

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/32971262>

# Circulation and behaviour of two major clones of *Trypanosoma cruzi* in Bolivian cycles

Article · January 1996

Source: OAI

---

CITATIONS

0

---

READS

7

3 authors, including:



[Simone Frédérique Brenière](#)

Institute of Research for Development

209 PUBLICATIONS 2,857 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



Study of wild *Triatoma infestans* populations [View project](#)

## RT6 - PATHOLOGY AND DIAGNOSIS OF CHAGAS DISEASE

### CHRONIC, CHAGASIC MYOCARDITIS IS *T. CRUZI*-ANTIGEN AND CD8+ T CELL-DEPENDENT

María de Lourdes Higuchi

Instituto do Coração do Hospital das Clínicas da FMUSP, Av. Dr. Enéas Carvalho de Aguiar 44, 05403-000 São Paulo, SP, Brazil

The role of *T.cruzi* in the pathogenesis of myocarditis in chronic phase of Chagas' disease is still a controversial matter, autoimmune mechanisms frequently being proposed. The main argument favouring this theory is the difficulty to find parasite in histopathological sections of chronic chagasic myocarditis and apparently lack of correlation between its presence and the intensity of inflammatory infiltrate. Many recent reports, mainly using endomyocardial biopsies, have demonstrated that the inflammatory infiltrate has an important role in the development of heart failure in chronic chagasic patients.

In order to clarify the real significance of the parasite in the development of chronic chagasic myocarditis we developed a series of works using polyclonal serum anti-*T.cruzi* and immunoperoxidase technique.

Initially we mapped 9 different regions from each of 8 necropsy hearts from chronic chagasic patients who died due to heart failure, semi-quantifying intensity of *T.cruzi* Ags and myocardial inflammation. We found that 7 (87%) out of 8 hearts exhibited at least one section positive for *T.cruzi* Ags and the septum was the region more frequently positive for parasites and myocardial inflammation. We amplified this study analysing only the septum of 24 chronic chagasic hearts and observed positivity in 58% of the cases and a good association between presence of parasites and moderate or severe myocardial inflammation. However, there was no direct correlation between intensity of Ags and intensity of inflammation. Scarce *T.cruzi* Ags frequently were associated with severe inflammatory infiltrate, suggesting hypersensitivity immune mechanisms involved. Several works have demonstrated that *T.cruzi* induces immunologic depression in the host during acute experimental infection. We characterized the phenotype of the inflammatory cells and the cytokines present in chronic chagasic myocarditis, looking for any correlation of them and presence of parasites. We found that T cells correspond to 96% of the lymphocytes with higher proportion of CD8+ cells. The CD4+ T cells were in reduced number and weakly stained, suggesting immunodepression (CD4+/CD8+ ratio was 0.3). The number of CD8+ T cells was higher in the cases positive for *T.cruzi* Ags. The quantity of CD4+ T cells remained low even in such cases reinforcing the concept that there is some immunological depression in chronic chagasic patients, as CD4+ T cells are expected to proliferate in response to parasites. We also searched for any correlation between different cytokines and the number of CD8+ and CD4+ T cells, in the presence of *T.cruzi* Ags. We observed a good correlation between numbers of CD8+ T cells and numbers of Interferon- $\gamma$  cells, in the groups with absent or scarce amount of *T.cruzi* Ags. The numbers of IL2+ cells were extremely lower even in cases positive for *T.cruzi* Ags. On the other hand, there was a good association between higher numbers of IL-4 + cells in the cases with +++ of *T.cruzi* Ags suggesting that Th2 response is associated with the dissemination and Th1 response with the control of the parasite.

Summarizing, our studies about the influence of the parasite on development of chronic chagasic myocarditis in hearts derived from chagasic patients (necropsies or receptors of heart transplantation) demonstrated that absence, scarce or many *T.cruzi* parasite Ags are correlated with different patterns of inflammatory infiltrate regarding number of CD8+ and CD4+ T cells and cytokines. More studies focusing on the cytokines and their relationship with the parasite survival may help to clarify different outcomes in chronic Chagas' disease.

1. Higuchi ML, De Brito T, Reis MM, Barbosa A, Bellotti G, Pereira-Barreto AC, Pileggi F. Correlation between *Trypanosoma cruzi* parasitism and myocardial inflammatory infiltrate in human chronic chagasic myocarditis: light microscopy and immunohistochemical findings. *Cardiovasc Pathol* (1993) 2(2): 101-106.
2. Higuchi ML, Gutiérrez PS, Aiello VD, Palomino S, Bocchi E, Kalil J, Bellotti G, Pileggi F. Immunohistochemical characterization of infiltrating cells in human chronic chagasic myocarditis: comparison with myocardial rejection process. *Virchows Archiv A Pathol Anat* (1993) 423: 157-160.
3. Reis MM, Higuchi ML, Benvenuti LA, Aiello VD, Gutierrez PS, Bellotti G, Pileggi F. An "in situ" quantitative immunohistochemical study of cytokines and IL-2R+ in chronic, human chagasic myocarditis: correlation with the presence of myocardial *T.cruzi* antigens (In press).

### CIRCULATION AND BEHAVIOUR OF TWO MAJOR CLONES OF *TRYPANOSOMA CRUZI* IN BOLIVIAN CYCLES

Brenière, S.F., Bosseno M.F. & Noireau, F.

UMR CNRS/ORSTOM n° 9926, Laboratoire de Génétique Moléculaire des Parasites et des Vecteurs, La Paz, Bolivia  
Mission ORSTOM Bolivia, CP 9214, La Paz, Bolivia.

Supported by TDR Chagas WHO, ORSTOM, French Cooperation.

On the basis of isoenzyme studies and population genetic interpretation (Tibayrenc *et al.*, *Proc. Nat. Acad. Sci. USA*, 83 : 115-119, 1986), we have previously designed a molecular identification tool of two major clones of *Trypanosoma cruzi* based on both kDNA PCR amplification and southern hybridization using clone specific probes (Veas *et al.*, *Cell. Mol. Biol.*, 37 : 73-84, 1991). These tools allow the direct identification of two genetic sub-groups of clones, genetically unrelated in biological

samples (feces of vectors and human blood, Brenière *et al.*, *Am. J. Trop. Med. Hyg.*, 46 : 335-341, 1992).

In the main vector of Chagas' disease in Bolivia (*Triatoma infestans*) we show that these two groups of clones are wide spread over large geographic distances and infect 92 % of triatomines. We also observe high rates of mixed infections, ranging from 7.7 % to 85.7 % according to the localities. In a high endemic area, we show a significant difference of clone distribution between vectors and chagasic children. We have some arguments to think that both groups of clones are not selected during the transmission to man but that later, the control of parasitemia would be more effective over one of the two groups of clone. The second potential vector in Bolivia is *Triatoma sordida* in some areas. Both groups of clones are occasionally found in this vector, but the large majority of clones are genetically unrelated to them. These data support, (i) a sylvatic origin of both groups of clones further catch by *T. infestans* domestic vector, (ii) the scarcity of present contacts between both cycles sylvatic and domestic.

**NITRIC OXIDE IS INVOLVED IN THE INTESTINAL DESNERVATION OBSERVED IN THE ACUTE PHASE OF EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION**

Garcia, S.B.; Paula, J.S.; Giovannetti, G.S.; Zucoloto, S.; Silva, J.S. Cunha, F.Q.

Departments of Morphology, Pathology, Immunology and Pharmacology of the Faculty of Medicine of Ribeirão Preto. University of São Paulo. Av.Bandeirantes 3900, Ribeirão Preto, SP, Brazil. CEP 14049900.

Chagas disease is a major public health problem in Latin America, caused by the flagellate protozoon *Trypanosoma cruzi*. Visceromegalies, mainly megaesophagus and megacolon are common consequences of this disease and are related with the intrinsic denervation of the organs, mainly in its acute phase (Koberle, 1968). Despite of this, the mechanism of this lesion are not fully known. Chagas (1916) and Koberle (1956) suggested that the neuronal lesions could be determined by the action of neurotoxins, which however have never been identified.

Nitric oxide (NO) is involved in a variety of biological functions in different systems (Moncada *et al.*, 1991). It is involved in endothelium-dependent vascular relaxation, platelet agregation and in central and peripheral neurotransmission. NO is also important anti-microbial effector molecule in macrophage against intracellular pathogens, including *T.cruzi* and its productions is increased in the murine *T.cruzi* infection (Gazzinelli *et al.*, 1992; Petray *et al.*, 1994; Vespa *et al.*, 1994). On the other hand, excessive NO production may cause host pathologic consequences associated with its cytotoxic effect in several cells types, including neurons (Chao *et al.*, 1992). So, the objective of this study was to investigate the possible involvement of NO in intestinal denervation in the acute phase of experimental *T. cruzi* infection.

Wistar male rats were infected with *T. cruzi* and were separated in two groups: group Nitro (9 animals) which was daily injected intraperitoneally with 50mg/kg of N<sup>o</sup>-nitro-L-arginine (NO synthase inhibitor) and group Inf (9 animals) that received daily intraperitoneal injection of saline solution. A control group of 9 animals was not infected and treated (C). After 18 days the animals were sacrificed and colon segments were removed and fixed in bouin for 24 hours and then embedded in paraffin. For each segment ten 7µm circular sections were obtained and stained with H&E and submitted to neuronal counting and histopathological analyze. The serum nitrate concentration was also determined. The statistical significance of the differences between data values was determined by Mann-Whitney-U-test (p < 0,05).

Table shows that the *T.cruzi* infection promotes an increase in the serum NO concentration which was reduced by the treatment of the infected animals with N<sup>o</sup>-Nitro-L-Arginine. Also the *T.cruzi* infection caused decrease in the neuronal number (74%) in the myenteric plexus compared with normal animals. This neuronal lesion was prevented by the treatment of the animals with N<sup>o</sup>-Nitro-L-Arginine. The histopathological analyze of the colon slices showed the presence of inflammatory reaction on the muscular layers and neuronal damage in the infected group. This last find was reduced by the N<sup>o</sup>-Nitro-L-Arginine treatment (data not shown).

Experimental Groups	Serum NO <sub>3</sub> (µM)	Ganglion cells (number/mm colon length)
Inf	24,8 ± 5,4 *	909,1 ± 95,2 *
Nitro	8,0 ± 0,9 †	2741,0 ± 156,6 †
C	4,7 ± 1,6	3486,5 ± 49,9

Ganglion cell counts in colon and serum NO concentrations in *T. cruzi* infected rats (group Inf) and infected and treated with 50mg/kg of Nitro-L-Arginine (group Nitro) and in normal animals (C).

\* p<0.05, compared with the control group

† p< 0.05, compared with the infected group.

These findings suggest that NO is involved in the neuronal destruction of myenteric plexus in acute experimental *T. cruzi* infection. Furthermore, they may explain the benefit effect of the glyocorticoids treatment in the nervous ganglion cells lesions observed in acute Chagas disease (Andrade & Andrade, 1966), since dexamethasone inhibits the induction of the enzyme responsible for NO production (Moncada *e cols.*, 1991).

**References**

Andrade, S.G. & Andrade, Z.A. Doença de Chagas e alterações neuronais no plexo de Auerbach. *Rev. Inst. Med. Trop. São Paulo*, 8(5): 219-224, 1966.  
 Chagas, C. Pathogenic process of American Trypanosomiasis. *Mem. Inst. Oswaldo Cruz VIII (N II): 3-36 (1916).*