

RESEARCH ARTICLE

Administration of artinm lectin reduces the severity of the acute phase infection with *Trypanosoma cruzi*

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Abstract

The acute phase of experimental *Trypanosoma cruzi* infection is associated with a strong inflammatory reaction, physiological changes, amastigote nests in tissues, and hematological alterations. ArtinM, a lectin extracted from *Artocarpus heterophyllus* seeds, is a homotetramer exhibiting immunomodulatory properties that promotes Th1 immune responses against intracellular pathogens, including the induction of neutrophil migration and increase in IL-12 production. This study aimed to evaluate the effects of ArtinM on experimental Chagas disease in mice. We evaluated mouse survival curves, parasitemia, hematological parameters including quantification of inflammatory infiltrates, and amastigote nests in cardiac tissue during infection. The results showed a reduced number of parasites in the blood, an increase in animal survival, improvements in hematological parameters, and decrease in inflammatory infiltrates and amastigote nests in the group treated with ArtinM. Collectively, these data suggest that the administration of ArtinM can lower the number of parasites in peak parasitemia caused by the Colombian strain of *T. cruzi* and can increase survival of infected mice. The observed reduction in cardiac tissue injury may be due to fewer *T. cruzi* amastigote nests and lower levels of inflammatory infiltrates. This study highlights the need for further investigation into the use of ArtinM as a potential alternative therapeutic for treating Chagas disease.

KEYWORDS

ArtinM, Chagas disease, parasitemia, *Trypanosoma cruzi*

Abbreviations: APC, antigen presenting cell; BZN, benzimidazole; CD, Chagas disease; CD11b+, cluster of differentiation 11b positive; CD14, cluster of differentiation 14; CD4+, cluster of differentiation 4 positive; CD8+, cluster of differentiation 8 positive; EDTA, ethylenediamine tetraacetic acid; IFN- γ , gamma interferon; IL, interleukin; NFX, nifurtimox; Pg, pictogram; *T. cruzi*, *Trypanosoma cruzi*; TBS, tris-buffered saline; Th, T helper cells; TLR, toll-like receptor; TNF- α , tumor necrosis factor alpha.

Camila Botelho Miguel, Thiago Aparecido da Silva, and Wellington Francisco Rodrigues contributed equally to this work.

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1 | INTRODUCTION

Chagas disease (CD), also known as American trypanosomiasis, is an anthroponotic infection caused by the parasite *Trypanosoma cruzi*, that can be transmitted to small wild mammals and humans by blood-sucking insects of the *Reduviidae* family.¹ CD is endemic to Central and South America, with approximately 8 million people infected with *T. cruzi* worldwide, and resulting in more than 10,000 deaths per year.^{2,3} Previous studies have reported that CD may be acquired congenitally by vertical transmission. However, in non-endemic areas, including the United States of America, Europe, Asia, and Oceania, CD is transmitted primarily by blood transfusion or organ transplantation.⁴

Several aspects of the pathogenesis and progression of CD during the acute and chronic phases of *T. cruzi* infection have not been fully elucidated. These include the following pathogen and/or host-associated features: the genetics/lineage of the strain, the infection phase, and the host immune response to infection.^{5–8} In contrast, *T. cruzi* is well known to invade host cells, where its amastigote form proliferates and creates pseudocysts. The rupture of pseudocysts induces inflammatory mediators, causing fibrosis. Subsequently released trypomastigotes infect other cells, thereby continuing the lifecycle of *T. cruzi*.^{2,9}

Nifurtimox (NFX; Lampit[®]) and benznidazole (BZN; Rochagan[®], Radanil[®]) are the current standard treatments for CD, but both promote cytotoxic effects and exert carcinogenic activities in the host. Moreover, these drugs have demonstrated low efficacy in treating chronic phase CD.^{10–12} Several strategies for treating CD have been studied, including *T. cruzi* ergosterol biosynthesis inhibitors, which have exhibited reduced host toxicity and greater efficacy in controlling CD.^{13–16} Some approaches to CD treatment focus on the inhibition and/or activation of pattern recognition receptors on innate immune cells.^{17–21} In this context, ArtinM, a D-mannose-binding lectin, exerts immunomodulatory activity by recognizing TLR2/CD14 N-glycans and inducing protection against intracellular pathogens.²²

ArtinM is a homotetramer of 16-kDa subunits extracted from *Artocarpus heterophyllus* seeds.²³ ArtinM possesses high affinity for Man α 1-3(Man α 1-6)Man, which constitutes the N-linked glycan core. The effect of ArtinM on antigen presenting cells (APCs) is mediated through its interaction with TLR2/CD14, which induces macrophage polarization to the M1 subtype (classically activated macrophages).²² The immunomodulatory activity of ArtinM also induces lymphocyte differentiation to CD4+ T helper (Th) 1 cells,²⁴ contributing to resistance against intracellular pathogens, such as *Paracoccidioides brasiliensis*,^{25,26} *Leishmania amazonensis*,²⁷ *Leishmania major*,²⁸ *Neospora caninum*,²⁹ and *Candida albicans*.³⁰ Recently, the capacity of ArtinM to induce IL-17

Highlights

- ArtinM activity extended the survival of mice infected with *T. cruzi*.
- Lower cardiac tissue impairment was observed in the ArtinM-treated acute Chagas disease model.
- ArtinM treatment in an acute Chagas disease model decreases the number of amastigote nests and inflammatory infiltration.

production in B and CD4+ T cells by carbohydrate recognition was discovered.³¹ To assess the importance of immune system modulation in controlling CD, we evaluated the effect of therapeutic ArtinM administration on the severity of the acute phase of *T. cruzi* infection. The immunomodulatory activity of ArtinM reduced the number of trypomastigotes and increased the number of leukocytes in the peripheral blood. Moreover, administration of ArtinM reduced *T. cruzi* amastigote numbers in the hearts of treated mice, accompanied by a balanced immune response.

2 | MATERIALS AND METHODS

2.1 | Cell viability assay with resazurin

The *T. cruzi* strain “Y-GFP” was kindly provided by the Department of Cellular and Molecular Biology and Pathogenic Bioagents at the University of São Paulo (Ribeirão Preto, SP, Brazil) for this assay. Ninety-six well microtiter plates were used for optimization of the experiments. After counting the parasites in a Neubauer chamber, 90 μ l of RPMI medium with 10% SFB and 5×10^3 trypomastigote forms per well were added. After 1 h, the following stimuli were added: 10% DMSO medium, and ArtinM at 5, 1, 0.5, and 0.1 μ g/ml. After 15 h, 0.015% resazurin was added. After 8 h, quantification was performed using a spectrophotometer (Power Wave-X microplate reader; BioTek Instruments, Inc., Winooski, VT) at 506 nM. The tests were performed in quadruplicate.

2.2 | Ethics statement

All animal experiments complied with the standards described in the Ethical Principles Guide for the Care and Use of Laboratory Animals adopted by the Brazilian College of Animal Experimentation. The protocols were approved by the Committee of Ethics in Animal Research of the Federal University of Triangulo Mineiro (UFTM; approval number 157/2010).

2.3 | Mice and *T. cruzi*

Mice were housed in the animal facility of the Department of Cell Biology, UFTM, under optimized hygienic conditions. Male BALB/c mice were used at 10 weeks of age. The Colombian strain of *T. cruzi* (MHOM/CO/00Colombiana (*T. cruzi* I))^{32,33} was maintained in the Department of Cell Biology of the UFTM.

2.4 | ArtinM purification and protocol for treatment of *T. cruzi* infections

ArtinM was purified from a saline extract of *A. heterophyllum* (jackfruit) seeds via affinity chromatography using D-galactose and D-mannose resins, as previously described.²³ These procedures were performed on an AKTA Purifier system (General Electric, Amersham). Before use, ArtinM aliquots were applied to Detoxi-gel columns according to the manufacturer's instructions to remove endotoxins (Catalog number 20339; Thermo Scientific).

BALB/c mice (5 per group) were subcutaneously injected with 3×10^3 *T. cruzi* trypomastigotes. Infected mice were treated with ArtinM (0.5 µg/animal) or vehicle (saline; 100 µl/animal) by intraperitoneal infusion on days 1, 2, and 3 prior to infection, and days 5, 10, and 15 post-infection. A group of uninfected mice was included as the control group.

2.5 | Parasitemia trypomastigotes of *T. cruzi* and survival curves

The number of *T. cruzi* trypomastigotes in the peripheral blood of mice was quantified on days 7, 14, and 21 post-infection by direct light microscopy. Five microliters of blood obtained from the tail vein was used to quantitate parasites, as reported by Brener.³⁴ The number of *T. cruzi* trypomastigotes was determined for each mouse by taking an average of triplicate counts. Parasitemia load was expressed as parasites/ml. Survival was assessed daily for a period of 60 days post-infection. Results are expressed as percent survival.

2.6 | Complete blood counts

Blood samples were drawn from the ophthalmic plexus at 23-days post-infection, and 5 µl of 10% EDTA was added to each collected blood sample. These samples were used to count erythrocytes and leukocytes, and quantify hemoglobin and hematocrit levels, using an automated cell counter (ABX MICROS 60; Horiba ABX Diagnostics). Differential leukocyte counts were performed in peripheral blood smears after staining with a kit for fast staining in hematology

(REAG-QUICK panoptic kit, Laborclin, Curitiba, Brazil). The results were expressed as absolute number/mm³. Reticulocyte counts were determined by mixing 15 µl of blood with 15 µl of brilliant cresyl blue (Laborclin, Curitiba, Brazil) for 20 min at 37 °C and using bright-field microscopy at 1000× magnification to quantify the number of reticulocytes.

2.7 | Cytokine quantification

Plasma was separated from the collected blood obtained from each mouse on day 23 post-infection, and was used to quantify levels of IL-12 p40, IL-10, INF-γ, and TNF using an enzyme-linked immunosorbent assay kit (OptEIA; BD Biosciences Pharmingen), according to the manufacturer's instructions. The results are expressed in pg/ml.

2.8 | Flow cytometric analysis of spleen cells

Mice spleen cell suspensions were prepared as reported by Da Silva et al.²⁴ The concentration of each cell suspension was determined using a Neubauer chamber. Subsequently, 5×10^5 cells from each mouse were incubated with anti-CD4 FITC (10 µg/ml; clone H129.19; BD Biosciences), anti-CD8 FITC (10 µg/ml; clone 53-6.7; BD Biosciences), anti-CD3 PE (10 µg/ml; clone 145-2C11; BD Biosciences), anti-CD11b PE (10 µg/ml; clone M1/70; BD Biosciences), or anti-CD19 PE (10 µg/ml; clone 1D3; BD Biosciences) antibodies for 45 min at 4 °C. Cells were then washed with phosphate-buffered saline (PBS) and resuspended in fresh PBS containing 0.5% (w/v) formaldehyde. An IgG isotype control antibody was used to calibrate the flow cytometry analysis. The frequency of CD4+ T cells, CD8+ T cells, CD 11b cells, and B cells were determined by flow cytometry (Guava easy-Cyte; Guava Technologies).

2.9 | Histopathological processing and quantification of inflammatory infiltrates

Mice hearts were sectioned longitudinally into three equal fragments. The middle fragment was fixed in methacarn, dehydrated in a series of ethyl alcohol solutions, diaphanized in xylol, and embedded in paraffin. Serial sections with a thickness of 6 µm were prepared at intervals of 60 µm. Tissues were stained with hematoxylin and eosin (Interlab, Contagem, Brazil).

Analyses were performed using a light microscope fitted with a digital camera (Evolution MP 5.0; Media Cibernetica Inc., USA) and Image-Pro Plus software (Media Cibernetica Inc., USA). Nine images were captured per slide from the

right and left ventricle for a total of 18 images from each animal. Images of inflammatory infiltrates were captured using 20× objective lenses and were analyzed using ImageJ software.³⁵ One image containing 393,622.66 μm^2 was separated into 25 grids (into each grid was 100 points; 155.20 μm^2 per point), and 3104.00 μm^2 (20,000 points) was considered for each ventricle. The number of points was determined using a formula from Hally.³⁶ Quantitation of the area of inflammatory cells in each mouse was calculated using $(\sum \text{points of inflammatory cells} / \text{total points analyzed}) \times 100$. Results are expressed as percentages.

2.10 | Immunohistochemistry analysis of *T. cruzi*

Slides of heart tissue, prepared as described above, were evaluated by immunohistochemistry for the presence of *T. cruzi*. Slides were incubated with xylene for 10 min, dehydrated in 100% alcohol, and hydrated in Tris-buffered saline (TBS). Blocking of endogenous peroxidase was performed with a 3% hydrogen peroxide solution in methanol. After 30 min, slides were incubated with 2% TBS/bovine serum albumin (BSA) for 30 min at 25°C in a dark chamber. Subsequently, tissues were probed for *T. cruzi* using a rabbit anti-*T. cruzi* polyclonal antibody (1:250 dilution) for 2 h at 37°C. Slides were washed three times with TBS and incubated with peroxidase conjugated protein A (1:100 dilution) for 1 h at 25°C. Slides were repeatedly washed three times with TBS, and finally developed with 3,3-diaminobenzidine tetrahydrochloride (DAB Chromogen Kit; Biocare Medical).

Immunohistochemistry analysis of *T. cruzi* was performed on three images each per slide from the right and left ventricles, for a total of six images per animal (1,550,883 μm^2 /image). Images of *T. cruzi* amastigote nests were analyzed with ImageJ software in the Color Threshold adjust mode. Quantification of the total area of amastigote nests in each mouse was calculated using $(\sum \text{area of nests} / \text{total area in the heart}) \times 100$. Results are expressed as percentages.

2.11 | Statistical analyses

Results were expressed as mean \pm standard error of the mean (SEM) or medians with ranges. All data were analyzed using Prism (Graph Pad Software). Normality and homogeneity of variance were assessed. When confirmed, parametric tests were used, including analysis of variance (ANOVA) with Turkey's multiple comparison test or unpaired *t*-tests for two-point comparisons. For non-Gaussian distributions, nonparametric tests were used, including Kruskal–Wallis tests with Dunn's multiple comparison tests or Mann–Whitney *U* tests for two comparisons. The Spearman nonparametric rank test

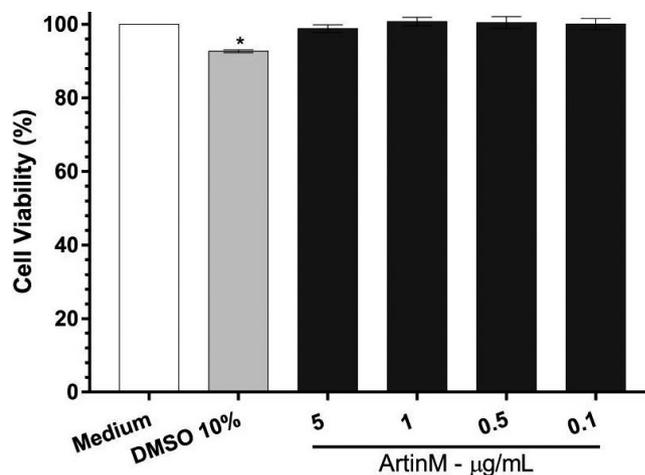


FIGURE 1 Viability assessment of *Trypanosoma cruzi* cells after stimulation with ArtinM. The *in vitro* cultivation of the *T. cruzi* “Y-GFP” strain was performed to evaluate the interaction between cell viability and stimulation with ArtinM. Stimulation with 10% DMSO, and ArtinM at 5, 1, 0.5 and 0.1 $\mu\text{g/mL}$ was performed with 5×10^3 trypomastigote forms in multi-well plates. The results are expressed as mean \pm SEM. Nonparametric tests were used for the analysis. * $p < 0.05$ represents a significant difference between the groups

was used to assess correlations in the data. Differences were considered significant at $p < 0.05$.

3 | RESULTS

3.1 | Cellular viability of *T. cruzi* does not change after stimulation with ArtinM

We evaluated the potential effects on the viability of *T. cruzi* cells (“Y-GFP”) after stimulation with different concentrations of ArtinM *in vitro*. The different treatments with ArtinM (5, 1, 0.5, and 0.1 $\mu\text{g/mL}$) did not influence the percentage cell viability when compared to the group that did not receive treatment (only medium). The group that received 10% DMSO showed significantly reduced cell viability (92.70%), $p < 0.05$ (Figure 1).

3.2 | Mice treated with ArtinM showed lower *T. cruzi* parasitemia loads

The immunomodulatory activity of ArtinM induces host immunity through the Th1-cellular immunity axis, which may protect against *T. cruzi* infection. Therefore, we studied the effect of ArtinM administration on *T. cruzi* trypomastigote parasitemia. Quantification of trypomastigotes in the peripheral blood on days 7, 14, and 21 post-infection demonstrated that mice treated with ArtinM reduced parasitemia by day 14 ($283,333 \pm 18,224$ parasites/ml) and 21

($2,815,333 \pm 309,159$ parasites/ml) compared to the saline group ($505,333 \pm 87,001$ and $5,217,333 \pm 510,332$ parasites/ml, in 14 and 21 days, respectively), in 56.07% and 53.96% mice at 14 and 21 days post-infection, respectively (Figure 2A). Mice that received ArtinM displayed 20% mortality after 47 days of infection, while the saline group displayed 60% mortality at 47 days post-infection (Figure 2B). Thus, the administration of ArtinM reduced parasitemia of *T. cruzi* in the acute phase of infection, which may play a role in the inhibition of CD progression.

3.3 | Treatment with ArtinM alters the number of leucocytes in peripheral blood and in spleens of *T. cruzi*-infected mice

The capacity of ArtinM to reduce the *T. cruzi* trypomastigote load led us to evaluate peripheral blood levels of erythrocytes, hematocrit, hemoglobin, and leukocytes on day 23 post-infection. Reductions in the levels of erythrocytes, hematocrit, and hemoglobin induced by *T. cruzi* infection were observed in both saline and ArtinM groups (Figure 3A-C), compared to uninfected mice. However, administration of ArtinM boosted peripheral blood leucocyte levels in mice infected with *T. cruzi* (7070 ± 1139) relative to levels in uninfected mice (4690 ± 902.4), in 57.75% (Figure 3D), whereas the numbers of leukocytes in infected mice treated with saline were not significantly altered compared to the control group (Figure 3D). In addition, the numbers of neutrophils (increased by 122.71%), lymphocytes (increased by 54.82%), and monocytes (increased by 75.62%) in mice infected with *T. cruzi* were significantly increased in animals treated with ArtinM compared to uninfected mice (Figure 3E-G) but not in infected mice that received saline. Thus, leucocyte levels in the peripheral blood of mice treated with ArtinM may be one mechanism of ArtinM protection against *T. cruzi* infection. We also investigated the phenotypes of leucocytes in the spleen on day 23 post-infection in infected mice treated with or without ArtinM. We found that frequencies of CD8+ T cells and CD11b+ cells were higher in mice

infected with *T. cruzi* than in uninfected mice (Figure 4B,D). In contrast, the frequency of CD4+ T cells and B cells in spleens decreased in *T. cruzi*-infected mice (Figure 4A,C). Animals treated with ArtinM had higher levels of B cells, 7.5%, when compared to untreated mice (Figure 4C).

3.4 | *T. cruzi*-infected mice showed balanced cytokine production after treatment with ArtinM

Cytokine profiles in hosts infected with *T. cruzi* are usually modified to establish the chronic phase of CD. Levels of cytokines in the peripheral blood of mice infected with *T. cruzi* and treated with ArtinM or saline were evaluated on day 23 post-infection. The levels of IL-12, IFN- γ , and TNF- α increased in 4.5, 1000, and 1200 \times , respectively, in *T. cruzi*-infected mice treated with saline relative to uninfected mice (Figure 5A-C), whereas infected mice that received ArtinM demonstrated a significant increase in IL-10 levels compared to infected-saline group mice (154.19%) (Figure 5D). These findings demonstrate that administration of ArtinM modulates the cytokine pattern in the peripheral blood of mice infected with *T. cruzi*, and a regulatory cytokine was also detected on day 23 post-infection. Measurement of cytokine levels in the heart showed that infected mice treated with saline had augmented IL-12 (98.51%) and IFN- γ (41.01%) levels compared to the control group (Figure 5E-F). The levels of IL-12, IFN- γ , TNF- α , and IL-10 in the heart homogenates from *T. cruzi*-infected mice treated with ArtinM did not change relative to other groups (Figure 5E-H).

3.5 | Reduction of inflammatory infiltrates and *T. cruzi* amastigote nests in the hearts of mice treated with ArtinM

Treatment of infected mice with ArtinM reduced the numbers of *T. cruzi* trypomastigotes in the peripheral blood and stimulated

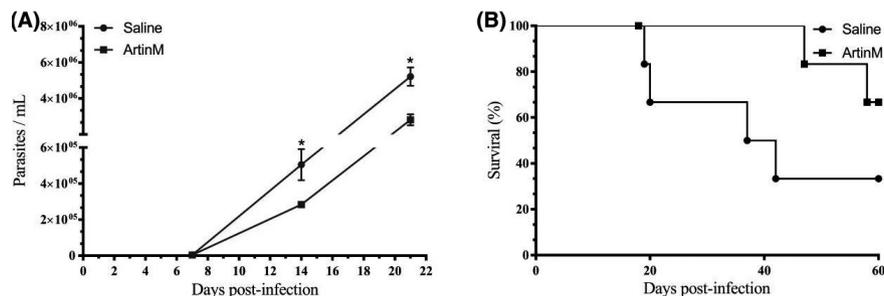


FIGURE 2 Effect of ArtinM administration on parasitemia and survival in mice infected with *Trypanosoma cruzi*. (A) BALB/c mice infected with *T. cruzi* were treated with ArtinM or saline. *T. cruzi* trypomastigotes were counted in peripheral blood on days 7, 14, and 21 post-infection. (B) Survival curves, expressed as percentages, of mice infected with *T. cruzi* that received ArtinM or saline were evaluated for 60 days. Results are expressed as mean \pm SEM, and * $p < 0.05$ represents a significant difference between the groups

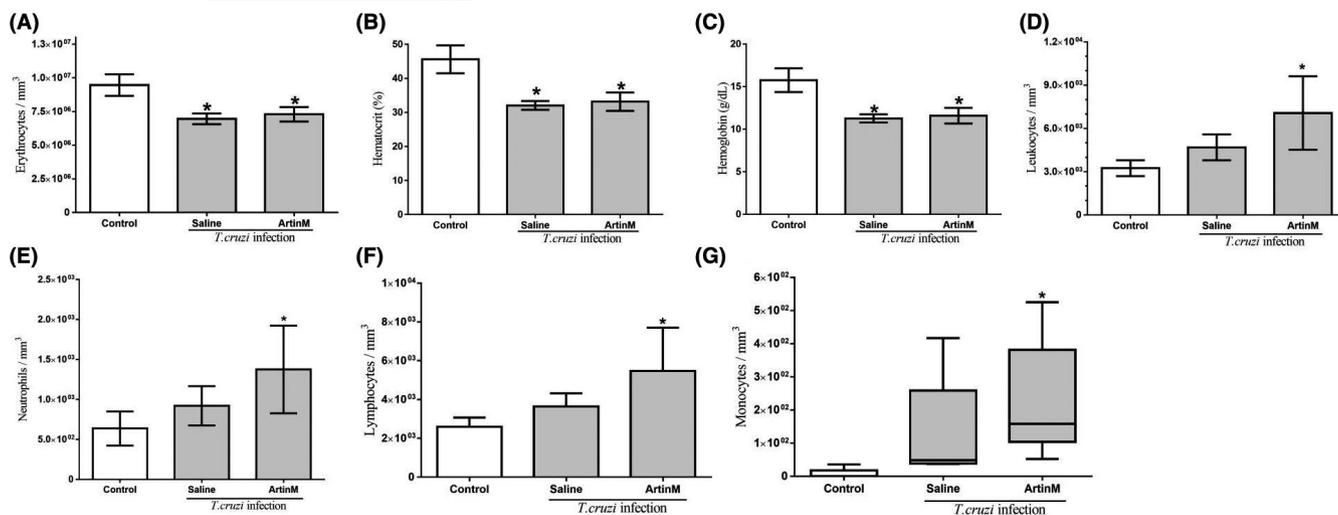


FIGURE 3 Quantification of hematological parameters after treatment with ArtinM in mice infected with *Trypanosoma cruzi*. Blood samples obtained from mice infected with *T. cruzi* (gray bar) and treated with ArtinM or saline were analyzed on day 23 post-infection using an automated cell counter and Neubauer chamber. Uninfected mice were included as a control group (white bar). Erythrocytes (A), hematocrit (B), hemoglobin (C), total leukocytes (D), neutrophils (E), lymphocytes (F), and monocytes (G) were quantitated. Results are expressed as mean \pm SEM, and * $p < 0.05$ represents a significant difference between the groups

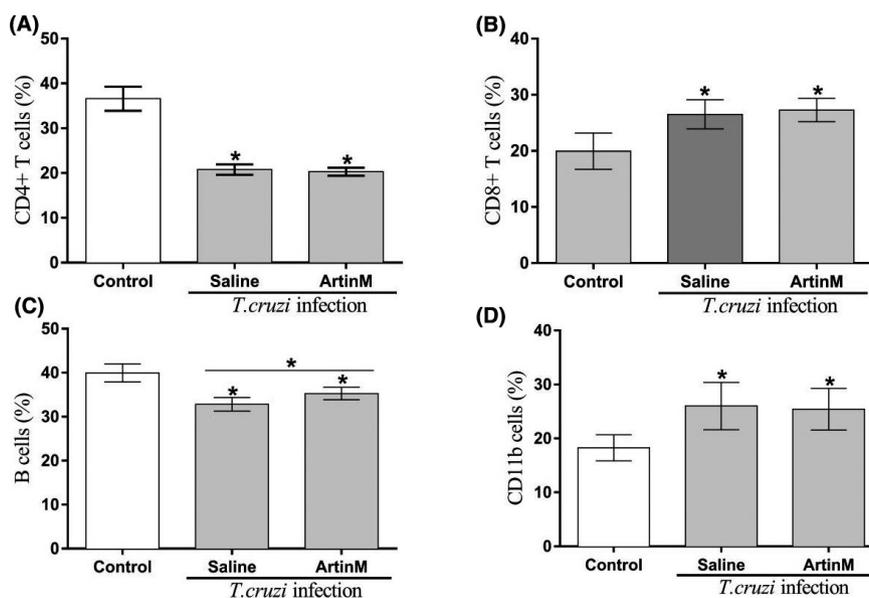


FIGURE 4 Determination of plasma pro- and anti-inflammatory cytokine levels after treatment of ArtinM infected mice. Cytokines were quantitated 23 days post-infection with *Trypanosoma cruzi* (gray bars) after treatment with ArtinM or saline. Plasma levels of the cytokines TNF- α (A), IFN- γ (B), IL-12 p40 (C), and IL-10 (D) were determined by enzyme-linked immunosorbent assay (ELISA). The control group included uninfected mice (white bars) subjected to the same conditions. The results are expressed as mean \pm SEM. Nonparametric tests were used for the analysis. * $p < 0.05$ represents a significant difference between the groups

a balanced immune response. Thereafter, we examined the inflammatory infiltrates in the hearts to evaluate the impact of treatment in the cardiac tissue microenvironment after *T. cruzi* infection. Inflammatory infiltrates were quantified in histological sections of hearts from mice treated with or without ArtinM after 23 days of *T. cruzi* infection. A significant difference between animals that received ArtinM (decrease of 59.78%) and those treated with PBS was observed (Figure 6). Moreover,

ArtinM treatment reduced the area of *T. cruzi* amastigote nest detection by immunohistochemistry in heart tissue, compared with PBS-treated mice by 73.79% (Figure 7A,B). Furthermore, we found a positive correlation between the inflammatory infiltrate and the number of amastigote nests (Figure 7C). These findings indicate that the immunomodulatory activity of ArtinM can control the *T. cruzi* parasite load in peripheral blood and in the heart during the acute phase of CD.

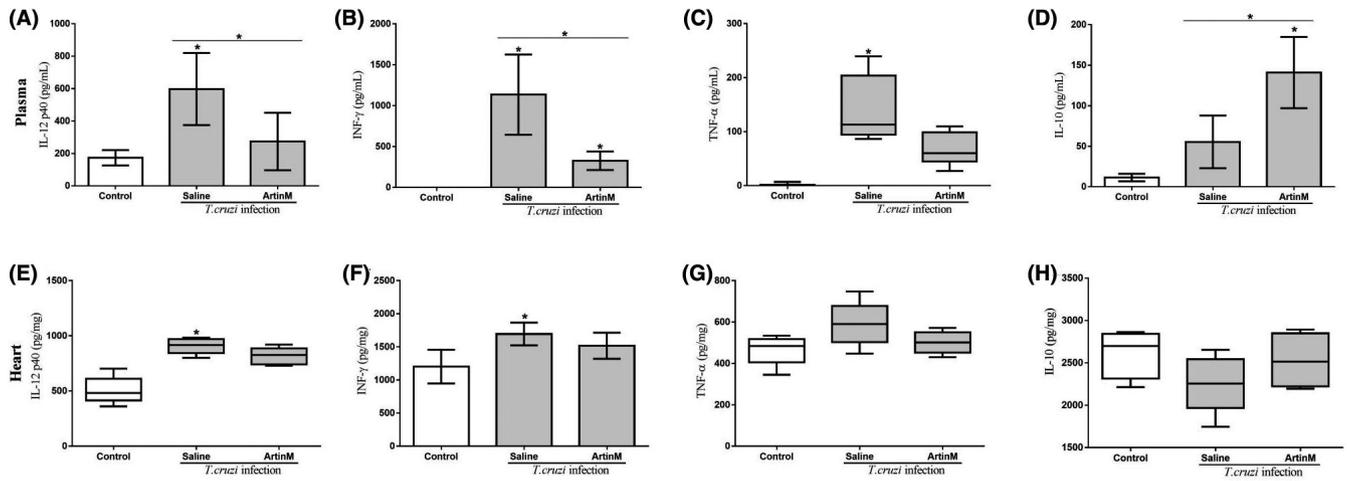


FIGURE 5 Phenotypic profile assessment of spleen cells from infected mice treated with ArtinM Cell markers were evaluated 23 days post-infection with *Trypanosoma cruzi* after treatment with ArtinM or saline. Flow cytometric analysis of spleen cells evaluated the presence of: CD4+ T cells (A), CD8+ T cells (B), B cells (C), and CD11b+ cells (D). The results are expressed as means \pm SEM (B and C) or medians with ranges (A and D). Parametric (B and C) and nonparametric (A and D) tests were used for analyses. * $p < 0.05$ represents a significant difference between the groups

4 | DISCUSSION

Virulence, pathogenicity, and tissue tropism are defining characteristics that differentiate the strains of *T. cruzi*. In this study, we used the Colombian strain, which exhibits tropism in skeletal and cardiac muscles. In this strain, severe immunopathology is noted in affected tissues 20–30 days post-infection, at the same time as parasitemia peaks.^{37–40} Thus, the Colombian strain allowed us to investigate the ability of ArtinM to protect against *T. cruzi* by cardiac tissue analysis at the peak of parasitemia.

CD is associated with several anatomic-clinical forms. Parasite load during the acute phase of infection influences these different forms by injuring specific tissues.^{41,42} The importance of proinflammatory cytokines^{43–45} and nitric oxide⁴⁶ in controlling parasitemia, and consequently in tissue protection, is well known. The immunomodulatory activity induced by ArtinM confers resistance to intracellular pathogens, such as *P. brasiliensis*,^{25,26} *L. amazonensis*,²⁷ *L. major*,²⁸ *N. caninum*,²⁹ and *C. albicans*,³⁰ and promotes the

control of parasitemia during the acute phase of *T. cruzi* infection. This environment promoted by ArtinM provides a basis for understanding the ability of ArtinM to increase the survival of mice infected with *T. cruzi* and confirms the deleterious effects of parasitemia during the acute phase of *T. cruzi* infection.

Inflammatory infiltration into cardiac tissue is an essential parameter for evaluating the effects of different trypomastigote strains on cardiac tissue injury. For this assessment, inflammatory infiltration was quantified after treatment with ArtinM and was shown to decrease in correlation with parasitemia. Mice treated with ArtinM exhibited a significant reduction in *T. cruzi* amastigotes in cardiac tissue. Moreover, a positive correlation was observed between the number of amastigotes and the degree of inflammatory infiltration in the heart within both ArtinM and saline infected groups, illustrating the protective effect of ArtinM during the acute phase of *T. cruzi* infection. These findings demonstrate that the reduction in parasitemia resulting from ArtinM directly protects cardiac tissue.

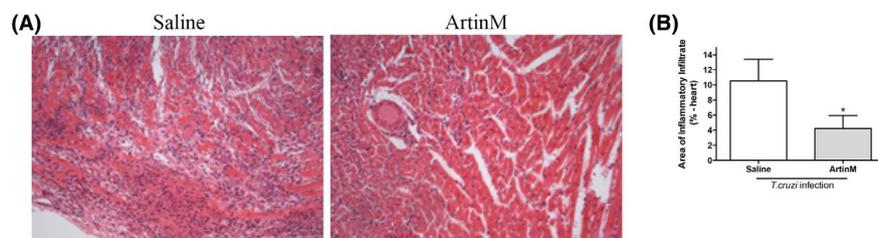


FIGURE 6 Histological analysis of inflammatory infiltrates in the hearts of infected mice treated with ArtinM Inflammatory infiltrates (A and B) in the ventricular walls of infected mice treated with saline (A) or ArtinM (B). After histological processing and staining with hematoxylin and eosin, images were obtained using 20 \times objective lenses and were then magnified by computer software for a larger view (ImageJ). Area of inflammatory infiltrate (%) in relation to the total area analyzed (C). Results are expressed as mean \pm SEM. * $p < 0.05$ represents significant differences between treatments

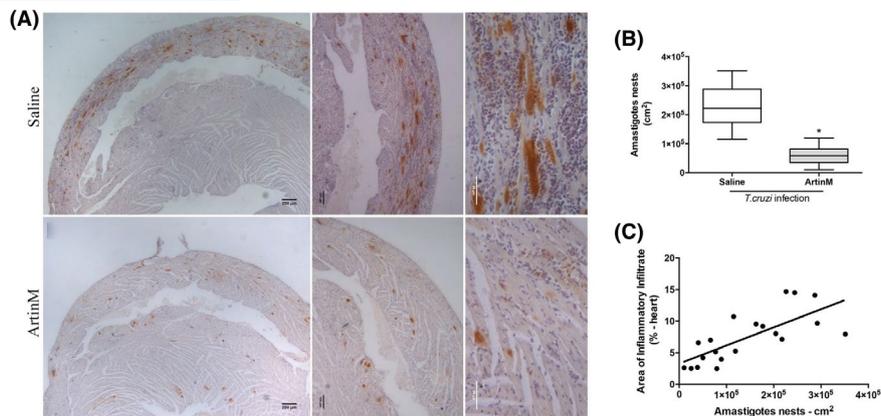


FIGURE 7 *Trypanosoma cruzi* amastigote nests in the hearts of infected mice treated with ArtinM. Amastigote nests (A and B) in the ventricular walls of infected mice treated with saline (A) or ArtinM (B). Immunohistochemical analyses with antibodies against *T. cruzi* and diaminobenzidine (DAB) as a substrate revealed immunocomplexes. Images were obtained using 20× objective lenses and were further magnified using a computer software (ImageJ). (C) Number of amastigotes per cm². Results are expressed as mean ± SEM. **p* < 0.05 represents significant differences between treatments

In vitro infection with *T. cruzi* has been demonstrated to destabilize erythrocyte membranes.⁴⁷ Similarly, *in vivo* infection with certain strains of *T. cruzi* affects erythrocyte membrane stability by inducing oxidative stress.⁴⁸ *T. cruzi* infection-induced anemia is associated with nitric oxide and TNF- α , and oxidative stress is associated with an increase in reticulocytes.⁴⁹ In addition, the deleterious effects on the hematological parameters of infected mice are associated with parasite burden.⁵⁰ Other studies have demonstrated that during the acute phase of *T. cruzi* infection in humans, the prevalence of anemia correlates with the presence of leukocytosis.^{51,52} In this study, we found that infection with the Colombian strain of *T. cruzi* promoted anemia in both infected groups, but ArtinM treatment prevented a significant decrease in erythrocytes. Instead, ArtinM administration increased the number of reticulocytes in mice infected with *T. cruzi*, maintaining close to normal erythrocyte levels.

Quantitation of leucocytes showed that ArtinM treatment of mice infected with *T. cruzi* results in leukocytosis, followed by monocytosis, lymphocytosis, and neutrophilia. The involvement of these cells in the control of *T. cruzi* infection reflects the induction of innate and adaptive immune responses to successfully reduce the deleterious effects of the parasite. Furthermore, effective immunomodulation, observed following treatment with ArtinM, confirms the importance of modulating the immune responses against infections by intracellular pathogens. Our finding that ArtinM administration favors a Th1 profile associated with the induction of IL-12, which can promote immunological control of experimental infections, confirms the results of previous studies.²⁸ Analysis of the cytokine production induced by ArtinM following *T. cruzi* infection, revealed a balanced profile of pro- and anti-inflammatory cytokines due to IL-10 induction

in the presence of the ArtinM-induced Th1 profile. In CD, the prevalence of proinflammatory cytokines is known to correlate with the induction of severe cardiomyopathy.⁵³ Thus, ArtinM exerts a protective effect against *T. cruzi* and prevents cardiac tissue injury by balancing the production of cytokines. Decreases in proinflammatory cytokines are associated with infection control, as demonstrated by Basso⁵⁴ after vaccine therapy with *T. rangeli* antigen.

The present study describes a newly discovered biological effect of ArtinM administration during experimental acute *T. cruzi* infection, besides allowing to indicate a great potential for new therapeutic strategies against CD. Thus, new pre-clinical studies, including a broad assessment of ArtinM binding selectivity, potentially combined with the use of nanocapsules and including the assessment of standard treatment for acute Chagas disease will enable advances in therapeutic or prophylactic use of Artin

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHORS' CONTRIBUTIONS

J. E. Lazo-Chica and M. C. Roque-Barreira conceived and designed the experiments. C. B. Miguel, T.A. Silva, P. K. M. Oliveira-Brito and W.F. Rodrigues performed the experiments. C. B. Miguel, T. A. Silva, W. F. Rodrigues, and J. E. Lazo-Chica analyzed the data. C. B. Miguel, T.A. Silva, W. F. Rodrigues, and M. C. Roque-Barreira wrote the paper. All authors have read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are included within the article.

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