiac* phenotype transition in Chagas disease. Different studies have shown the relevance of effector or memory CD4/CD8 lymphocytes and their effector cytokines not only in controlling parasite multiplication over the course of acute and chronic infection, but also participating in the pathogenesis of chronic Chagas disease [12]. The Th1 lymphocytes are critical in the control of Chagas disease during the acute stage unlike the chronic phase when they could be harmful. The adoptive transfer of *T. cruzi*-specific CD8+ T cells confers mice partial protection from *T. cruzi* inoculation [13], while cardiac damage may still develop. Conversely, the human being develops myocarditis in the presence of $CD4^+$ and $CD8^+$ T cells, and parasite components as well [14].

6.1.2. Cruzi virulence and immunomodulatory factors

Cruzi parasites cause pathology depending on factors like the parasite species and strains, the route of infection [15], and the host genetic background. Different parasite strains coexist in infected patients, and in natural reservoirs in domestic and peridomestic areas. The strain-dependent immunomodulatory effects of the parasite might influence parasite-host interactions [14]. Mucin-like sialic acid-acceptor glycoproteins and other parasite cell membrane products determine *Cruzi* virulence [15]. One of the hallmark parasite-derived molecules are the glycol-inositol-phospholipids (GIPLs) covering the parasite cell surface that alter the B cell compartment, work as TLR4 agonists, and mediate proinflammatory effects [16, 17]. In sum, *T. cruzi* virulence factors actively subvert the host immune system leading to chronic infection [18–21].

6.1.3. White adipose tissue is an immune active endocrine organ

Another survival strategy of *Cruzi* parasites is targeting the adipose tissue (AT) [22], both brown (BAT) and white (WAT), the largest endocrine organ in the body shaped by adipocytes, fibroblasts, macrophages, and endothelial cells. The adipose tissue is involved in many

physiologic functions including energy homeostasis and immunity [23], and might warrant long-term parasite persistence by providing a safe reservoir to avoid the host-defense mechanisms. Then, immune system suppression would result in parasite recrudescence and multiple tissue invasion [24]. Besides, the AT might be the major site for parasite reactivation as indicated by the finding of parasite-derived DNA in AT [25] in patients with CCC [25]. Likely, the AT serves as a parasite reservoir favoring opportunistic reinfection upon immunosuppression, as observed in chagasic transplanted or HIV patients, or those under immunosuppressive therapies [26]. As infection increases the level of TLR4 and TLR9, and of the mRNA of cytokines, chemokines, and of their receptors, the adipose tissue appears to be both a target and a sensor of parasitic infection even in the early, latent stage of *T. cruzi* infection. Recently, the *T. cruzi* was detected in the adipose tissue of chronically infected individuals [25].

6.2. Host-protective and pathogenic anti-*T. cruzi* immune-mediated-response

6.2.1. The innate immune system

Innate, nonspecific, immunity involves any pathogen-eliminating mechanism triggered promptly without memory requirement. The acute nonspecific inflammatory molecules serve not only as 'gateway' signals, generating conditions unfavorable for the invading agent, but they are also implicated in chronic inflammatory diseases. Chronic sustained inflammation actually contributes to cardiac hypertrophy.

6.2.1.1. Toll-like receptors agonists expressed by T. cruzi activate inflammatory pathways

Nonspecific immune system cells modify their functional repertoire (phagocytic activity, activation, antigenic presentation, migration, and adhesion) through pathogen-associated molecular pattern recognition receptors (PAMPs) like the toll-like receptors (TLRs), many of which can recognize several types of structurally unrelated PAMPs. These receptors trigger proinflammatory pathways' activation interacting with their pathogen-derived ligands, and even with endogenous molecules, and releasing effector molecules like cytokines. This signal cascade triggers the expression of cytokine genes, where the type of TLRs bound determines the type of response.

Different *T. cruzi*-derived molecules belong to the PAMPs family and act as TLR agonists inducing the secretion of inflammatory cytokines, chemokines, and the production of nitric oxide (NO) by cells of the monocytic lineage. In this regard, the first evidence was the identification of the trypomastigotes-derived glycosylphosphatidylinositol (tGPI) that anchors mucin-like glycoproteins (tGPI-mucins), as a potent agonist of the human TLR2 [27] inducing proinflammatory responses on cells which express normal levels of TLR2 and TLR4 (**Figure 2**).

Another epimastigote-derived GPI family member, the glycoinositolphospholipid characterized by a lipid moiety, induced TLR4-mediated NF-κB activation. Many GIPLs freely anchor at the surface membrane of all the parasite life-cycle stage forms, whether infective metacyclic trypomastigotes or epimastigotes forms and have pleiotropic properties [28]. The variable lipid moiety composition of different GPI anchors determines the TLR type specificity for TLR2 (alkylacylglycerol) or TLR4 (dihydroceramide).

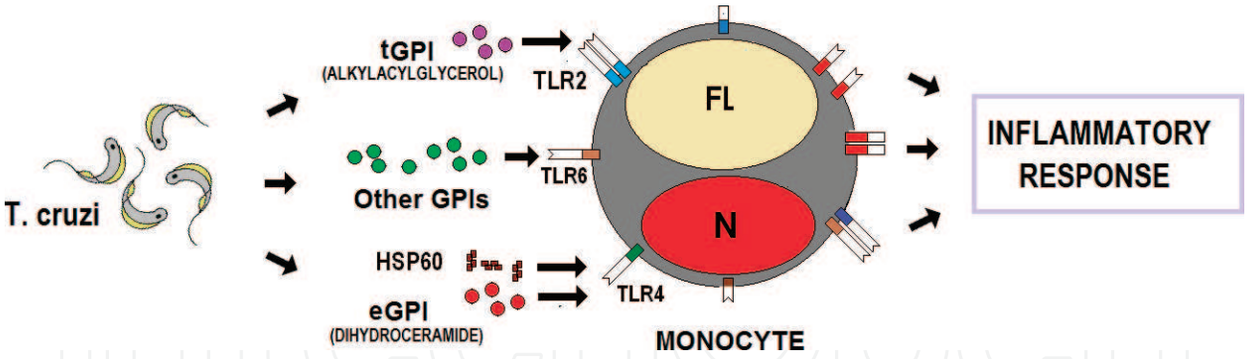

Figure 2. *T. cruzi* molecules PAMPs members are TLR agonists. The variable lipid moiety composition of different GPI determines whether their recognition is mediated by TLR2 (alkylacylglycerol) or TLR4 (dihydroceramide). TLR4 agonist triggers powerful proinflammatory molecules release. FL: Phagolysosome; GPI: Glycosylphosphatidylinositol; tGPI: Trypomastigotes-derived glycosylphosphatidylinositol; eGPI epimastigote-derived glycosylphosphatidylinositol, HSP: Heat shock protein.

6.2.1.2. Toll-like receptors and resistance to infection

Direct testing of the hypothesis that TLR triggering by PAMPs is crucial for host resistance to infection but is currently not possible due to unavailability of *T. cruzi* strains lacking the expression of any TLR agonist. Nevertheless, studying the course of infection in TLR-encoding genes-deficient mice, evaluating mortality, parasitemia, and several parameters of the innate and acquired immune responses have brought an additional understanding of the impact of impairing TLR-mediated recognition of *T. cruzi* in developing host susceptibility to the infection. In this regard, MyD88-deficient mice lacking the transducer of multiple TLR-signaling pathways first evidenced the crucial involvement of TLRs in host resistance to *T. cruzi* [29, 30].

6.2.1.3. Cardiac toll-like receptors increase in ischemia/reperfusion-induced cardiac hypertrophy

The development of cardiac hypertrophy involves TLR signaling so that MyD88 blockage attenuates cardiac hypertrophy and extracellular heat shock protein 70 (HSP70) induces cardiomyocyte inflammation [31, 32].

6.2.2. The adaptive immune system

The immune-mediated-pathology (IMP) links to parasite persistence inducing protective effector and autoimmune response and has been a subject of debate in CCC for years. Immune effector cells along with autoantibodies participate in both protective and pathogenic adaptive responses in CCC. Typically, histopathological examination in chronic myocarditis reveals inflammatory polymorphism with macrophages, eosinophils, mast cells, B and T lymphocytes, and granuloma cells, and a predominance of B cells and plasma cells in the epicardium and T cells in the myocardium, which progresses towards fibrosis. The mononuclear infiltrate and its mediators would be, at least partly, primarily responsible for myocardial damage [33].

6.2.2.1. Autoimmunity

At first post-infectious autoimmune myocarditis was proposed to reasonably explain the mismatch between myocardial areas showing parasite invasion and those with myocardial damage revealed by pathology examination that hampered reliably establishing Chagas disease pathogenesis.

- Cardiac epitopes share amino acid sequences with *T. cruzi* epitopes.
- The transfer of lymphocytes to syngeneic recipients produces inflammatory lesions in cardiac and nervous tissue.
- Chronic chagasic patients have autoantibodies in the bloodstream.
- Plasma cells obtained from murine myocardial lesions release anti-cardiac antibodies.
- T-lymphocytes obtained from human biopsies show cardiac muscle reactivity.

6.2.2.1.1. Evidence derived from the autoreactive immune response

While activation of autoreactive clones (possibly by polyclonal activation) occurs during the acute primary infection phase, autoantibodies appear to be generated during both the acute and chronic phases of the disease, likely perpetuated due to myocardial reactivity regardless of the etiologic agent. Both humoral and cellular cardiac autoimmunity might develop upon acute *T. cruzi* infection in the genetically susceptible host [34]. Another hypothesis sustained that autoimmunity develops only after sustained low-level stimulation of self-reactive cells over the chronic phase. Altogether, self-reactivity was initially proposed as a mechanism of tissue damage.

Many publications have mentioned the presence of cardiac tissue-parasite cross-reactivity. *T. cruzi* may induce antibodies and T cells also reactive to host antigens causing autoimmune reactions.

Certain antigens might induce nonspecific polyclonal activation, expanding clones that were in the anergic state as the polyclonal B cell activation associated with hypergammaglobulinemia and delayed specific humoral immunity reported in *T. cruzi* experimental infection in mice [35].

All in all, self-reactivity is accepted in Chagas disease though only subsidiarily contributing to myocardial tissue damage and deterioration as argued for the presence of autoantibodies and lymphocytes at the site of the lesion. Actually, immunosuppression not only does not cause improvement but rather aggravation of the course of the disease. Indeed, was self-reactivity relevant to injury, immunosuppression should be beneficial. Immunosuppression during acute infection reduces tissue inflammation while the parasite load increases in the infected tissues in mice. However, immunosuppression results in a generalized tissue invasion aggravating the disease. In chronically infected individuals, parasitemia is undetectable but any induced or acquired immunosuppression condition including pharmacological treatments,

AIDS, a transplant, an autoimmune disease, leukemia or pregnancy, may trigger reactivation. When immunosuppression occurs during the chronic stage, skeletal and cardiac muscle inflammation increases, allegedly explaining why a subset of patients presenting an insufficient or suboptimal immune response develop heart disease [36].

6.2.2.1.2. Evidence derived from molecular mimicry and the formation of cardioactive autoantibodies

Cardioactive substances from sera from chagasic patients. Subsequently they were characterized as antibodies with specificity towards the cardiac β-adrenergic receptors that acting as partial agonists increased mechanical tension and sinus beating frecuency in chagasic patients. The Cardiology Service of the Ramos Mejía Hospital contributed to the characterization of the anti-cardiac receptor reactions, and the results indicated that self-reactivity of this kind was caused by parasite epitopes with low affinity for cardiac receptors, later proved by the identification of anti-*T. cruzi* P ribosomal proteins in chagasic individuals. In *in vitro* or *ex vivo* experiments, IgG-enriched serum fractions obtained from chagasic patients modify baseline heart beating frequency in cultured cardiomyocytes, increase cardiac inotropism, and trigger atrioventricular blockage in isolated hearts. Rabbits immunized with the major immunogenic region of β-adrenergic receptors develop cardiomyopathies and malignant arrhythmias, and cardioactive antibodies have also been observed in other pathologies. Patients with ethiologically different chronic heart diseases like the idiopathic dilated cardiomyopathy or presenting primary electrical alterations produce chagasic IgG-like enriched fractions with antibody activity. Circulating autoantibodies to cardiac beta-adrenergic and muscarinic receptors may affect cardiac function in chagasic patients. The prevalence of anti-autonomic receptors antibodies was higher in patients with chronic chagasic heart disease and other forms of heart disease than in the healthy counterparts [36].

6.2.2.2. Immune origin of the disease and the localized inflammatory response

The 'tissue load of parasites' is associated with the 'severity of the lesion' and might predict not only the characteristics of the immune response in the acute or chronic (reactivation by immunosuppression) stages of the disease of the disease but also of the localized inflammatory response. Accordingly, tissue specificity would result from the local 'parasites persistence' in the site of inflammation. Whole, fractionated, or recombinant parasites immunization triggers inflammatory lesions and electrocardiographic alterations. The spectrum of clinical presentations might result from both the efficacy of the immune response during the acute stage and the parasite strain involved. Individuals infected with less virulent strains and immunocompetent hosts should become asymptomatic over the chronic stage (**Figure 3**). The immune response so results from the net balance of the *T. cruzi* strain immunogenic potency and the regulatory T cells and effector lymphocyte subpopulation Th1/Th17 during the infection. The IL-17 produced during experimental *T. cruzi* infection regulates Th1 cells differentiation and parasite-induced myocarditis. A low regulatory T cell activity and the frequency of IL-17-producing T cells correlate with CCC severity [37], not precluding a minor participation of the self-reactive immune reactions in producing injury.

Induced immunosuppression fails to cause relevant autoimmune-mediated damage for it aggravates rather than ameliorates the disease. Adoptive lymphocytes transfer to syngeneic

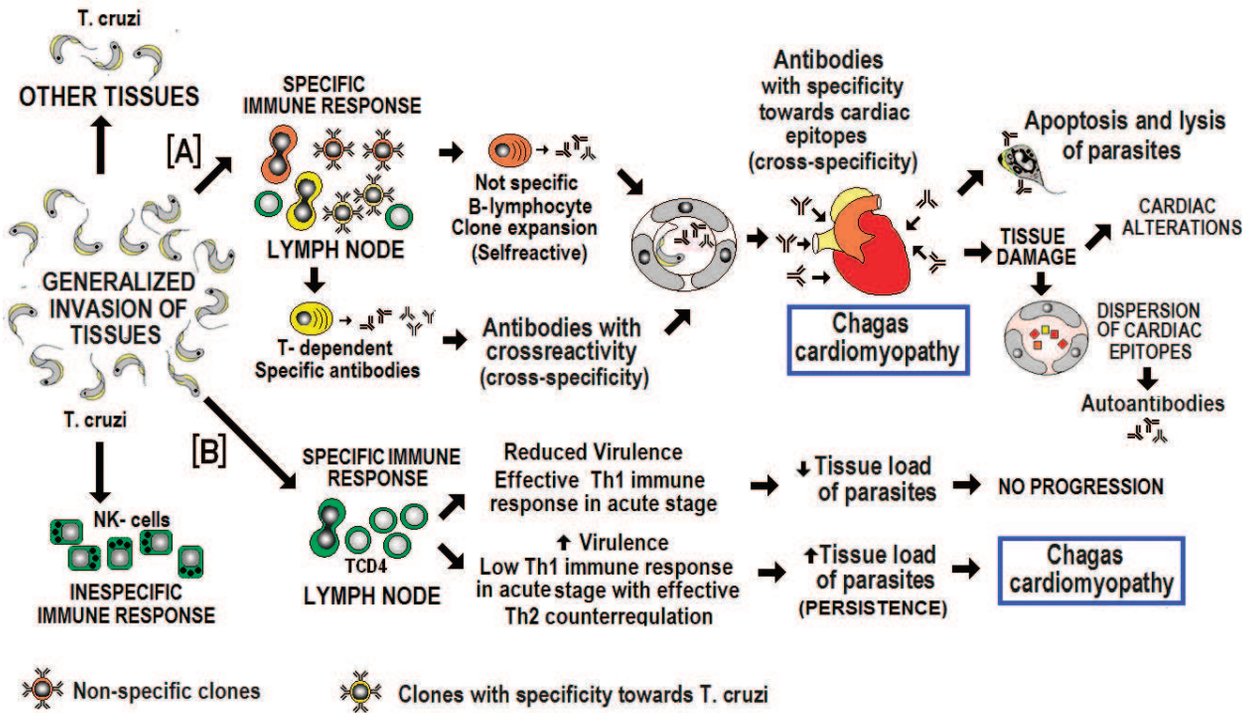

Figure 3. Possible mechanisms involved in the immunopathogenesis of Chagas disease. Although the immune system protects man from massive infection, he is unable to completely eliminate all parasites. (A) The humoral protective and immunopathogenic immune response to *T. cruzi* has been intensely studied. Although many questions remain, the antibody response is always present in infected individuals and many of the antibodies have a protective capacity. Several aspects are related to the presence of autoreactive antibodies in chagasic patients and in experimental chagasic models. The presence of similarities between the epitopes of the parasite and cardiac tissue leads to the expansion of autoreactive clones. Some authors argue that some autoantibodies should be due to the existence of polyclonal activation. Other autoantibodies may be the result of the autoantigen release of damaged tissue (epitopes dispersion epiphenomenon). (B) Th1 lymphocytes have great relevance in the control of the disease. The importance of the T-dependent response is evidenced by the observations recorded in immunosuppressed patients in whom the disease worsens. In vitro studies would indicate that the NK cell-mediated immune response (ADCC and natural cytotoxicity) could participate in the in vivo response. According to some authors the antigenic persistence would be the main mechanism inducing the inflammatory immune response in the chronic stage and IL17 seems to be involved in CCC. This interpretation postulates that the sustained activation of the immunoinflammatory response is the main cause of tissue damage.

recipients produces inflammation indicating that the T-response can effectively control the disease. The cellular immune response is critical in controlling *T. cruzi* infection, and the developed vaccine so far exacerbates damage and progression of the disease. Then, the vaccine either failed in enhancing the adequate response and/or its design requires further adjusting the epitopes. Of note, despite cellular control the infection, immune response persistence and cardiac infiltrated cells may cause injury. Finally, the contribution of autoantibodies is complex as it is likely involved in further enhancing cardiovascular damage.

7. Suppressor of cytokine signaling proteins, immune regulation, and dilated cardiomyopathy

Cell-cell signaling is an essential hallmark of multicellular organisms for communicating different cell populations [38] and is particularly crucial for the immune system function. The 'suppressor of cytokine signaling' (SOCS) plays a critical role in the regulation of all

SOCS type	Factor	Effect	References
SOCS3	G-CSF	Hematopoietic neutrophilia/inflammatory conditions	Chen et al. [35]
	IL-23 (↑IL17), (↑IFN-γ)	Enhance Th1 and Th17 polarization	Chen et al. [35]
	Leptin	Resistance to diet-induced obesity	Yang et al. [39]
	IL-6, (+IL-27)	Reduced CD8+ T cell proliferation	Yang et al. [39]
	TNF-γ, IL1β	Hypertrophy, fibrosis and inflammation	Liongue et al. [33]
	Global action	• Critical role in regulation of cytokine signaling • Control of hyperproduction of IL-10 and TGFβ • Promote Th2 response • Indirect sustained Th1 activity	Kinjyo et al. [34], Chen et al. [35]

Table 1. Main action of SOCS3 as a critical regulator.

crossroads of the cytokine-induced pathogenesis of dilated cardiomyopathy. The SOCS3 transgene induces Th2 responses, and SOCS3 gene deletion did not enhance Th1 polarization as expected but induced a negative regulator of the Treg subset with increased IL-10 and TGFβ production in mice [39, 41]. Not only SOCS3 is essential for G-CSF, IL-6, LIF, and leptin signaling, but is also an indirect regulator of IFNγ signaling and a negative regulator of IL-23 signaling, inducing IL-17-secreting T cells (Th17) polarization (**Table 1**).

The SOCS3 pathways are important, altering cardiac physiology by affecting molecular targets associated with myocardial changes implicated in structural pathologies. The SOCS3 proteins regulate specific cytokine pathways related to cardiac growth and enlargement and seem to be consistent with their roles critical regulators of hypertrophy, contractile dysfunction, and ventricular arrhythmias. They were, as their name suggests, first described as cytokine signaling inhibitors as observed for the Janus kinase/Signal Transducer and Activator of Transcription/Suppressor of Cytokine Signaling (JAK/STAT/SOCS) signaling pathway (JSS-SP). The remodeling by cytokine receptor signaling mediated by the JSS-SP provides a morphological basis explaining the pathogenesis of myocardial hypertrophy, fibrosis, and inflammation [41] in CCC. The inflammatory markers TNFα, and IL1-β represent potential targets in cardioprotection and therapy [42].

The SOCS3 protein is a key negative-feedback regulator of the gp130 receptor involved in signaling pertaining cardiac hypertrophy and survival. Activation of the gp130 without SOCS3 regulation leads to cardiac hypertrophy, in line with their roles as negative regulators of cardiac growth. Also, SOCS3 regulation on cardiac gp130 signaling participates in the pathogenesis of contractile dysfunction and ventricular arrhythmias. Consistently, human CCC is characterized by segmental left ventricular wall motion abnormalities (WMA), mainly in the early stages of the disease.

The failure of the SOCS3 protein, also a major negative regulator of both leptin, and insulin signaling, might participate in the pathogenesis of obesity, and associated metabolic abnormalities as found for diet-induced and genetic obesity, systemic inflammatory burden, and

hyperlipidemia. In sum, the SOCS3 may be critically negative regulators of inflammation, cardiac hypertrophy, contractile dysfunction, and ventricular arrhythmias. However, our understanding of the origins of the individual pathway components and their assembly into a functional pathway has remained limited.

8. Long-lasting systemic inflammatory burden and clinical Chagas progression

The immune-inflammatory response plays a key role in cardiovascular damage [40, 41], and *T. cruzi*-derived molecules may sustain the TLR-mediated innate immune response inducing inflammatory cytokines and chemokines secretion. The adaptive immune response to Chagas antigens may protect the host from secondary reinfection, though damaging the CV system due to inflammation, and the associated connective repair (fibrosis).

Crossroads between "natural, and specific immunity effector cells and molecules" and "parasite persistence strategies in blood, adipose tissue, the heart, and other infected tissues" seem to contribute to cardiovascular risk. Tissue damage induces inflammatory reactions. Immune-activated pathways are the main contributors to systemic inflammation in human CCC by active crosstalk between different CVRFs, metabolic and immune-mediated innate and adaptive host-parasite interactions. The contribution of natural and specific immunity against Chagas antigens enhance systemic inflammatory burden (SIB). Certainly, the immune system is not the only source of inflammatory molecules, but other tissues also contribute to enhancing systemic inflammatory burden as the WAT which releases inflammatory cytokines.

In a recent study, the levels of IL1β, IL6, IL10, TGFβ, IL12, IL17, TNFα, and serum IFNα were different in either chronic asymptomatic or cardiac chagasic patients compared with healthy controls. The asymptomatic patients had a higher plasma TNFα concentration (eightfold) and IL10, and lower IFNα than in normal controls, suggesting a process of immune regulation. Neither the interaction with traditional CVRFs and their contribution to CVR nor control-matching for age, sex, weight, or BMI were considered in this study. The advent of noninvasive imaging techniques allows studying the relationship between inflammatory markers in subclinical atherosclerosis development. The association of many systemic diseases with an increase in the prevalence of cardiovascular diseases involves immunoinflammatory mediators related to chronic inflammation and cannot be explained by the classic CVRFs. Unlike the classical CVRFs like the lipid profile, the approaches based on the contribution of the inflammatory milieu to cardiovascular disease development have not yet allowed identifying clinically validated biomarkers regardless the evidence suggesting their association with increased morbidity, and mortality except CRP [43].

Scientific research is encouraged to delve into the important role suggested for the inflammatory response in the metabolism and control of the atherogenic potential [44]. Hypercholesterolemia and inflammation are certainly considered contributive partners in atherosclerosis.

Only lipid-related indicators like LDL- and HDL-cholesterol fractions, and triglycerides are currently recommended in predicting cardiovascular risk. Paradoxically, plasma total

cholesterol level is accepted as a marker of relative CVR, though more than 50% of all cardiovascular events can occur in individuals with concentrations below the accepted normal total cholesterol level [56].

With a complete understanding of atherogenesis, the atherothrombotic markers, and the already mentioned inflammatory markers [45] are potentially available for CVR estimation.

Recent data suggest that the measurement of related inflammatory markers may improve cardiovascular risk assessment. Certain markers being evaluated include a group termed as "cellular" cytokines (e.g., IL1, IL2, IL12p40, IL15, IL17, TNF, IFN, and IFN), and "humoral" cytokines (e.g.IL4, IL5, IL6, IL10, and IL13), growth factors and angiogenic [e.g., EGF, VEGF, FGF, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage-colony-stimulating factor (GM-CSF)], and chemokines [e.g. CCL2 (MCP1), CCL3 (MIP1), CCL11 (Eotaxin), and CXCL8 (IL8)]. The role of some of them in cardiovascular pathogenesis is described below.

8.1. Soluble ICAM1

Inflammatory stimuli trigger a dramatic increase in ICAM1 expression on the vascular surface. Later on, inflammatory cytokines induce the endothelial expression of VCAM1 which, like ICAM1, interacts with leukocyte integrins promoting the firm adhesion of leukocytes to the surface of the endothelial cells. After proteolytic cleavage, the endothelial ICAM1 molecules can be released into the circulation as soluble molecules (ICAM1s), the level of which correlates with CVRFs like smoking, hypertension, hypercholesterolemia, and hypertriglyceridemia and with acute phase reactants like the PCR, and increases in patients with coronary disease. The circulating level of the soluble forms of ICAM-1, VCAM-1, selectin, and CD44 are remarkably high during the acute *T. cruzi* infection, while the soluble forms of VCAM-1 and P-selectin increase in chronic infection [46–52]. As inflammation markers, the soluble forms of ICAM-1 might be notably higher in patients with DCM [53–55].

8.2. Chemotactic monocyte-1 protein

An important early step in atherosclerosis is the adhesion of monocytes to activated endothelial cells. The endothelium produces several molecules critical in the proatherogenic events like the endothelium-released MCP1 which contributes to increased monocyte recruitment, and activation of nuclear factor kappa B (NF-kB) involved in the transcription of many functional genes in the inflammatory process [64]. Recently, CCL2/MCP-1 has emerged as a critical factor in infectious and autoimmune myocarditis, is largely produced in *T. cruzi*-loaded mice hearts, and it promotes macrophages infiltration and parasite destruction. *T. cruzi*-infected CCL2$^{(-/-)}$ mice developed higher parasitemia, dying prematurely, and showed increased levels of TNF, IFN-gamma, and IL-10 in plasma, clinical signs of systemic inflammatory response. Cardiac density of amastigote nests was associated with leukocytes infiltrates. Other studies demonstrated that CCL2 contributes to the reduction of parasite growth by controlling the distribution, cellular composition, and the status of inflammatory infiltrates in acute *T. cruzi* infection [21]. More recent evidence shows that polymorphisms involved key molecules related to innate immunity, and that cell migration plays a critical role in genetic susceptibility to CCC [56–58].

8.3. Interleukin 10

Within the group of the so-called anti-inflammatory cytokines, the human IL10 is associated with an anti-atherogenic action reducing inflammation. Cytokine knockout of the Th1 inhibitor IL10 increase vascular lesions [59]. IL10 has strong regulatory properties on macrophages and T cells, and negatively regulates many cellular processes involved in atherosclerotic plaque development and stability.

A recent study revealed comparable serum cytokine levels in cardiac chagasic and asymptomatic patients though IL10 level was higher, and IFNγ level was lower in the former suggesting a greater regulatory activity in cardiac patients. Presumably, the IL10 level is ineffective to restore homeostasis. Likewise, circulating IL10 increases in patients with DCM.

8.4. Transforming growth factor beta (TGFβ)

The transforming growth factor beta is produced by different cell types including adipocytes, macrophages, endothelial cells, smooth muscle cells, platelets, and regulatory T cells. The TGFβ1 factor stimulates PAI-1 release and suppresses leptin release from the human adipose tissue. It inhibits atherogenesis modulating T-lymphocyte activity rather than modulating the prothrombotic and fibrinogenic activity, as confirmed in TGF II receptor type-KO models. It is also involved in regulating host tissue fibrosis. The oral administration of GW788388, a novel kinase inhibitor type associated with TGFß I and II receptors, remarkably increased cardiac cells' survival time and decreased cardiac fibrosis, offering a potential alternative to the current asymptomatic Chagas treatment. However, the cost-benefit balance is uncertain since circulating TGFβ modulates the pathogenic effector immune response avoiding immune damage but exacerbating fibrosis. Recent studies indicate that the deep alterations induced by circulating TGFβ increase in patients with DCM with or without cardiac fibrosis [60–62].

8.5. Interleukin-17A

The interleukin-17A cytokine released by Th17 cells is elevated in plasma in atherogenesis mice models. Increased serum levels of IL17 and IFNγ are found in patients with coronary atherosclerosis. The proatherogenicity of IL17A results from the monocytes/macrophages recruitment into the aortic wall [63]. The differentiation of Th17 depends on IL23 and IL6 released by myeloid dendritic cells, IL1, IL6, and IL21 derived from macrophages and T lymphocytes [64]. In humans, TGF*α* acts as a negative regulator of IL17. For years, Chagas-associated cardiac damage has been attributed to immunological dysregulation, including an imbalance between pro- and anti-inflammatory cytokines, Th1-Th2 immune deflection, and regulatory T cell activity. Recently, IL17 produced during experimental *T. cruzi* infection regulated Th1 differentiation, and parasite-induced myocarditis. The decrease in IL10 and IL17 cytokines' production in association with high levels of IFNγ, and TNF*α* correlates with the severity of human chagasic cardiomyopathy. This immunological imbalance might be causally related to a poor suppressor activity of the regulating T cells controlling myocardial inflammation. Finally, the derived IL17A-fibroblast and the derived -GM-CSF-macrophage axis are potential targets for the treatment of DCM and related inflammatory cardiac disorders [64].

8.6. C-reactive protein

The CRP marker level is used as a predictor of future cardiovascular events, being a good estimator of mortality risk in different contexts, particularly in metabolic syndrome in the general population. It has also been suggested as a direct stimulator of plaque formation decreasing endothelial nitric oxide synthase (eNOS) activity [65], and implicated in other deleterious effects. *In vitro* studies provide evidence of the proatherogenic direct effects of CRP as found in endothelial dysfunction, Also, an increase CRP serum level in chagasic patients has been associated with a greater progression towards heart failure. High hs-CRP level is associated with a higher incidence of the long-term combined endpoint of all-cause mortality and hospitalization in patients with DCM. Besides, oxidative stress molecules and hs-CRP are both associated with heart failure and damage severity in patients with DCM [66, 67].

8.7. Tumor necrosis factor alpha (TNFα)

The pleiotropic TNFα cytokine is one of the most potent mediators of inflammation. It is associated with an increased CVR. It can induce proatherogenic lipid alterations, including increased LDL-cholesterol and HDL-cholesterol [68] and promote hypercoagulability inducing tissue factor (TF) expression in endothelial tissue and suppressing anticoagulant activity through activated thrombomodulin-activated protein C [69]. It also induces endothelial dysfunction through decreasing nitric oxide and regulating adhesion molecules, an early critical step in atherogenesis. Several investigations in animal and human models provide convincing evidence on the action of TNFα as one of the major regulators of vascular homeostasis. Blocking of TNFα results in a significant decrease in Lp (a), homocysteine levels and increases in Apo AI, triglycerides, and Apo B concentration. The prolonged use of TNFα blocking agents interferes with proatherogenic action, reducing the incidence of cardiovascular events. Taken together these studies confirm a critical role of TNFα at the prothrombotic, proinflammatory and metabolic level. An association between TNFα levels with heart failure was observed in chagasic patients. Autopsies specimens confirmed the presence of cardiac inflammatory cell infiltrates showing a Th1 cytokines pattern. Chronic asymptomatic chagasic patients have TNFα plasma concentrations roughly eightfold higher than healthy controls, and TNFα may play a role in progression to heart failure, The increased level of TNFα might also be related to disease severity in chronic Chagas disease as found in patients with DCM. Interestingly TNFα blockage aggravates experimental CCC [70, 73].

8.8. Interleukin-1 (IL-1)

The IL-1 interleukin is a crucial proinflammatory mediator in acute and chronic inflammation, and also a powerful innate immune response inducer. It induces the synthesis and expression of several hundreds of secondary inflammatory mediators in different diseases [74, 75]. The inflammatory response is associated with the expression of numerous cytokines [interferon gamma, interferon alpha, tumor necrosis factor (TNF), and interleukin-3 (IL-3)], which stimulate xanthine oxidoreductase (XOR). The main circulating form of IL-1 is the IL-1β, initially synthesized as the pro IL-1β a precursor which is activated by caspase-1 cleavage in the setting of a macromolecular structure known as the inflammasome [71]. Many potential triggers

of the inflammasome have been identified, including microbial agents, ischemia, damaged cells, cholesterol crystals, and TLRs ligands such as danger-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs). The IL-1 molecule has been associated with endothelial dysfunction, hypertension, heart failure, and diabetes [75]. Recent studies indicate that XOR and XO serum levels are considerably increased in both cardiac and asymptomatic patients following *T. cruzi* invasion. Serum levels of IL-1β could be used in predicting the long-term outcome of patients affected by idiopathic DCM [71, 76]. Cardiac fibrosis and heart failure progression in inflammatory dilated DCM might be related to the myeloid differentiation factor-88/IL-1β signaling pathway [72, 77].

8.9. Interleukin-6

IL-6 provides a link between innate and adaptive immunity through the regulation of leukocyte activation, differentiation, and proliferation. During the acute and chronic inflammatory response, macrophages release TNFα in the presence of a variety of stimuli including atherogenic factors. In the macrophages, TNFα triggers the release of TNFα, and of more IL1B, which stimulates endothelial cells to produce IL6 and IL8. To date, both the scientific outcome of experimental studies, and the abundant clinical evidence in atherosclerosis indicate that low-intensity sustained inflammation plays a key role in atherosclerotic plaque formation, progression, and destabilization leading to clinical endpoints like myocardial infarction, sudden death, or stroke. The underlying mechanisms are still unclear, despite the intense research over the past two decades. Both IL6 and its signaling events contribute to atherosclerotic plaque development and destabilization. Increased levels of IL6 [78, 79] and CRP, an accepted CVRF, can also contribute to atherosclerosis and arterial thrombosis by activating tissue factor production, increasing adhesiveness of endothelial cells, fibrinogen and factor VIII and stimulating platelet production, and aggregation [91]. In addition, smooth muscle cells (SMC) also produce abundant IL-6. Other inflammatory factors generated by adipocytes like IL6, CRP, and TNFα are also implicated in the pathophysiology of the metabolic syndrome. The polymorphism of IL6 genes correlates with the severity of coronary artery disease, and with myocardial infarction risk [80, 81], but not with carotid atherosclerosis, which seems to be independent, These findings clearly suggest a strong association between IL6 levels, atherosclerosis, and risk of cardiovascular death. Produced locally IL6 in the endothelial vasculature and by SMC, IL6 induces ROS production, proliferation, and SMC migration. IL6 is an important autocrine and/or paracrine regulator of SMC proliferation and migration, critical steps in atherosclerosis progression. Besides, circulating IL-6 levels (in parallel with an increase in circulating CRP) increase with progression to heart failure in Chagas disease. These observations agree with the polymorphisms analyzed in patients with idiopathic dilated cardiomyopathy (IDCM) that relates to TNF, IL-6, and CRP profile. A recent study shows that a rough increase in serum IL-6 is incidental with chronic IDCM [82].

9. Conclusion

Search results illustrated that immune-activated pathways are the main contributors to systemic inflammation in human CCC due an interplay and active crosstalk between different

traditional risk factors, mainly metabolic and inflammatory factors derived from immune-mediated host-parasite interactions, both innate and adaptive.

Tissue damage induces inflammatory reactions leading to dilated cardiomyopathy in genetically predisposed persons. The DCM represents an essential hallmark of CCC. Evidence suggests that the pathway of inflammation in DCM culminates in altered concentrations of various markers in peripheral blood, including oxidative stress molecules and markers of vascular and systemic inflammation. These scenarios necessarily require a means of communicating between different cell populations. Crossroads between "natural and specific immunity effector cells and molecules" and "parasite persistence strategies in blood, adipose tissue and heart and other infected tissue" would appear to contribute to cardiovascular risk. The Suppressor of Cytokine Signaling (SOCS) plays a critical role in the regulation of all crossroads of cytokine inflammatory network that induced pathogenesis of dilated cardiomyopathy and seems to play a critical role as negative regulators of inflammation, hypertrophy, contractile dysfunction and ventricular arrhythmias. However, our understanding of the origins of the individual pathway components and crossroads and their assembly into a functional pathway is limited so far. Unlike classical risk factors, approaches based on inflammatory status needs clinically validated biomarkers and its contribution to the development of cardiomyopathy in chagasic and IDCM needs additional studies, even when there is strong evidence suggesting increased morbidity and mortality associated with the systemic inflammatory burden and inflammatory cardiomyopathy.

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