FEMS Pathogens and Disease, 73, 2015, ftv082

doi: 10.1093/femspd/ftv082
Advance Access Publication Date: 5 October 2015
Minireview

MINIREVIEW

Immunity and immune modulation in *Trypanosoma cruzi* infection

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One sentence summary: This article reviews the immune mechanisms responsible for the control of parasite numbers as well as the mechanisms that control the immune response, avoiding tissue damage and disease.
Editor: Patricia Bozza

ABSTRACT

Chagas disease is caused by the protozoan *Trypanosoma cruzi*. The parasite reaches the secondary lymphoid organs, the heart, skeletal muscles, neurons in the intestine and esophagus among other tissues. The disease is characterized by mega syndromes, which may affect the esophagus, the colon and the heart, in about 30% of infected people. The clinical manifestations associated with *T. cruzi* infection during the chronic phase of the disease are dependent on complex interactions between the parasite and the host tissues, particularly the lymphoid system that may either result in a balanced relationship with no disease or in an unbalanced relationship that follows an inflammatory response to parasite antigens and associated tissues in some of the host organs and/or by an autoimmune response to host antigens. This review discusses the findings that support the notion of an integrated immune response, considering the innate and adaptive arms of the immune system in the control of parasite numbers and also the mechanisms proposed to regulate the immune response in order to tolerate the remaining parasite load, during the chronic phase of infection. This knowledge is fundamental to the understanding of the disease progression and is essential for the development of novel therapies and vaccine strategies.

Keywords: *Trypanosoma cruzi*; memory T cell; regulatory T cell; gamma delta T cell; interleukin-17; myeloid-derived suppressor cell (MDSC)

INTRODUCTION

The intracellular protozoan parasite *Trypanosoma cruzi* causes Chagas' disease in humans (Koberle 1968; Andrade, Gollob and Dutra 2014). The infection is characterized by an acute phase resulting in parasitemia that resolves upon the appearance of an effective immune response (Cardillo *et al.* 2002). In humans and mice, the acute infection is characterized by high parasitemia that increases after 1–8 weeks following infection, depending on the *T. cruzi* strain (Cardillo *et al.* 1996). However, the immune response induced during the acute infection is not sufficient to completely eradicate the pathogen, thus resulting in chronic infection (Albareda *et al.* 2006). The chronic form of the disease mainly affects the peripheral autonomous nervous system in the gastrointestinal tract and heart and the heart muscle in approximately 30% of the infected patients (Koberle 1968;

Received: 1 June 2015; **Accepted:** 30 September 2015

Andrade, Gollob and Dutra 2014). The chronic infection may be accompanied by additional autoimmune mechanisms triggered by the parasite and its persistence (dos Santos *et al.* 1992; Mengel and Rossi 1992; Bonney and Engman 2008, 2015; Cunha-Neto *et al.* 2011). Yet, the majority (about 70%) of the patients that progress to the chronic phase remain clinically asymptomatic in the chronic phase of the infection. This condition characterizes the indeterminate form, known as early-indeterminate disease, usually seen in infected children and adolescents, and late-indeterminate disease, generally observed in infected adults (Umezawa *et al.* 2001).

In this review, we discuss the molecules, cells and possible mechanisms involved in the potentiation and/or the control/downregulation of the immune response during *T. cruzi* infection.

CELLS AND MOLECULES THAT PROMOTE IMMUNITY IN THE ACUTE PHASE OF *T. CRUZI* INFECTION

Initial IFN-γ production prior to the generation of T-cell-mediated adaptive immunity is known to occur during the course of many infections and may be important in the development of resistance to many intracellular infections such as *Leishmania*, *Salmonella*, *Toxoplasma* and *T. cruzi* (Locksley and Scott 1991; Ramarathinam, Niesel and Klimpel 1993; Sher *et al.* 1993; Cardillo *et al.* 1996). Natural killer cells may be the major cell type responsible for IFN-γ production in the early stages of *T. cruzi* infection and their activation requires the presence of live parasites (Cardillo *et al.* 1996). In addition, innate or adaptive immune cells, such as dendritic cells, macrophages, NKT lymphocytes, $\gamma\delta$ T cells and B cells, may contribute to host resistance (Locksley and Scott 1991; Sher *et al.* 1993; Cardillo *et al.* 1996; Galli *et al.* 2003, 2007; Takahashi and Strober 2008).

Dendritic cells (DCs) and/or macrophages act as professional antigen-presenting cells and are central in the initiation and development of immunity or tolerance (Lanzavecchia and Sallusto 2001; Steinman, Hawiger and Nussenzweig 2003). Trypomastigotes are responsible for the generation of regulatory DCs *in vitro* (Sher *et al.* 1993; Poncini *et al.* 2008; da Costa *et al.* 2014). The production of TNF-α, IFN-γ, IL-12, IL-22, IL-6, IL-10 and CCL2 and the expression of CD40, CD80, MHC-II, PD-L1, CCR5 and CCR7 may be different, depending on the *T. cruzi* strain used for stimulation (Poveda *et al.* 2014; da Costa *et al.* 2014). These results strongly argue that DCs, monocytes and macrophages are active players in the modulation of the adaptive immune response to *T. cruzi* (Rezende-Oliveira, Sarmento and Rodrigues 2012; Pinho *et al.* 2014) and may be useful to manipulate immunity/tolerance either before or during the infection (Poncini *et al.* 2015; Rampazo *et al.* 2015).

NK1.1+ cells may participate in generating memory T cells, since their depletion in acute infection diminished the generation of the activated/memory T cells in the spleen of *T. cruzi*-infected mice (Cardillo *et al.* 2002). In fact, this resulted in larger numbers of T cells expressing CD69, without the corresponding formation of effector T cells (Cardillo *et al.* 2004). Furthermore, the depletion of NK1.1+ cells caused an earlier appearance of anti-*T. cruzi* IgM antibodies and less isotype switching to IgG at later time points, suggesting a diminished T-cell-helper response (Cardillo *et al.* 2002). However, these studies did not discriminate between CD3– and CD3+ NK cells, as they were performed with depleting monoclonal antibodies to the NK1.1 molecule.

Other studies using CD1 and/or V alpha 14 knockout mice have shown that natural killer T cells (NKT cells) may play more discrete or opposing functions during the acute phase of *T. cruzi* infection (Duthie *et al.* 2002; Procopio *et al.* 2002; Miyahira *et al.* 2003; Duthie *et al.* 2005a,b), but the net results point to a role of these cells in resistance to *T. cruzi* infection. However, the mechanisms by which these cells contribute to the immune response to acute infection are not clear yet and might also involve a regulatory function, dampening T-cell hyperactivation, and IFN-γ and NO production (Cardillo *et al.* 2004). Activated NK cells, bearing a particular phenotype (CD16 + CD56–), were found to increase during the acute phase of Chagas' disease in children (Sathler-Avelar *et al.* 2003). However, the exact mechanism used by NK cells to help in the control of the infection in humans is not clear. The mechanism might rely on the secretion of cytokines such as IFN-γ and TNF-α by parasite activated NK cells, thus amplifying the innate and/or the adaptive immune responses (Andrade, Gollob and Dutra 2014).

Another T-cell lineage that might be involved in up- or down-regulation of the immune response to *T. cruzi* during the acute phase of the infection is the $\gamma\delta$ T lymphocyte (Cardillo *et al.* 1993; Nomizo *et al.* 2006). $\gamma\delta$ T cells are not homogeneous and their functions may vary depending on T-cell receptor usage and different stimulatory conditions (Chien and Hampl 2000; Chien, Meyer and Bonneville 2014). A small subpopulation, bearing the Vγ1 chain, is found in the thymus and in the secondary lymphoid organs such as the spleen, lymph nodes and the GALT of the adult mouse (Azuara *et al.* 1997, 2001; Azuara, Lembezat and Pereira 1998; Azuara and Pereira 2000). Part of this subset also expresses NK1.1 molecules (Azuara *et al.* 1997) and the administration of a monoclonal antibody to the Vγ1 chain results in increased susceptibility to *T. cruzi* infection (Nomizo *et al.* 2006). These cells appear to function as helpers for conventional CD4 T cells, increasing the formation of memory T cells and their IFN-γ production. Taken together, the previously mentioned studies show that the NK1.1+ cell subset is composed by different lineages, having complex functions (Werner *et al.* 2011). In spite of this, these cells may function by helping conventional T cells to fully differentiate into memory cells, since in their absence conventional T cells might accumulate in an early stage of activation. This would lead to elevated production of inflammatory cytokines and oxygen-containing toxic molecules in peripheral lymphoid organs, since these cells do not migrate efficiently to infected tissues and are ineffective parasite killers in infected tissues. The overall result would be immune hyperactivation accompanied by poor parasite growth control and early death of the host (Cardillo *et al.* 2004).

B lymphocytes are also required to mount an effective immune response to *T. cruzi*, helping in the control of the infection (Cardillo *et al.* 2007; Sullivan *et al.* 2015). The disease in C57BL/6 muMT KO mice is more severe than in control mice, with higher parasitemia levels and a poor generation of central and effector memory CD4+ and CD8+ T cells in the spleen. During early stages of the *T. cruzi* infection, B cells are fundamental to trigger T-cell functions related to the Th1 pathway that favor the control of parasite growth (Cardillo *et al.* 2007). Therefore, in the absence of mature B cells, the immune system is unable to generate and/or maintain central and effector memory CD8+ T cells and to instruct a Th1 functional pattern of T-cell cytokines, since the levels of proinflammatory cytokines such as IFN-γ and IL-12 are reduced in spleen cell supernatants from mice lacking mature B cells (Cardillo *et al.* 2007). Tissue inflammatory responses in these mice are much less intense in the acute phase of the infection, which is consistent with a deficit in the generation of

effector T cells. Furthermore, the preponderant cell type in the skeletal muscle inflammatory infiltrate is the CD4+ T cell, contrary to what is observed in B-cell-sufficient mice, where CD8+ T cells dominate in the inflammatory infiltrate (Cardillo *et al.* 2007). Adoptively transferred splenic B cells induce increased numbers of both effector/memory splenic CD4 and CD8 T cells, during early chronic infection (Cardillo *et al.* in preparation). Accordingly, it has been reported that the development, maintenance and functional activities of memory CD8+ T cells during immune responses are dependent on the generation of memory CD4+ T cells and B cells (Williams *et al.* 2006; Sullivan *et al.* 2015). Besides, it has been described that B cells may themselves produce many different cytokines upon stimulation, including IFN-γ, IL-10, IL-12, IL-17 and BAFF/BLyS (O'Garra *et al.* 1990; Mengel *et al.* 1992; Pang *et al.* 1992; Veras *et al.* 2006; Wojciechowski *et al.* 2009; Amezcua Vesely *et al.* 2012; Bermejo *et al.* 2013). In addition, in the chronic phase of *T. cruzi* infection one may speculate that B cells modulate the immune response through IL-10 production, since the transfer of B cells from IL-10 knockout mice to mu knockout mice helps to control the acute infection, but also leads to an increased inflammatory heart disease in the chronic phase (Cardillo *et al.* in preparation). Regarding this topic and until recently, virtually no report addressed the real phenotypic markers of B cells producing IL-10, in humans. Thus, the CD19+ CD5+ CD1d+ IL-10+ B cells were found to be increased in chronic chagasic patients. In addition, a higher expression of CD21 and CD24 on the surface of circulating CD19+ B cells has been shown in those patients. The study also showed that the expression of MHC-II (HLA-DR), CD80, CD86, caspase-3, granzyme B and intracellular IL-10 and TGF-β by CD19+ B cells was higher in patients with chronic Chagas disease (Fares *et al.* 2013).

In summary, in *T. cruzi* experimental infection, with the Tulahuen strain, the development and function of memory CD8+ and CD4+ T cells are greatly modulated by NK1.1+ and B cells, since lower numbers of memory T cells are formed in acute infection when these subsets are absent. In addition, high parasite load has been observed in NK1.1 cell- or B-cell-depleted mice, indicating that the conversion of activated to effector memory cells is an important step in the control of infection levels and mortality.

The initial magnitude of CD8+ T-cell responses appears to be one of the critical factors in determining the final size of the antigen-specific memory T-cell pool (Olivieri, Cotta-De-Almeida and Araujo-Jorge 2002; Williams *et al.* 2006; Bixby and Tarleton 2008; Bustamante, Bixby and Tarleton 2008; Miyahira 2008). Central memory CD8+ T cells, expressing high levels of CD44 and CD62L and reduced expression of KLRG1, a marker of repetitive antigen stimulation and cell exhaustion (Bustamante, Bixby and Tarleton 2008), are detectable in the late acute phase among the parasite-specific CD8+ T cells, being related to the low parasite load found in the chronic phase of *T. cruzi* infection. Therefore, it seems that the central memory CD8+ T-cell population increases as the infection becomes chronic and this pool may be important to dynamically replace cells in the memory/effector T-cell pool, as previously suggested (Sallusto *et al.* 2010). Interestingly, treatment with benznidazole during the acute phase of the infection lowers the parasitemia and also induces a stable pool of central memory CD8+ T cells (Olivieri, Cotta-De-Almeida and Araujo-Jorge 2002; Bixby and Tarleton 2008). In addition, an increase in total effector/memory CD8+ T cells in *T. cruzi*-infected subjects has been reported (Leavey and Tarleton 2003; Fiuza *et al.* 2009). However, the authors also claimed that these cells would be dysfunctional and this could be a consequence of a gradual clonal exhaustion in the CD8+ T-cell population, perhaps as a result of continuous antigenic stimulation by persistent parasites (Leavey and Tarleton 2003). This study also showed an increase in the numbers of effector memory CD8+ T cells as the disease progresses, suggesting that the central memory T-cell pool could be, in fact, a source of effector memory T cells. Consequently, its depletion may worsen the disease by increasing tissue parasite load during the chronic phase of the infection, since the accumulation of effector/memory CD8 T cells would be less effective in controlling the infection because they would be functionally exhausted. However, in another series of experiments, resistance in the acute phase of murine *T. cruzi* infection correlated with higher percentages of effector/memory T cells prior to infection (Cardillo *et al.* 2002). This was the case for mature/aged or thymectomized mice, where T cells are submitted to homeostatic expansion due to thymic hypofunction and acquire memory/effector markers (Cardillo, Nomizo and Mengel 1998; Cardillo *et al.* 2002). In fact, the levels of effector/memory T cells could be inversely correlated with host susceptibility, since the higher the numbers of effector/memory T cells found in mature/aged or thymectomized mice, the lower their susceptibility (Cardillo *et al.* 1993, 2002). Therefore, it appears that effector/memory T cells are required to control infection, but a pool of central memory T cells is also important to replenish exhausted effector/memory T cells. The restricted availability of reagents to follow parasite specific T cells has hampered more detailed studies, aiming at the evaluation of memory T cells during the acute Chagas' disease. However, after culturing mononuclear cells from chronic patients with *T. cruzi* extracts, we have found a preferential *in vitro* expansion of CD4 + Vβ5+ T cells. In addition, we have shown a decrease in Vβ5 expression in the CD4± T-cell population freshly isolated from acutely infected chagasic individuals, probably reflecting tissue redistribution rather than depletion, whereas CD4 + Vβ5+ T cells were found to be increased in a subset of chronic chagasic patients (Costa *et al.* 2000). As a whole, the results showed a differential Vβ-TCR usage in different stages of the disease, and that parasite antigens stimulate a portion of the T-cell repertoire with preferential usage of Vβ5-TCR. Therefore, CD4 + Vβ5+ T cells are a unique population of CD4 T cells to be analyzed in future studies, regarding the dynamics of memory T-cell formation in humans.

HOW (AUTO)IMMUNITY IS CONTROLLED DURING *T. CRUZI* INFECTION

As pointed out above, *T. cruzi* induces a strong immune response against its own components, but the infection also induces a measurable immune response to host self-antigens (dos Santos *et al.* 1992; Bonney and Engman 2008; Bonney and Engman 2015). Antigenic mimicry between parasite antigens and host antigens may underlie the reasons for the anti-host autoimmune response (Wood *et al.* 1982; Duranti *et al.* 1999; Cunha-Neto *et al.* 2006). However, antigenic mimicry and immune cross-reactivity among parasite antigens and host antigens are not always deleterious and may even be beneficial to a balanced parasite/host relationship (Pontes-de-Carvalho *et al.* 2013; Massilamany, Gangaplara and Reddy 2015). Therefore, a malfunction of regulatory immune mechanisms may also be involved in the autoimmune responses during the infection (Cardillo *et al.* 1993, Cardillo, Nomizo and Mengel 1998; Mariano *et al.* 2008). It is debatable whether the autoimmune response found during the *T. cruzi* infection is actually the causative factor leading to organ damage during the chronic phase of the disease (Cunha-Neto *et al.* 2011). However, both the immune response

to parasite antigens and host self-antigens are not dissociated and occur concomitantly (Gattass *et al.* 1988; Cunha-Neto *et al.* 2011), and therefore should both be considered as promoters of tissue lesion during infection. A similar condition is found in autoimmune inflammatory bowel diseases (IBD), which ameliorate with the use of antibiotics, suggesting a role for bacteria as an important factor for the disease initiation or persistence (Sokol 2014). Yet, in IBD there is a considerable overlap between immunity and autoimmunity (Cassinotti *et al.* 2014). This means that it is not just an infection per se that may trigger autoimmunity, but an infection that is inappropriately dealt with (Sester *et al.* 2015). Nevertheless, in most of the chronically *T. cruzi*-infected patients, a perfectly balanced immune response is achieved and pathology is never manifested (Rassi, Rassi and Marin-Neto 2010). In addition, it has been described that susceptibility to *T. cruzi* infection might reflect an overreactive host immune response that kills most of the susceptible individuals without being effective in the control of the parasite load (Nascimento *et al.* 2002; Cardillo *et al.* 2004). In any of the cases described above, regulatory mechanisms are at the core of the problem, either dealing with resistance/susceptibility in acute infection or health/disease during the chronic phase.

Many cells and molecules have being described to have regulatory/suppressor activity during *T. cruzi* infection (Cardillo *et al.* 1993; Lopes and Reis 1994; Abrahamsohn and Coffman 1995; Pinge-Filho *et al.* 1999; Cuervo *et al.* 2011). For instance, splenic adherent cells or macrophages were described to suppress T-cell responses *in vitro*, by the release of mediators such as prostaglandins (Pinge-Filho *et al.* 1999) and nitric oxide (Abrahamsohn and Coffman 1995). More recently, myeloid-derived suppressor cells (MDSCs) were claimed to be responsible for controlling or suppressing immune responses during acute *T. cruzi* infection (Cuervo *et al.* 2011; Goni, Alcaide and Fresno 2002; Arocena *et al.* 2013).

In another series of experiments, using mice of the BALB/c genetic background, we were the first to demonstrate that $\gamma\delta$ T cells were involved in the suppression of immune responses during the acute phase of *T. cruzi* infection *in vitro* and *in vivo* (Cardillo *et al.* 1993; Cardillo, Nomizo and Mengel 1998). The *in vivo* depletion of $\gamma\delta$ T cells by an anti-δ monoclonal antibody (Costa *et al.* 2015) raised the levels of IFN-γ produced by $\alpha\beta$ T cells in the acute infection similarly to the *in vivo* blocking of IL-17 (Matta Guedes *et al.* 2010), and promoted the recovery of a third party humoral immune response to ovalbumin during the acute *T. cruzi* infection (Cardillo, Nomizo and Mengel 1998). The $\gamma\delta$ T-cell suppressor activity was absent in the spleens of thymectomized and aged mice, suggesting that those cells were dependent upon an intact thymic function (Cardillo *et al.* 1993; Cardillo, Nomizo and Mengel 1998). Thymic output of naïve T cells clearly downmodulated effector responses, since the continuous administration of thymocytes to either aged mice, young thymectomized mice or total spleen cell-reconstituted athymic mice markedly decreased splenic cell proliferation to non-specific stimulation and increased parasitism in recipient *T. cruzi*-infected mice (Cardillo, Nomizo and Mengel 1998). More recently, one study described that T cells bearing the Vγ4 TCR chain were responsible for producing large amounts of IL-17, resulting in an increase of MDSCs that had the ability to downregulate pathogen-responsive T cells, contributing to parasite persistence (Kong *et al.* 2014). Additionally, these Vγ4 T cells are produced and exported by the thymus as IL-17 producers (Schmolka *et al.* 2013). Some $\gamma\delta$ T-cell subpopulations, again through the production of IL-17, were also implicated in the augmentation of MDSC numbers in tumor microenvironments, either in mice or humans, promoting tumor growth by opposing cancer immunosurveillance (Rei *et al.* 2014; Wu *et al.* 2014). It should be pointed out that most, if not all, $\gamma\delta$ T cells in lymphoid organs of the murine BALB/c background produced only IL-17 and no IFN-γ, in contrast to other mouse strains where these cytokines are produced by different $\gamma\delta$ T-cell subpopulations (Wakita *et al.* 2010). Consequently, the overall function of $\gamma\delta$ T cells during *T. cruzi* infection might be dependent on the genetic background where both *T. cruzi* and mouse strains should be considered. Therefore, it seems that a subpopulation of $\gamma\delta$ T cells, producing high levels of IL-17, is the candidate to modulate the numbers of MDSCs that might be the final suppressor cells in the acute phase of *T. cruzi* infection.

It should be pointed out that the mechanisms described above are easily detected along the acute phase of the infection and vanish after parasite growth is controlled or during the chronic phase of the disease. Therefore, they are less likely to perform these regulatory functions during the chronic infection and in fact, there is no evidence that these mechanisms are operative after the infection is controlled.

The contribution of other regulatory cell populations such as Tr1 and Treg (CD4 + CD25 + Foxp3+) T cells to the immune response modulation, during the acute infection, is not clear yet. It has been shown that IL-10 may function to increase host survival and also to help in the control of parasite load in some models (Hunter *et al.* 1997; Roffe *et al.* 2012). However, the exact source of IL-10 has not been studied in detail. In addition, high levels of IL-10 were recently related to protection against cardiomyopathy in human subjects, indicating that this cytokine is of critical importance in the regulation of the immune response during *T. cruzi* infection (Dutra *et al.* 2014). The role of CD4 + CD25 + Foxp3+ Treg cells has also been evaluated and early mouse studies where these cells were depleted by monoclonal antibodies to CD25 indicated that their role in the regulation of immunity during the acute phase of the *T. cruzi* infection is rather limited (Kotner and Tarleton 2007; Sales *et al.* 2008). On the contrary, recent findings in humans have shown an increased percentage of Treg cells in chagasic subjects in the indeterminate chronic phase (free of disease) when compared to patients with heart damage, suggesting an important role for Tregs in Chagas disease (de Araujo *et al.* 2011). Moreover, it has been recently demonstrated, using a nondepleting monoclonal antibody to CD25, that regulatory CD4 + CD25 + Foxp3+ T cells may also help to control the adaptive immune response, during the acute infection, in mice (Nihei *et al.* 2014). The immunomodulatory activity of the nondepleting monoclonal antibody to CD25 was similar to that which has been described for humans (Huss *et al.* 2015) and encompassed a delayed increase of Treg frequencies and an augmented production of IL-10 and TNF-α by T cells. Interestingly, it was demonstrated that TNF-α levels are significantly higher in Chagas' disease patients with severe ventricular arrhythmias and in patients with dilated cardiomyopathy, suggesting that this cytokine could be detrimental to the heart (Ferreira *et al.* 2003). However, in one study, using the mouse model, *in vivo* blockade of TNF-α during the chronic phase of *T. cruzi* infection aggravated cardiomyopathy, suggesting that TNF-α would have a protective role (Bilate *et al.* 2007). In fact, it has been recently shown that TNF-α is cardioprotective in both mice and humans (Papathanasiou *et al.* 2015). In addition and more importantly, there was a clear indication that the functional activity of Treg cells might be of crucial importance during the acute and chronic phases of the infection, decreasing tissue destruction and pathology (Nihei *et al.* 2014; Bonney *et al.* 2015). Therefore, the notion concerning the manipulation

of Treg cells either by antibodies or even interleukins such as IL-2 (Kosmaczewska 2014) might open up a new avenue for therapeutic strategies in Chagas' disease.

CONCLUDING REMARKS

The study of acute and chronic phases of infection with intracellular pathogens, such as *T. cruzi,* allows the elucidation of the mechanisms and conditions that may be targeted to reprogram the host immune system, either by using tools that interfere with components of the regulatory arm of the immune system machinery (Nihei *et al.* 2014) or by improving therapeutic vaccine strategies (Pereira *et al.* 2015). This knowledge would certainly result in a better understanding of the necessary balance to achieve or reestablish the health of the host during *T. cruzi* infection, thus providing new strategies to treat Chagas' disease, besides the use of drugs that kills the parasite *in vivo*, sterilizing the host—a difficult task to achieve because the available treatments are not always efficient, having many toxic collateral effects, so that clinical researchers have not reached a consensus about them after more than 30 years of their clinical use and in addition, parasite resistance to these drugs is common and well documented (Bestetti and Restini 2014; Molina, Salvador and Sanchez-Montalva 2014; Molina *et al.* 2014; Rassi, Rassi and Marin-Neto 2014; Zingales *et al.* 2015). Of note, the authors of a randomized, double-blind, placebo-controlled trial in which trypanocidal therapy with benznidazole was evaluated in patients with established Chagas' cardiomyopathy concluded that this type of treatment significantly reduced serum parasite detection but did not significantly reduce cardiac clinical deterioration through 5 years of follow-up (Morillo *et al.* 2015).

ACKNOWLEDGEMENTS

We thanks Drs Owen Williams and Lain Pontes de Carvalho for their critical review of the manuscript.

FUNDING

This work was supported by CNPq, PAPES/FIOCRUZ and FOG-FMP/FASE. PRZA is granted with CNPq and FAPERJ research fellowships (PQ-2 and JCNE, respectively).

Conflict of interest. None declared.

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