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Chagas disease: Immunology of the disease at a glance

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ABSTRACT

Chagas disease is an important neglected disease that affects 6–7 million people worldwide. The disease has two phases: acute and chronic, in which there are different clinical symptoms. Controlling the infection depends on innate and acquired immune responses, which are activated during the initial infection and are critical for host survival. Furthermore, the immune system plays an important role in the therapeutic success. Here we summarize the importance of the immune system cytokines in the pathology outcome, as well as in the treatment.

1. Introduction

Chagas disease is an important public health problem because it affects mainly a portion of the population that has lower socioeconomic resources and less access to health care. There are about 6–7 million people worldwide with the disease [1], which is caused by the protozoan *Trypanosoma cruzi* and was discovered in 1909 by the physician Carlos Chagas. Benznidazole (Bz) and nifurtimox (Nx) are the current treatment of the disease, which are effective in the acute stage of the disease, but not in the chronic stage [2]. Furthermore, these drugs have a high toxicity that leads to different adverse effects. Despite being discovered 111 years ago there is still much to be understood about it [3], especially concerning the host immune response and cytokines.The life cycle of *T. cruzi* begins when the vector — different species of triatomines [4] — makes the blood meal in an infected host. In the vector, the parasite differentiates into epimastigotes. After that, epimastigotes migrate to the hindgut of the vector where they differentiate into metacyclic trypomastigotes (infective form). These forms are eliminated in the urine and feces of the vector during blood meal. In the mammalian host, trypomastigotes infect cells and later turns into amastigotes (intracellular form). These amastigotes will turn into trypomastigotes, which rupture cells and spread the infection in the host. In the Fig. 1, it is possible to see the vector and the mammalian stages of the parasite.

The disease has two phases: acute and chronic. The acute phase may present in some ways: asymptomatic, symptomatic with nonspecific symptoms (fever, apathy, hepatosplenomegaly, etc.). After this acute phase, about one-third of patients progress to the chronic phase. At this stage, there may be progression to the indeterminate form - without evidence of organic impairment - or to the symptomatic (cardiac, digestive or cardio-digestive), decades after the initial infection [5].

Patients with the indeterminate form have positive serology for anti-*T. cruzi* antibodies, normal electrocardiogram, and normal radiology of the chest, esophagus and colon [6]. Individuals with the digestive form have alterations in the esophagus and colon motility, which can lead to problems of deglutition, regurgitation and constipation [7]. Most patients with the chronic form have the cardiac manifestation, which is extremely debilitating. This disease presentation has as its main histopathological characteristic myocardial inflammation, which can lead to

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enlargement of the heart (megalocardia), together with myocytolysis and fibrosis [8].

There are two antithetic strands of thoughts for chronic chagasic pathogenesis. The first one brings the idea that *T. cruzi* induces a response of the immune system to normal tissues of the host and this is independent of its presence in the tissue, that is, it induces an autoimmunity [9]. The second supports the hypothesis that the continuous presence of the parasite in the tissues is responsible for the inflammation and damage observed [10].

Controlling *T. cruzi* infection depends on innate and acquired immune responses, which are activated during the initial infection and are critical for host. Although there is a conflict about the mechanism of Chagas' disease pathology, the role of the cytokines has been reported in the acute and chronic phases of the disease. Due to the importance of the immune system in the pathogenesis of the disease, it is also important to understand how treatment can influence in disease progression.

2. Immune response in the acute phase

The innate immune response plays an important role in the initial fight against the parasite, and toll-like receptors (TLRs) help to orchestrate this response. Since cells of the innate immune response are directly involved in combating the initial infection with *T. cruzi*, it is interesting to understand the interaction of the parasite compounds with the TLRs in order to stimulate the capacity of this immune response to fight the infection [11]. TLRs are a family of transmembrane proteins, evolutionarily conserved between insects and humans that are part of a group of molecules called pattern recognition receptors (PRRs). When interacting and recognizing molecules called the pathogen-associated molecular pattern (PAMPs) they can induce an immune response, both innate and acquired. Several molecules of the parasite have been identified as PAMPs for TLRs. The main ones are the glycosylphosphatidylinositol-anchors derived from *T. cruzi* mucin-like glycoproteins (GPI-mucins) found in large quantities in the cell membrane of the trypanosomatids. Studies have shown that GPIs derived from trypomastigotes are recognized by TLR2, which is active after the dimerization with TLR6 [12,13]. Another molecule of the parasite, glycoinositolphospholipids (GIPLs) is recognized by TLR4 [12,14,15]. The first confirmation of the importance of TLRs in the mechanisms of resistance to *T. cruzi* was achieved using knockout mice for the adapter molecule MyD88, essential for signaling to almost all TLRs. These mice, when infected with trypomastigotes, showed high parasitemia and mortality that were associated with a low production of IFN-γ and IL-12 [16,17]. In addition, TLR4 knockout mice had severe parasitemia, confirming the role of TLR4 in resistance to *T. cruzi* infection [15,18]. Related to this, knockout mice for TLR2 and TLR9 (which recognizes the parasite's DNA) were more susceptible to infection by *T. cruzi* in addition to having a low Th1 response [19]. According to previous studies, TLR9 plays a crucial role in establishing the Th1 response, while TLR2 appeared to act as an immunoregulator in the early stage of infection. Gravina and co-workers [20] investigated the role of TLRs 2 and 9 in experimental acute infection by *T. cruzi*. They observed that dendritic cells, macrophages and monocytes use these two TLRs differently during the acute phase of infection - leading to different production of TNF and IL-12 between these cells - and that only TLR9-deficient mice are susceptible to *T. cruzi* infection.

Furthermore, studies show that macrophages, dendritic cells and natural killer (NK) cells trigger a strong inflammatory response along with increased production of cytokines and chemokines [21]. Chemokines are highly conserved proteins that are involved in a high variety of biological process, especially in leukocyte migration [22]. Yet not largely explored, a great range of chemokines and their receptors have been described in the pathogenesis of Chagas disease. In the acute phase, augmented production of inflammatory cytokines (IL-12, TNF-α, and IFN-γ), and chemokines (CCL2, CCL3, CCL4, CCL5, and CXCL10) have been shown [23]. In mice infected with *T. cruzi* in the acute phase, CXCL9 and CXCL10 seems to be important in the control of the parasite burden [24]. Also, in the chronic phase, it was demonstrated that Chagas disease patients also had higher serum levels of CXCL9, CXCL10 and IL-1β with lower serum levels of CCL5 than non-infected subjects [25]. However, another study demonstrated that chronic patients that showed higher levels of CXCL9 and CXCL10 mRNA also had higher intensities of myocarditis [26].

The chemokine receptors CCR5 and CXC3 are known as immunological markers of Th1 response, whereas CCR3 is associated with Th2 response [27]. The CCR5 receptor, receptor of CL2, CCL3, CCL4 and CCL5, has a dual role in the pathogenesis of the disease: on the one hand, it is extremely important in the control of acute infection, on the other hand, its exacerbated expression may maintain the inflammation leading to myocardial tissue damage [28]. Together with CCR5, the CCR2 receptor is also related the different clinical manifestations of the disease [29]. It is clear that chemokines play a relevant role in Chagas disease both in the initial infection and in the chronic form of the disease.

The control of the disease also involves antibodies, CD8 + (cytotoxic) T lymphocytes and CD4 + T lymphocytes (helper, Th1 type) producing high levels of IFN-γ [30]. Studies on acute experimental models of the disease have shown the role of proinflammatory cytokines such as IFN-γ, TNF and IL-6 in resistance to *T. cruzi* infection [31,32]. On the contrary, the Th2 profile is related to disease susceptibility and the main cytokine involved is IL-4 [33].

After the initial interaction of the parasite with the cells of the innate immune response, different intracellular signals are presented, culminating in the activation of NF-kB, production of inflammatory cytokines, connecting the innate response to the adaptive response [34]. Besides

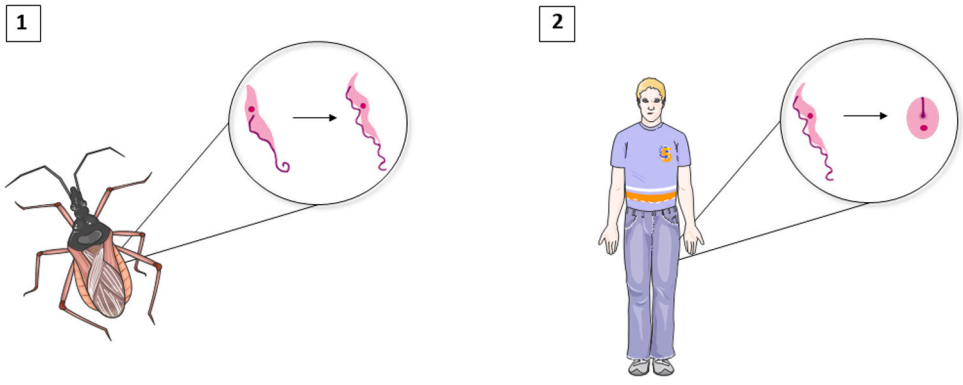

Fig. 1. Vector (1) and mammalian stages (2) of *Trypanosoma cruzi*. In the vector stages, epimastigotes and metacyclic trypomastigotes are show. In the mammalian stages, trypomastigotes and amastigotes are shown. Some components of this figure were created using modified templates from Servier Medical Art, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com.

that, the increased production of IL-12 induced by *T. cruzi*, mainly by macrophages, interferes in the production of IFN-γ by activation of NK cells and induction of a Th1 response cells [35]. In addition, these IL-12 activated NK cells lead to the expansion of CD4 + and CD8 + T cells by the production of IFN-γ, which then produce more IFN-γ. The IFN-γ cytokine, together with TNF, also produced by innate immune response cells, and IL-12 stimulates macrophages infected with *T. cruzi* to produce nitric oxide (NO) and the expression of the induced nitric oxide synthase enzyme, which contributes to control of the intracellular division of the parasite and its elimination [36]. However, the excess of NO may also lead to tissue damage, showing its dual role and the importance of control/regulation of immune response [37]. It is important to note that IFN-γ and TNF seem to play a dual role according to the course of the disease. In the early phase of the disease, these cytokines are important to combat the parasite, but they can be harmful when produced in excess in the chronic phase [38,39].

Other cytokines may be important in the initial stages of the infection. TGF-β is one of these cytokines that regulates the parasite invasion and cardiac fibrosis. Thus, in the initial phase of infection, mice treated with GW788388 – an TGF-β inhibitor – showed decreased cardiac fibrosis and improved survival rates [40]. In mice infected with *T. cruzi* but deficient in the production of IL-6, there was an increased production of IL-1β (important cytokine involved in NO production), NO, and inflammatory monocytes, leading to higher mortality upon infection, suggesting that IL-6 is able to modulate the inflammation [41]. Corroborating with this, orally infected mice showed significantly higher TNF, IL-6, and IFN-γ levels than controls, demonstrating that a potent pro-inflammatory systemic response can be trigged by the infection with the parasite [42].

Despite all the efforts of the immune system to fight against the infection, some molecules of the parasite can help in the scape of the immune response. One of the ways that the parasite can do it is by blocking the activation of NF-kB, which leads to less production of the pro-inflammatory cytokine IL-12 by macrophages, through the action of the cruzain parasitic enzyme [43]. Another molecule of the parasite that interacts with the immune system and modulates it is the enzyme trans-sialidase, which is considered a parasitic virulence factor. During antigen presentation, trans-sialidase limits the pro-inflammatory response and stimulates the anti-inflammatory response through increased production of IL-10, favoring infection and persistence of the parasite in the tissues [44]. The parasite also expresses mucin-like molecules on its surface that participate in the initial parasite-host interaction, as well as evading the immune system. The sialylated form of these proteins protects the parasite from the action of antibodies, from the complement system [45], in addition to preventing the initial events of T lymphocyte activation [46]. The Fig. 1A summarizes the effort of the immune system to control the initial infection, highlighting the leading cells and the molecules evolved, apart from the clinical features in each stage of disease.

Another factor that is involved in the initial infection and, consequently, with the immune system is the strain of the parasite. A study with two strains of two different DTUs (TcI and TcII) in acute infection showed that the strain Col cl1.7, belonging to TcI, led to a greater activation of monocytes and production of IL-10, while Y strain, belonging to TcII, activates less monocytes, however, it causes a greater production of pro-inflammatory cytokines by peripheral mononuclear cells [47]. DTU is a set of stocks genetically more similar to each other than to any other unit and can be identified by common genetic/molecular/immunological markers [48]. In other words, the strains lead to diverse immunological outcomes and, consequently, may have different impacts on the clinical course of the disease.

In addition to the innate immune response, the adaptive immune response is extremely relevant even in the early stage of infection. A research by Menezes and co-workers [49] showed myocarditis related to the presence of CD4 + and CD8 + T lymphocytes in the acute phase of the disease, as well as of *T. cruzi* antigens. This study shows that these cells may be involved in the pathology of acute myocarditis. CD4 + and CD8 + T lymphocytes mainly secrete IFN-γ, the main cytokine responsible for Th1 polarization in relation to Th2. On the other hand, CD8 + T lymphocytes are seen in processes related to the control of parasites in acute infection and during disease progression, and also in the chronic phase of the pathology, being likewise associated with a possible absence of activity that helps in establishing the disease [50–52]. Because of this range of influences described for these cells, it is speculated that distinct populations of CD8 + T cells may exist during disease, related to different functions[34]. One of the cytokines that influence these lymphocyte effector mechanisms, the expansion of CD4 + T and CD8 + T lymphocytes, and acute infection is IL-2 [53].

In addition to the Th1/Th2 dichotomy, Th17 cells have also been shown to help control acute infection and cardiac inflammation in the experimental model of the disease, modulating the Th1 response [54]. A study with mice in the acute phase of the disease showed that, in the absence of the IL-17 cytokine at that stage, the animals became more susceptible to infection, with lower expression of cytokines such as IFN-γ, IL-6 and TNF [55].

The acute phase lasts 2–3 months and ends when the immune system is able to control parasitemia and the level of parasites in the tissues. Despite all the immune response developed to combat the parasite, many patients evolve to the chronic form of the disease. Some of the factors involved in this evolution are the evasion of *T. cruzi* to the immune system, the strain involved in the initial infection and the host immune system [56,57].

3. Immune response in the chronic phase

In the chronic phase of Chagas disease, the clinical signs and symptoms of the patients, or the absence of these, appear to be related to the individual's immune response. It is known that there is a Th1 profile of cytokines (IFN-γ, TNF, IL-2, IL-6, IL-9, IL-12) with low level of a Th2 profile of cytokines (IL-4, IL-5, IL-10, IL-13) in patients with the cardiac and digestive signs. On the other hand, the opposite is seen in the indeterminate form of Chagas, suggesting that the balance of these cytokines could play a key role in the development of the disease.

Two of the major cytokines of the proinflammatory profile, TNF and IFN-γ, are directly involved in the chronic cardiac pathology of Chagas disease in human and experimental models [11,58–60]. Due to the importance of cytokines in the development of the disease, some of them, such as TGF-β, IL-1β and TNF, have been proposed as biomarkers of myocardial fibrosis [61]. Conversely, patients with cardiac form of the disease produce higher levels of IFN-γ – a really essential pro-inflammatory cytokine – whereas indeterminate patients showed higher levels of IL-10, one of the key regulators of IFN-γ production [62]. In addition, IL-6 was a highly expressed cytokine by patients with the cardiac form in comparison to patients with the indeterminate form, who expressed IL-10 in greater quantity [63]. Corroborating with these studies, Gómez-Olarte and co-workers [64] found that patients with the cardiac form of the disease have an increased quantity of inflammatory monocytes and IL-6 production compared to asymptomatic individuals. Thus, the type 1 immune response, while important in containing parasite replication during the acute phase as already described, may also be involved in the development of severe cardiac disease.

Regarding the indeterminate form, there is an increased expression of cytokines and transcription factors related to Th2, Th9, Th22 and Treg profiles, associated with reduced expression of proinflammatory cytokines such as IFN-γ and TNF [65,66]. Studies show a correlation between the production of inflammatory cytokines by CD4 + T cells and monocytes from patients with the cardiac form and the production of IL-10 by the same cells from asymptomatic patients [67,68]. This indicates that anti-inflammatory cytokines may help neutralize the action of pro-inflammatory cytokines and consequently may lead to reduction of tissue damage and the absence of symptoms.

Emphasizing the relevance of the immune system regulation, the

Treg cells are mostly found in patients with the indeterminate form of the disease and it is the main type of cell involved in the IL-10 production, showing the importance of these cells in the regulation of the immune response in these patients [69]. Due to this, it has been suggested that a treatment for Chagas disease should enhance Treg cells [70]. In addition, a study with patients with the cardiac form showed a higher production of IL-10 and IL-17 in the milder form of the disease and that a decrease in the function of CD4 +CD25 + T cells and lower levels of IL-17 are linked with the most severe form of disease [71]. A study corroborated these findings and also verified that in the cardiac form there is a smaller number of circulating Th17 cells in relation to the indeterminate form and to the uninfected patients [72]. A recent study of the experimental model of the disease showed that Th17 cells are more protective against *T. cruzi* infection than Th1 cells, in addition to showing that these cells can act on both extracellular and intracellular immunity [73]. The Fig. 2B shows a summary of the cytokine profile of the cardiac and indeterminate form.

It is not known what leads to an increase of the anti-inflammatory profile observed in the indeterminate form in relation to a pro-inflammatory observed in the cardiac form. This may depend on the host's genetic characteristics and age-dependent changes in the immune system, for example [74].

4. Treatment and immunological response

The treatment with Bz or Nx is limited by the stage of the disease and the high toxicity. Finding a new treatment for Chagas disease is one of the main goals to achieve its control [75]. In the Table 1, it is summarized the Target Product Profile of a new drug candidate for Chagas disease suggested by de Drug for Neglected Diseases *Initiative* (DND*i*).

Aiming to find a new compound for the disease, different classes of compounds have been obtained based on a range of approaches, such as planned synthetic compounds and drug repurposing [76]. Much has been said about the importance of the treatment, mainly taking into account clinical manifestations, serology and parasitemia [77,78]. However, little is investigated about the influence of the treatment on the immune system, although it exerts great influence on the success of chemotherapy in many parasitic diseases, including Chagas [79]. This has been seen mainly in experimental acute *T. cruzi* infections; however, there is no strong support of this correlation in humans [80].

Table 1
Target product profile of a new drug candidate for Chagas disease suggested by the DND*i*.

	Ideal	Acceptable
Target population	Chronic and acute	Chronic
Geographic distribution	All regions	All regions
Contraindications	No contraindications	Pregnancy
Treatment regimen	< 30 days	Oral: any duration Parenteral: < 7 days
Cost	Lowest as possible	Current treatments

The presence of high levels of IFN-γ in peripheral blood mononuclear cells of patients cured after treatment suggests a beneficial effect of this cytokine on the efficacy of chemotherapy [81]. That is, during the acute phase of infection, IFN-γ may act in synergy with the specific treatment to eliminate the parasites [82,83]. The beneficial relationship between cytokine balance and treatment, as found in the acute phase, was also seen in the chronic phase in patients with indeterminate and cardiac form. In the cardiac form, Camara and co-workers [84] measured the plasma of 66 patients, treated or untreated with Bz, and found out that there is a positive correlation between the treatment with Bz and myocardial function, strengthening the idea that the treatment with this drug can bring benefits. In addition, Bz seems to upregulate cell activation, antigen presentation, phagocytosis receptor and macrophage activation [85]. How it was previous discussed, in the cardiac form of the disease there is an important inflammatory component. In line with this, the cytokine profiles of chronic infected subjects treated with Bz showed lower levels of inflammatory mediators than those of the untreated *T. cruzi*-infected subjects [86], suggesting the role of Bz in regulating inflammation.

In the indeterminate form, treatment with Bz led to an induction of a pro-inflammatory profile by NK and CD8 + T cells with maintenance of IL-10, emphasizing the relevance of the regulatory environment, while in cardiac form led to lower levels of IFN-γ and higher levels of IL-10 [87]. A study conducted by Mateus and co-workers [88] with chronically infected individuals, showed that treatment with Bz improved the antigen-specific T CD8 + response, decreasing the coexpression of inhibitory molecules in these cells and increasing the functional capacity of these cells (observed by increasing production of cytokines and

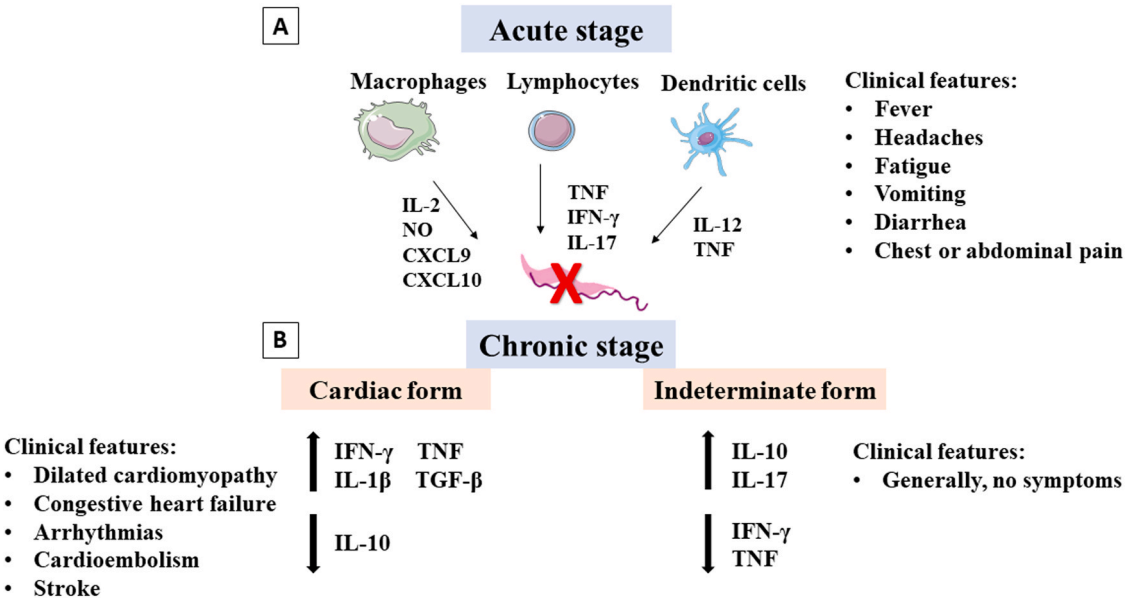

Fig. 2. Immunological and clinical profile of acute and chronic cardiac stages of Chagas disease. Some components of this figure were created using modified templates from Servier Medical Art, which are licensed under a Creative Commons Attribution 3.0 Unported License; https://smart.servier.com.

cytotoxic molecules). In line with this, a study by Pérez-Antón and co-workers concluded that treatment with benznidazole in chronically infected people reversed the process of exhaustion of CD4 + and CD8 + T cells and increased the responsiveness of these lymphocytes (increase in cells expressing IL-2 and TNF) [89,90]. This process of exhaustion of T lymphocytes has been reported in the literature. It occurs due to continuous exposure to parasitic antigens, triggering a dysfunctional response of T lymphocytes by decreasing the ability to produce cytokines and cytotoxic molecules against the infectious agent, in addition to inducing a progressive increase in the expression and coexpression of inhibitory molecules in the cell membrane [91]. In an analysis with 4 years after Bz-treatment subjects, patients who had progressed to the cardiac form, or had a worsening in the cardiac symptoms, showed elevated levels of IFN-γ and Th1 cells, while those subjects who remained in the indeterminate form were associated with lower IFN-γ levels and inflammatory/anti-inflammatory balance favoring IL-10 and Tregs [92]. Altogether, this reinforces the importance of treatment regulation between pro- and anti-inflammatory profiles in the context of Chagas disease.

Despite all that, a multicentric study conducted recently to assess if there is advantage in treating chronic cardiac patients with Bz (BENEFIT) showed that there is no profit in using this treatment in this group of patients [93]. It is important to note that a wild range of genes, including these related to the immune system, are dysregulated in chronic Chagas disease, especially in the cardiac form [94], and this may justify the difficulty in obtaining benefits from treatment at this stage of the disease [95]. However, another multicentric study is being conducted aiming to know if different dosages, times of treatment, and association of Bz with fosravuconazole could lead to clinical benefits in adults with chronic Chagas disease. Initial results have shown that Bz showed antiparasitic activity in all treatments, not depending on duration, dose, or combination with fosravuconazole [96]. This shows the importance of continuing to research the influence of treatment on the chronic phase of the disease, including the participation of the immune system, to better understand how it influences in treatment success.

On one hand, the range of immunological profiles involved in the pathology of the disease reinforce the importance to investigate how a possible new treatment can act on the immune system, helping to combat the development of the disease. On the other hand, this immunological aspect is poorly investigated when it comes to new drugs for the disease. A variety of compound classes has been tested for Chagas disease and some privileged structures, especially those related to phthalimide, thiosemicarbazones, thiazoles and 4-thiazolidinones are being considered promising anti-*T. cruzi* and immunomodulatory scaffolds [97]. Gomes and colleagues tested both phthalimido-thiosemicarbazones and phthalimido-thiazole derivatives in vitro and found 3 compounds with anti-*T. cruzi* activity lower than 5 μM [98]. In a work that tested thiosemicarbazones derivatives, the C3 compound showed anti-*T. cruzi* activity in vitro and in vivo, in addition to decreasing the release of trypomastigotes from cardiomyocytes without depending on NO, TNF, and IL-6 production [99]. Álvarez and collaborators synthetized and tested thiazole derivatives and it was possible to find a molecule that diminishes mononuclear inflammatory infiltrates in the heart of mice infected with *T. cruzi* [100]. Two of the 4-thiazolidinones chlorine derivatives tested against *T. cruzi* – compounds 2c and 3a – showed antiparasitic activity independent of NO release [101].

Due to all this, it is clear that immunotherapy and the development of immunomodulatory compounds is an important approach in the context of Chagas disease. It is important to note that immunological parameters, such as changes in cytokines/chemokines production and cell activity, can be useful in the drug discovery field and to monitor treatment efficacy [80,102,103].

5. Conclusion

The existing antithesis between autoimmunity and inflammation resulting from parasitic persistence promoted a delay in the study of new drugs to treat the disease and, consequently, delayed the development of studies on the influence of treatment on the immune system, that is, immunomodulation studies in Chagas. Although there are immunological studies in animals and humans that show differences between the immunological profile in the acute and chronic stages, there is no consensus regarding the pathogenesis of Chagas disease and what is the ideal immune response to promote its cure or permanence in the indeterminate chronic form. The design and obtention of immunomodulatory compounds have grown in parallel as a strategy for therapeutic approaches in parasitic diseases, since the immunological profiles involved in the pathology of Chagas disease show that the involvement of the host's immune response can play an important role in the efficacy of chemotherapy. Therefore, it is important to investigate how a drug candidate might act on the immune system, and the complexity of the possible immunological responses. These gaps must be fulfilled in order to achieve more effective strategies for therapeutic interventions. It is known that a range of immunological profiles are involved in the pathology of the disease and there is a great need for further studies in the area to help in the development of more efficient strategies for its treatment and prophylaxis. In addition, little research is done on the influence of treatment on the immune system, although the number of studies suggesting that its involvement may play an important role in the efficacy of chemotherapy is gradually growing. With this in mind, it is extremely important that researchers start to give focus on the immune system in the drug discovery strategies for Chagas disease.

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Author contributions

ACCS and MCABC wrote the manuscript. VRAP and MZH contributed to the discussion of the draft and made final corrections.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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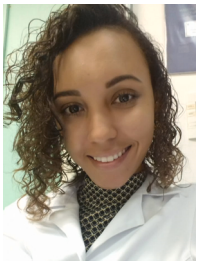

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