Chapter 8

Trypanosoma cruzi Infection in Non-Human Primates

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Abstract

For decades, non-human primates (NHPs) have been employed as experimental models to study many aspects of human diseases. They are the closest genetically to humans of any of the models applied in biomedical research; therefore, many authors have published scientific work regarding these animals and infectious diseases, including tuberculosis, AIDS, and tropical diseases. Among these, Chagas disease has caught the attention of many researchers all over the world. Recent studies have demonstrated great similarities with the human pathology, including cardiomyopathy and exacerbated pro-inflammatory response. Besides being genetically close to humans, NHP have a great probability to be naturally infected by *Trypanosoma cruzi*, which turns them into more interesting models to study Chagas disease mechanisms.

Keywords: non-human primates, Chagas disease, *T. cruzi*, immunology, infectious diseases

1. Introduction

The haemoflagellate *Trypanosoma cruzi* causes Chagas disease, one of the most relevant neglected tropical diseases of humankind. The World Health Organization estimates that there are 6–7 million people infected over the world [1–3] as shown in **Figure 1**. Nevertheless, other mammals are also at risk of becoming infected, such as marsupials, armadillos, sylvatic and domestic dogs, racoons and non-human primates (NHPs) [4–8]. The most common Chagas disease transmission is the vectorial via by several species of triatomine. The

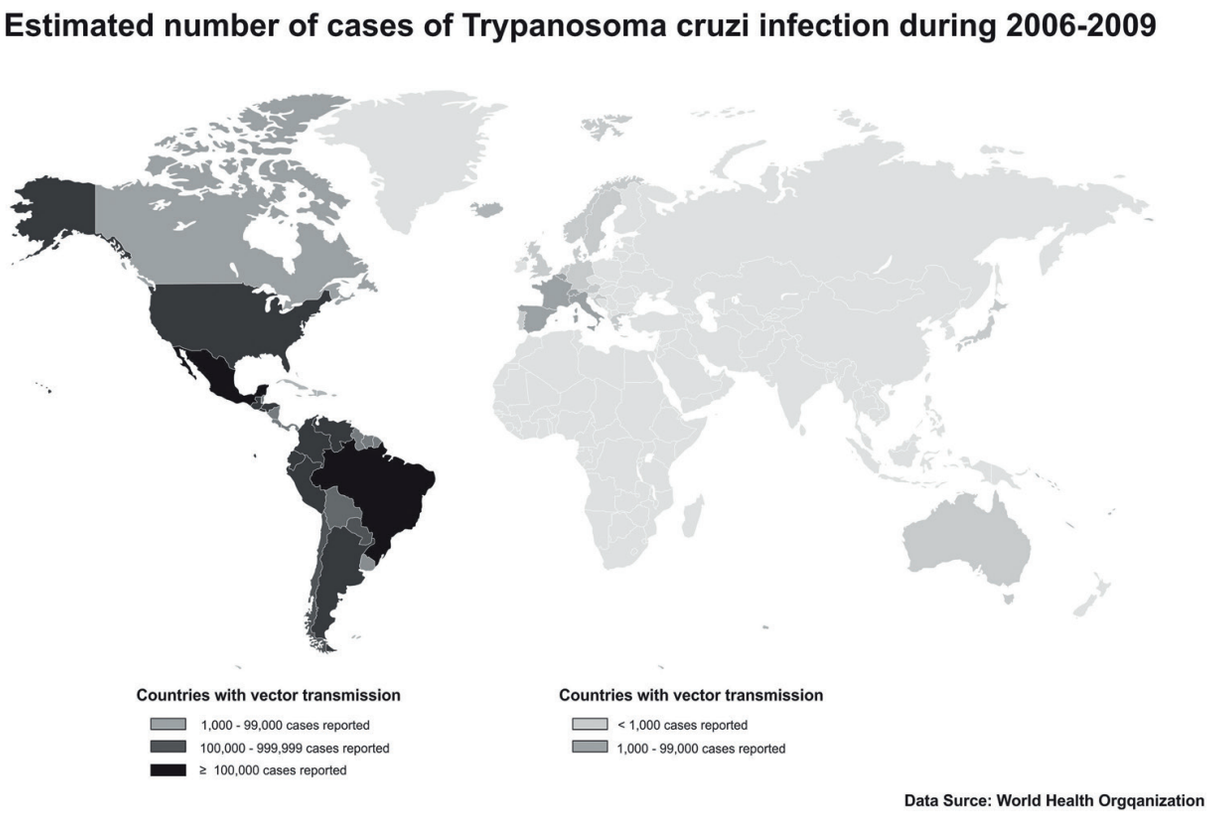

Figure 1. Descriptive map of infection areas in the world.

vector insect ingests a blood meal containing bloodstream trypomastigotes which later, in the insect's gut, the parasite differentiates into epimastigotes and replicates. When the vector defecates, metacyclic trypomastigote forms are released and invade the host through broken skin or mucosal membranes. A brief schematic mechanism of *T. cruzi* infection is shown

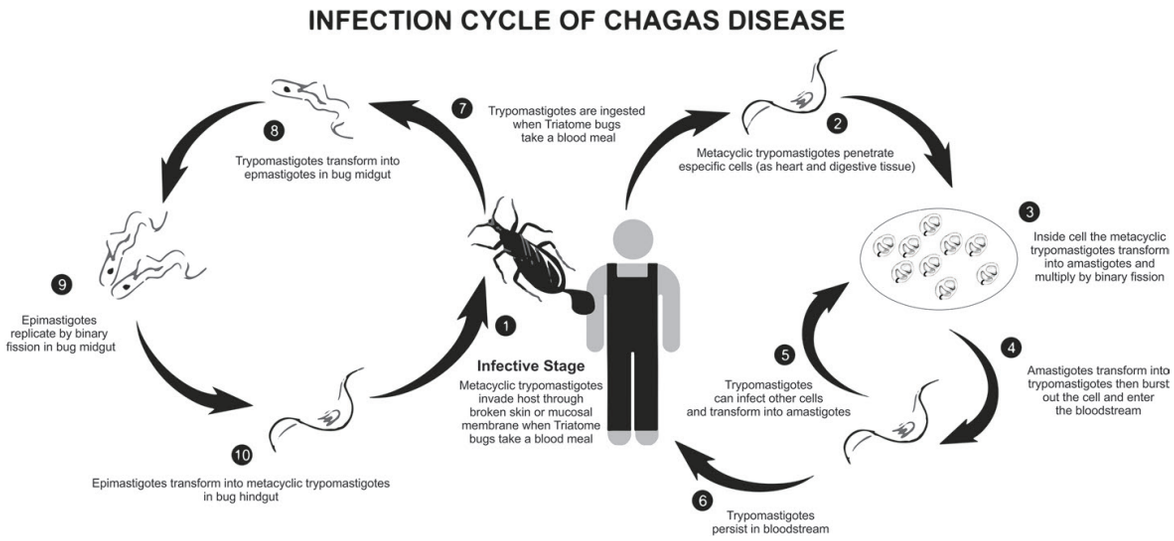

Figure 2. Schematic life cycle of *Trypanosoma cruzi.*

in **Figure 2**. In addition, the oral and congenital transmissions are other important ways of becoming infected [6]. Out of these, the oral transmission seems more relevant, especially by non-human species. The habit of consuming bugs may predispose these animals to ingest infected triatomines [9]. Amongst the non-human species presented above, non-human primates are the greatest species comparable to human beings, leading researchers all over the world to employ NHP in biomedical-related studies. Interestingly, Carlos Chagas was the first to describe both experimental and natural *T. cruzi* infections in non-human primates [10]. After that, many others have portrayed the disease in these animals, and studies are still being produced nowadays. From small college laboratories to huge pharmaceutical industries, the purpose is the same: to find better options to diagnose and to treat patients. Our group has been working with NHP for a few years, and so far, our findings are similar to those from many researchers all over the world. The aim of this work is to explore the most relevant findings regarding *T. cruzi*-infected NHP.

2. Clinical manifestations of NHP *T. cruzi* infection

Several studies have demonstrated that NHP develop clinical manifestations highly similar to what is observed in both acute and chronic human Chagas disease [9, 11–14]. In the acute phase, several signs and symptoms can be observed, such as inoculation chagoma, patent parasitemia, *T. cruzi*-specific IgM and IgG antibodies, and leukocytosis and lymphocytosis. Histopathological data revealed intense heart parasitism and pronounced inflammatory infiltrate, along with myocardial fibrosis with collagen deposits [12]. Besides that, cardiac alterations have also been found in these animals, such as abnormal electrocardiogram and heart muscle cells presenting degrees of damage [13]. Those findings reinforce the results found by Bonecini-Almeida et al. [11] who described electrocardiographic patterns detected in *T. cruzi*-infected rhesus monkeys during the acute phase. The results have evidenced atrioventricular block, right bundle branch block—first-degree His bundle, low voltage QRS complex, and abnormal ventricular repolarization. Interestingly, these alterations disappeared at the fourth month post infection. Controversially, Bommineni et al. [14] demonstrated that the acute phase in NHP may be lethal. Despite that, *T. cruzi* infection usually evolves from an acute phase to a chronic phase that may manifest itself in a variety of ways.

In contrast to the acute phase, during the chronic stages of the disease, the trypomastigotes in the peripheral circulation are extremely difficult to detect microscopically. However, more detailed histopathological studies have shown nests of *T. cruzi* amastigote forms in host cardiac tissue from naturally infected baboons. The majority of individuals that progress to the chronic phase remain clinically asymptomatic for many years, characterizing the indeterminate clinical form of the disease. Usually the disease confirmation requires the application of several diagnostic techniques, such as microscopic examination of blood smears, serological assays, xenodiagnosis, hemoculture and PCR-based assays for direct detection and quantification of parasite DNA [15–17]. After long years of infection, individuals may progress to the cardiac and/or digestive chronic phase, which usually represents the most severe clinical damages [18]. Researchers have demonstrated numerous alterations in the electrical conduction system, ventricular arrhythmias,

cardiomegaly, enteromegaly, myocardial fibrosis, and edema, along with other clinical signs of chronicity [9, 12, 13, 19, 20]. In several cases the infected NHP develop aggressive chronic Chagas disease and die, usually due to cardiac damages. In this context, a NHP animal model with these features could contribute significantly to better comprehend the disease outcome as well as to improve the therapies for Chagas disease.

3. Immunological features of NHP *T. cruzi* infection

In addition to pathophysiological changes, NHP also manifest alterations in their immunological system. It is well known that the immune system plays an important role in the pathogenesis of the disease. When it comes to NHP, it is not different. Recent studies, including ours, have shown that their immunological response resembles what is observed in human Chagas disease, as briefly schemed in **Figure 3** [1, 6, 18, 21].

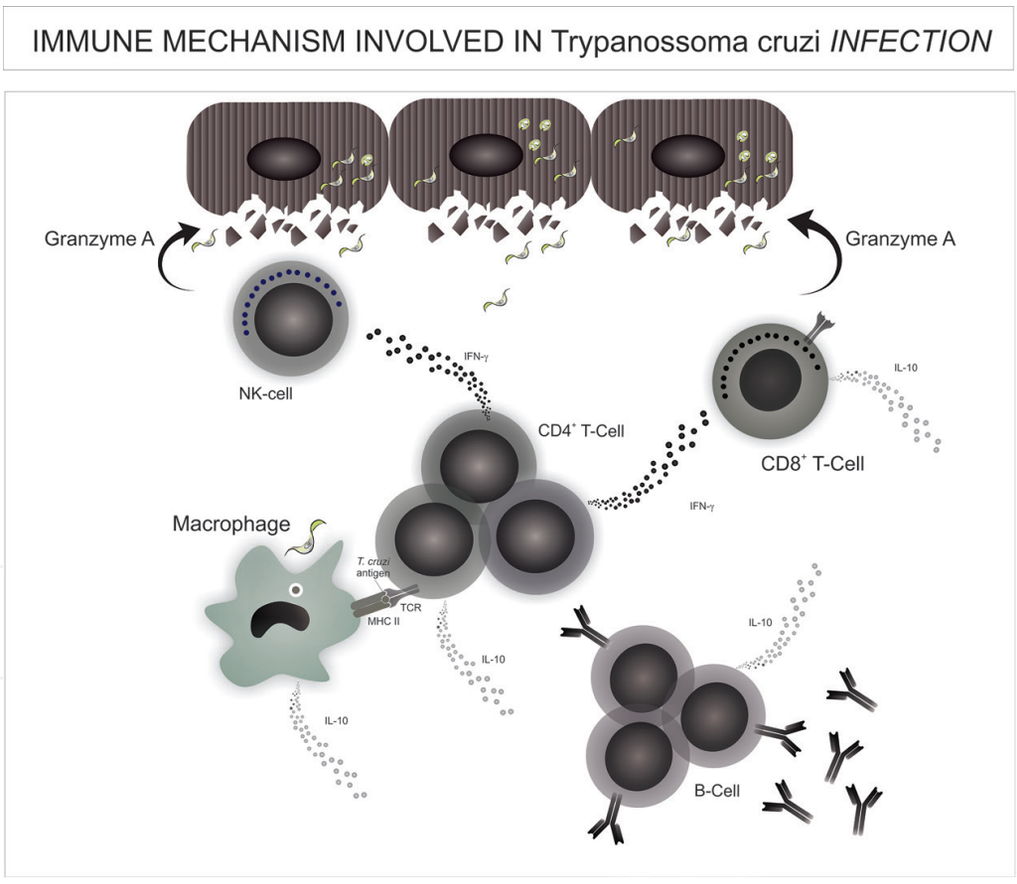

Figure 3. *Trypanosoma cruzi* naturally infected non-human primate displays, in the peripheral blood, a high activity of cytotoxic cells (Granzyme A^+ NK cells and Granzyme A^+/Perforin $CD8^+$ T cells) and expansion of macrophages and activated T-cell subsets. Furthermore, the infected animals exhibit an overall mixed pro-inflammatory/regulatory cytokine milieu, with $CD4^+$ T cells, the most important source of IFN-γ, as well as $CD4^+$ T cells, $CD8^+$ T cells, macrophage/monocytes and B-cell producers of IL-10.

Our group has recently published a research on *T. cruzi* naturally infected cynomolgus macaques which displayed, in the peripheral blood, a similar immunological profile to that observed in humans, with high activity of cytotoxic cells and expansion of macrophages and activated T-cell subsets [21]. The infected animals exhibit higher frequency of NK Granzyme A^+ cells. Furthermore, this cell population was able to increase the pro-inflammatory cytokine secretion afterwards *T. cruzi* antigen stimulation [22]. These data reinforce the important role of NK cells as a source of IFN-γ to activate macrophages and increase the nitric oxide production to inhibit the intracellular parasite growth [23, 24]. Moreover, the NK cells mediate a relevant cytotoxic mechanism that kill infected host cells or even free parasites throughout a lytic perforin-independent mechanism [24]. It is important to mention that the higher expression of inducible nitric oxide synthase by monocytes/macrophage has been correlated with loss of connexin43 in cardiopathic *T. cruzi*-infected rhesus monkeys [12]. Connexin43 is the major protein responsible for the electrical synchrony of cardiomyocytes [25]. In this context, any injury in this protein may result in arrhythmias and heart failure during the chronic chagasic cardiomyopathy.

It is well known that the adaptive immune response plays a critical role in Chagas disease progression in humans; however, in NHP its mechanisms remain unclear. Recently, our group showed that the *T. cruzi*-infected NHP developed a pattern of activated T lymphocytes as observed in the human infection. In fact, higher expression of CD54 and HLA-DR by T cells, especially within the $CD8^+$ subset, along with outstanding expression of Granzyme A and Perforin, emphasized the enhanced cytotoxicity-linked pattern of $CD8^+$ T lymphocytes. These data reinforce the role of $CD8^+$ T lymphocytes in the pathogenesis of Chagas disease. Additionally, Pisharath et al. [6] while evaluating *T. cruzi* naturally infected cynomolgus macaques demonstrated by immunohistochemistry that the inflammatory infiltrate from cardiac tissue had mild to moderate multifocal areas, composed predominantly by $CD8^+$ T cells and $CD68^+$ monocyte/macrophage with fewer $CD4^+$ T lymphocytes. In agreement with these data, Mubiru et al. [20] showed a focal and multifocal collection of lymphocytes and plasma cells, as well as rare granulocyte infiltration within the myocardium and epicardium. Moreover, their study revealed a positive correlation between PCR positivity and lymphocytic myocarditis in both baboons and cynomolgus macaques infected with *T. cruzi*, reinforcing the hypothesis of direct parasite-induced damage and *T. cruzi*-specific immune responses, in myocardial injury.

It is known that B lymphocytes play a crucial role in protecting against *T. cruzi*. This is due to the fact that these cells synthesize anti-*T. cruzi* antibodies, establish the functional pattern of T-cell cytokines and still are involved in the maintenance of $CD8^+$ memory cells [26, 27]. In addition, it has been displayed that NHP infected with *T. cruzi* presents a high frequency of B-cell population associated with upregulated expression of Fc-γRII (CD32), enhancing the potential of this biomarkers' high expression, in counterbalancing the $CD8^+$ T-cell cytotoxic activity and influencing the degree of myocardiopathy.

It has been clear that cytokines are integral components of the complex intercellular system required to mount and control disease morbidity [28, 29]. However, little is known about the cytokine profile during NHP infection with *T. cruzi*. In order to further understand the

mechanisms of *T. cruzi* infection in NHP, Vitelli-Avelar [22], for the first time, characterized the ex vivo cytokine pattern of cynomolgus macaques naturally infected with *T. cruzi* and observed an overall mixed pro-inflammatory/regulatory cytokine milieu, mediated by IFN-γ from $CD4^+$ T cells counterbalanced by IL-10 produced by $CD4^+$ T cells and B cells. This microenvironment resembles that previously described for chronic Chagas disease in humans, mainly in indeterminate clinical form [24]. It has been proposed that this pro-inflammatory/regulatory pattern represents a key element to control deleterious antiparasite immune-mediated inflammatory mechanisms [30].

T. cruzi strains are currently classified into six discrete typing units (DTUs) named TcI to TcVI. It is known that these DTUs have different biological and geographical features [31]. In South American isolates, all of the strains have been characterized from a variety of host species. In contrast, isolates from the Central and North America have been characterized only as TcI or TcIV [32]. Several researchers have discussed the characteristics of different *T. cruzi* genotypes, and it seems like that the strain diversity is associated with the distinct immunological patterns observed in Chagas disease, which might be associated to disease severity [31, 33–36]. While working with a North American NHP colony, we intended to provide insights pertinent to the higher prevalence of TcI natural infection observed amongst these animals by interpreting the differential impact of TcI and TcIV antigen priming in vitro on circulating leukocytes. In this context, our data showed that NHP presents distinct cytokine profile in the presence of TcI and TcIV antigen. While the TcIV antigen triggered an outstanding response, characterized by high levels of TNF- and IFN-γ-producing $CD8^+$ T cells, along with low levels of IL-10, the TcI antigen elicits a predominant regulatory microenvironment, mediated by IL-10 derived from HLA-DR^{++} monocytes and T cells with low levels of TNF^+CD8^+ T cells [22]. The prominent pro-inflammatory milieu, mediated by TNF, seems to be relevant to control the *T. cruzi* infection NHP. The role of TNF in protective mechanisms has been already reported, underscoring its ability to activate macrophages and induce nitric oxide production [37]. Additionally, the enhanced frequency of IFN-γ^+ T cells beside low levels of IL-10-producing cells may also account as a relevant trypanocidal event favoring the TcIV clearance. Conversely, the IL-10-mediated microenvironment observed upon TcI-antigenic recall in vitro represents a critical event to support the ongoing infection with the TcI genotype. These findings may support, at least in part, the predominance of TcI infection amongst cynomolgus macaques in Southern part of the United States. **Figures 4** and **5** present a synthesized scheme of our newest cytokine findings and reinforce the distinct cytokine pattern produced upon *T. cruzi* TcI and TcIV antigen recall in vitro [22]. Furthermore, other studies have confirmed the presence of both TcI and TcIV isolates from Amazonian primates, TcI being more predominate strain than TcIV [38–40].

Regardless the relevance of therapeutic intervention to control morbidity and clinical progression of Chagas disease, currently, there are only two drugs available to treat infected hosts, benznidazole and nifurtimox. Several studies have demonstrated that the effectiveness of therapeutic agents against *T. cruzi* is influenced by the parasite load, genotype as well as by intrinsic features of the host immune response. Studies focusing on aspects related to the synergic effect of the immune response and chemotherapeutic agents in humans and NPH are still scarce. Sathler-Avelar and colleagues [21, 22, 24] have provided insights about

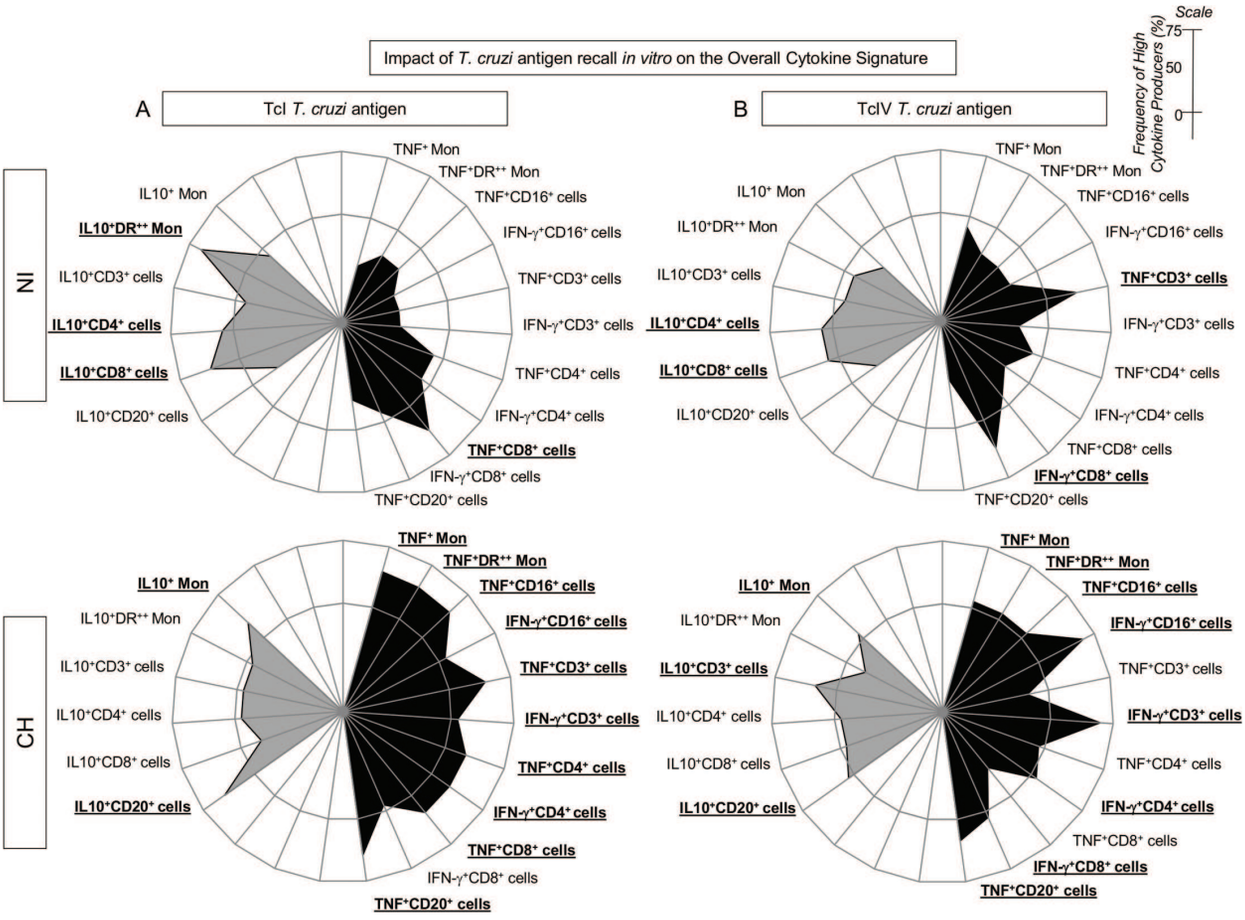

Figure 4. The cytokine milieu in *T. cruzi*-infected and non-infected non-human primates upon antigen recall from TcI and TcIV *T. cruzi* strain. The radar charts illustrate the changes on the pro-inflammatory (black background) and regulatory cytokine microenvironment (gray background) connecting circulating leukocytes of *T. cruzi*-infected cynomolgus macaques (CH) and non-infected controls (NI) upon TcI (A) and TcIV (B) *T. cruzi* antigenic recall in vitro. Relevant data comprising biomarkers with frequency of producers above the 50th percentile are underscored by bold/underlined font.

the relevance of a balanced immune response elicited after chemotherapeutic intervention to mediated parasite killing but minimize tissue damage. There are evidences supporting that a pro-inflammatory response mediated by IFN-γ acts synergistically with the drug treatment to accomplish effective trypanocidal events [24, 41] and that simultaneous regulatory mechanisms elicited by IL-10 are relevant to control deleterious effects of therapeutic intervention [21, 22, 24].

The urgent need of novel drugs to treat Chagas disease has stimulated scientific community to validate appropriate experimental model or in vitro tolls to conducted studies during preclinical trials. These studies that can contribute and elucidate drug mechanisms are still unknown, in an attempt to find a more effective therapeutic agent. In this context, NHP models have been considered one of the most appropriate tolls, especially due to the similarities between the disease aspects and the immune response observed in NPH as compared to humans. Vitelli-Avelar and colleagues have recently provided data focusing on the immune response of NPH infected with *T. cruzi* that can be used to shed light on this issue. Using an in vitro system of antigen recall to mimicry the endogenous booster of parasite-derived antigens that

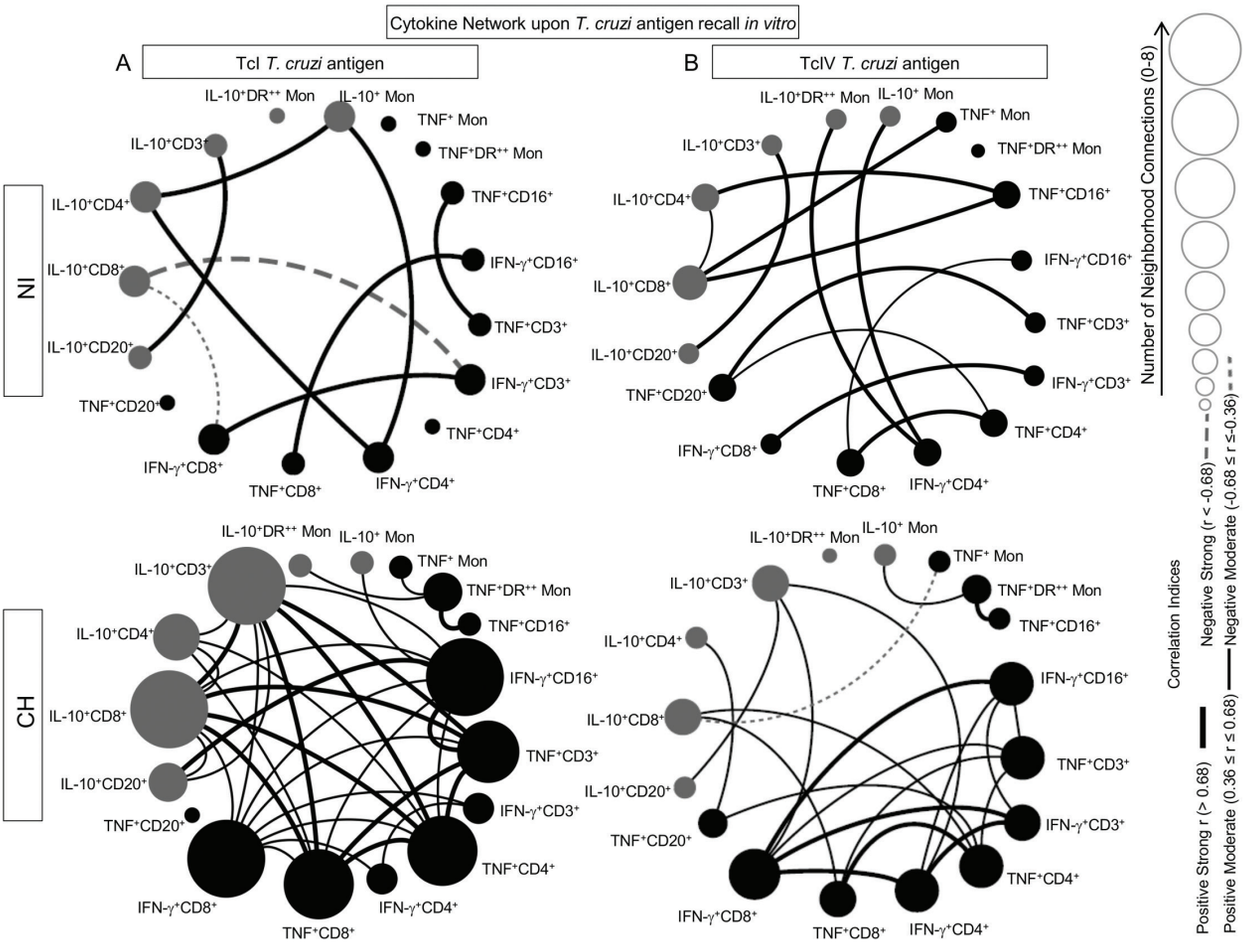

Figure 5. Cytokine network analysis upon *T. cruzi* antigen (TcI/TcIV) stimulation in vitro. Correlation matrices for cytokine producers were constructed to illustrate the distinct cytokine pattern upon (A) TcI and (B) TcIV antigen stimulation. Cytokine$^+$ cell networks for non-human primate naturally infected with *T. cruzi* (CH) and control animals (NI) are shown by clustered distribution of nodes for pro-inflammatory (black) and modulatory (gray) cytokine patterns.

occur throughout chronic infection or upon the extensive antigen release mediated by therapeutic intervention, these authors have demonstrated that similarly to what was found in human Chagas disease patients, NPH-infected host also exhibited a pro-inflammatory/regulatory cytokine signature triggered by *T. cruzi*-antigenic restimulation in vitro, These findings suggest the ability of these hosts to mount an appropriate immune response with putative balanced profile that may contribute for parasite killing, by IFN-γ release, modulated by IL-10 to prevent deleterious idiosyncrasy.

4. Final remarks

The urge for an experimental model that resembles all medical disorders observed in humans is of great importance. Non-human primates are great models to study Chagas disease. It is clear that these mammals present clinical, immunological, and histopathological resemblances to humans. All studies conducted so far lead to believe that as shown in humans, primates

naturally infected with *T. cruzi* also evolve to chronic phase and that is probably associated to the extension of the immune response they develop. With an experimental model that develops clinical and immunological manifestations closely comparable to humans, innovative therapeutic strategies may be deeper studied and new drugs may be developed. There are still much more to comprehend; however, the scientific advances and better comprehension of the mechanisms of *T. cruzi* infection may contribute to find hope to Chagas disease patients.

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