RT6 - PATHOLOGY AND DIAGNOSIS OF CHAGAS DISEASE

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

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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 policlonal 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 envolved.
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-γ+ 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.

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

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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 triatomes. 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 scarity 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

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Chagas disease is a major public health problem in Latin America, caused by the flagellate protozoon *Trypanossoma cruzi*. Visceromegalies, mainly megaoesophagus 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) sugested 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 invetigate 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^{ω}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 histophatological analyse. 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_3^- concentration which was reduced by the treatment of the infected animals with N^{ω}-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^{ω}-Nitro-L-Arginine. The histophatological 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^{ω}-Nitro-L-Arginine treatment (data not shown).

Experimental Groups	Serum NO_3^- (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_3^- 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 glycocorticoids 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

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