Rev. Inst. Med. trop. São Paulo
33 (3): 187-192, maio-junho, 1991

IMMUNOMODULATORY EFFECT OF CIMETIDINE ON THE PROLIFERATIVE RESPONSES OF SPLENOCYTES FROM T. cruzi-INFECTED RATS

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SUMMARY

The immunomodulatory effect of cimetidine (CIM), a histamine type-2 receptor antagonist, was evaluated in respect to the blastogenic response to Con A of Wistar Furth (WF) rats infected by the Y strain of **Trypanosoma cruzi (T.cruzi)**. Enhancement of blastogenesis of normal splenocytes was observed at a concentration of 10^{-3}M. However, the splenocytes from infected animals responded to concentrations of CIM ranging from 10^{-8} to 10^{-3}M. The mitogenic response to Con A of cells from infected animals was restored in the presence of CIM. The results show that CIM modulates the "in vitro" proliferative response of cells from **T.cruzi**-infected rats and suggest an immunoregulatory role of histamine and/or of cells that express H2 receptors in this infection.

KEY WORDS: Trypanosoma cruzi; Rat; Inbred strain; Cimetidine; Immunoregulation.

INTRODUCTION

Experimental infection with the protozoan parasite **Trypanosoma cruzi** induces a marked suppression of the humoral and cellular immune responses possibly mediated by suppressor T cells[12], suppressor macrophages[6], a suppressive substance produced by T cells[7] or by a deficiency in the production of IL-2[17].

Some of these mechanisms, e.g., T suppressor cells, could be mediated by hormones as has been already described in other systems[2,15]. Histamine has been shown to suppress cellular immune responses to antigen[14] and mitogen[18], T cell cytotoxic activity[16], lymphokine production[13] and immunoglobulin synthesis[8]. These inhibitory effects of histamine are selective and can be abrogated by H-2 receptor antagonists like Cimetidine (CIM)[1]. This drug can inhibit the stimulatory effect of histamine on suppressor T lymphocytes[11], increase cell-mediated cytotoxicity[5] and the response to mitogens, bacterial antigens and alloantigens[4]. The role of histamine receptors antagonists, as modulators of cell mediated suppression in **T. cruzi** infection has not yet been investigated.

We have previously demonstrated that Wistar Furth rats are moderately resistant to **T.cruzi**. The parasitemia levels, the histopathologic findings and the survival rates were dependent on the size of the inoculum. During the acute phase of infection a three to fourfold increase in the numbers of T cells (W3/13+) of both cytotoxic/suppressor (OX8+) and helper (W3/25+) phenotypes and of B cells and class II expressing cells was observed in the spleen[9].

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The present study has investigated whether the blockage of histamine receptors of spleen cells by Cimetidine can modulate the mitogenic response of these cells in the acute **T.cruzi**-infection in WF rats.

MATERIALS AND METHODS

ANIMALS

Wistar Furth (WF, RT1 ^{u,u}) rats 5 to 6 weeks old of both sexes, 90-120g of weight, from colonies bred and kept at our animal facilities, were used.

PARASITES

Bloodstream trypomastigotes of the Y strain of **Trypanosoma cruzi** were maintained by serial passages in WF rats (3,5-4,5 weeks old). For infection of the animals, heparinized blood was drawn by cardiac puncture from infected rats and inoculated intraperitoneally (i.p.) with $2x10^5$ trypomastigotes/0,2 ml.

CELL SUSPENSIONS

Spleens of WF rats were minced and pressed through a 60 gauge stainless steel mesh and converted to single-cell supensions into ice-cold RPMI 1640 medim (Flow) supplemented with 40ug/ml gentamicin, 25mM N-2-hydroxyethylpiperazine-Ni-2-ethanesulfonic acid (HEPES, Sigma), 20mM sodium bicarbonate and 10% heat inactivated fetal calf serum (FCS, Cultilab), pH=7.2. The spleen cell suspension was centrifuged over a Ficoll-Hypaque (Sigma) density gradient (u=1096 g/ml). The mononuclear cell suspension obtained was then washed three times by centrifugation with RPMI/FCS, counted in a hemocytometer and adjusted to the appropriate concentrations of viable cells per milliliter.

CULTURE SYSTEM

Mononuclear spleen cells (MSC) from normal and trypanosome-infected rats (12 days post infection) were cultured at the concentration of $5x10^5$ cells or $2,5x10^5$/per well, in the presence or not of Cimetidine (CIM, Smith Kline and French Laboratories) at concentrations from 10^{-8} to 10^{-3} M and Concanavalin A (Sigma) at 1,25 ug/ml or pokeweed mitogen (Sigma) at 12,5 ug/ml , in a final volume of 0,2ml of RPMI/FCS. In some experiments, the mononuclear cells from infected animals were co-cultured with mononuclear cells from normal rats at a 1:1 proportion, to a final concentration of $5x10^5$ cells/well, stimulated with Con A with or without 10^{-3} M of CIM. The cultures were incubated at 37 C, an atmosphere of 95% humid air and 5% CO_2 for 72 hours and labeled with 1,0 uCi of ^{3}H-thymidine (^{3}H-Tdr, New England Nuclear) per well. Cultures were harvested on glass fiber filters and processed for liquid scintillation. Cell bound radioactivity was measured in a beta scintillation counter (Beckman LS 3150T).

STATISTICAL ANALYSIS

Statistical significance ($p<0,05$) of the data was determined by Student's "t"-test.

RESULTS

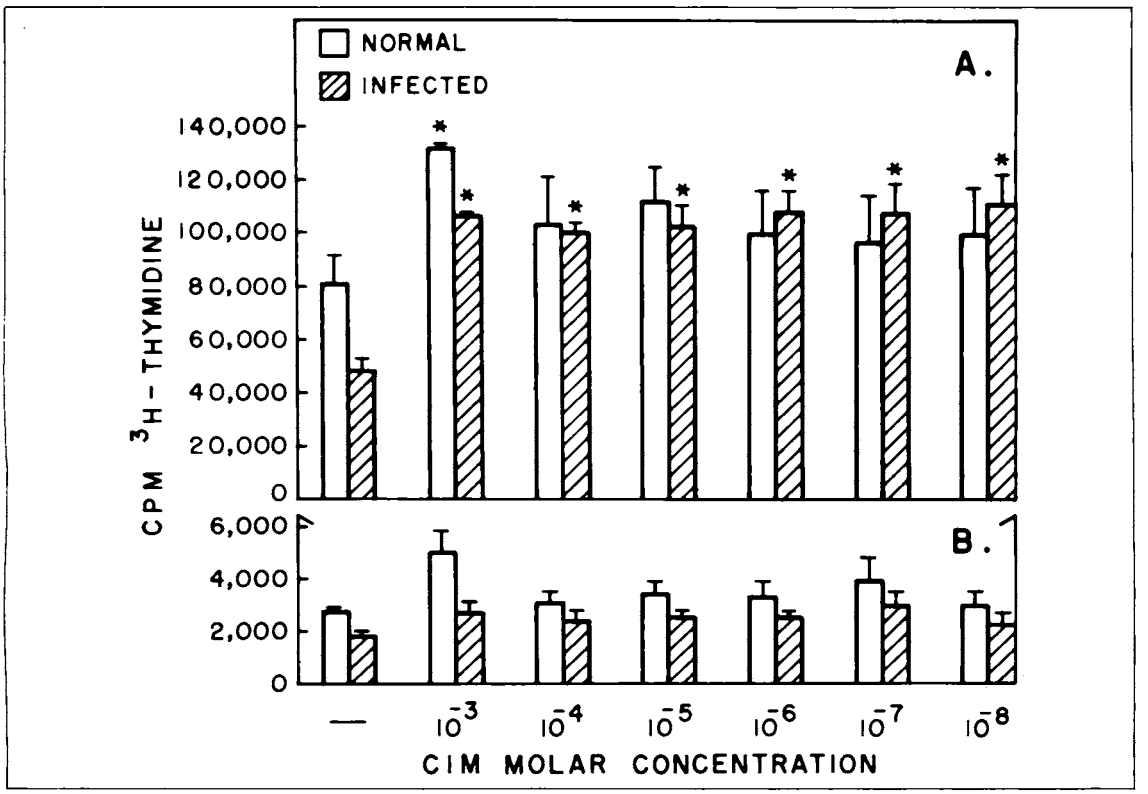
THE EFFECT OF CIMETIDINE (CIM) ON THE PROLIFERATIVE RESPONSE OF MONONUCLEAR SPLEEN CELLS (MSC) OF NORMAL AND **T.cruzi** - INFECTED RATS

The **T.cruzi** infection in WF rats infected with 200.000 bloodstream trypomastigotes induced an exponential increase of parasitemic levels, that achieved $3\text{-}4x10^6$ parasites/ml of blood, near to the death of the animal (22,2±7,2 days post infection).

The results presented here have shown that CIM, at the concentration of 10^{-3} M, induced an enhancing effect ($p<0,05$) of the Con A response of cells from normal animals at the density of 2.5 x 10^5 cells/well (Fig. 1A). Furthermore, the mitogenic response to Con A of MSC from infected animals increased in the presence of any concentration of CIM used (10^{-8} to 10^{-3} M) ($p<0,01$). The addition of the drug in the absence of stimulus did not modify the baseline levels of ^{3}H-Tdr incorporation by the cells (Fig. 1A).

On the other hand, CIM did not interfere with the proliferative response to PWM of MSC from normal and infected animals (data not shown).

Fig. 1. Effect of Cimetidine on mononuclear spleen cells blastogenesis induced (A) or not (B) by Con A of 8-10 normal or **T.cruzi**- infected rats. *p<0,05 compared to control values.



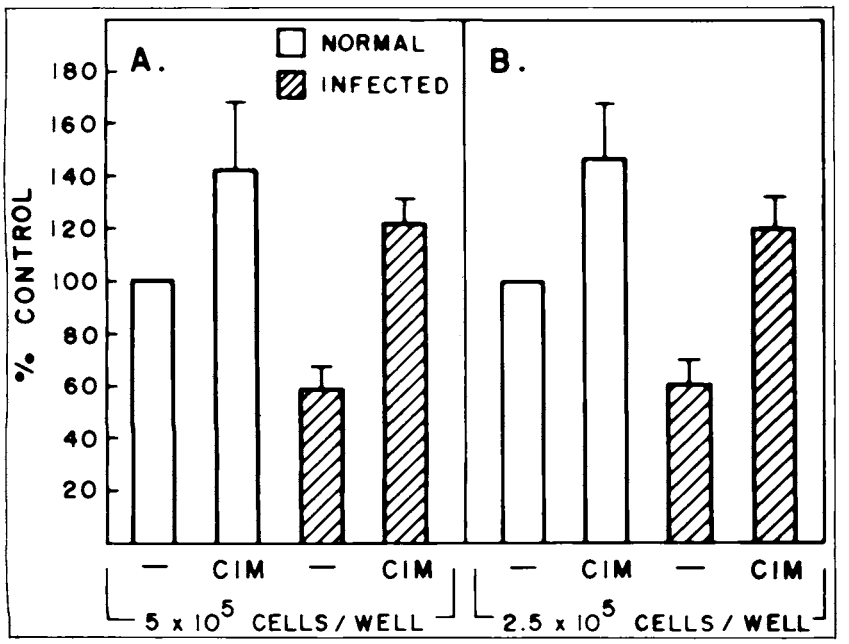


Fig. 2. Restoration of Con A responsiveness of mononuclear spleen cells from **T.cruzi**-infected rats by Cimetidine. Splenic lymphocytes from normal or infected-rats at densities of $5x10^5$ (A) or $2,5x10^5$ (B) cells/well, were cultured for 72h in the presence of 10^{-3} M of CIM and Con A. The results of 8-13 animals are expressed as percentage of the normal cells' response in the absence of the drug.

REVERSIBILITY OF THE BLASTOGENIC RESPONSE OF CELLS FROM T. cruzi INFECTED RATS BY CIM

In Fig. 2A it can be observed that the proliferative response to Con A of spleen cells from infected animals at the density of $5x10^5$ cells/well was significantly diminished (54275±2003; $p<0,01$). This response corresponded to 59,0% of normal blastogenic levels (91178±3423). The addition of the drug (10^{-3}M) not only restored but amplified the mitogenic capacity of **T.cruzi**-infected cells at levels higher than that showed by normal untreated cells. Similar results were observed with cultures at the density of $2,5x10^5$ cells/well (Fig.2B).

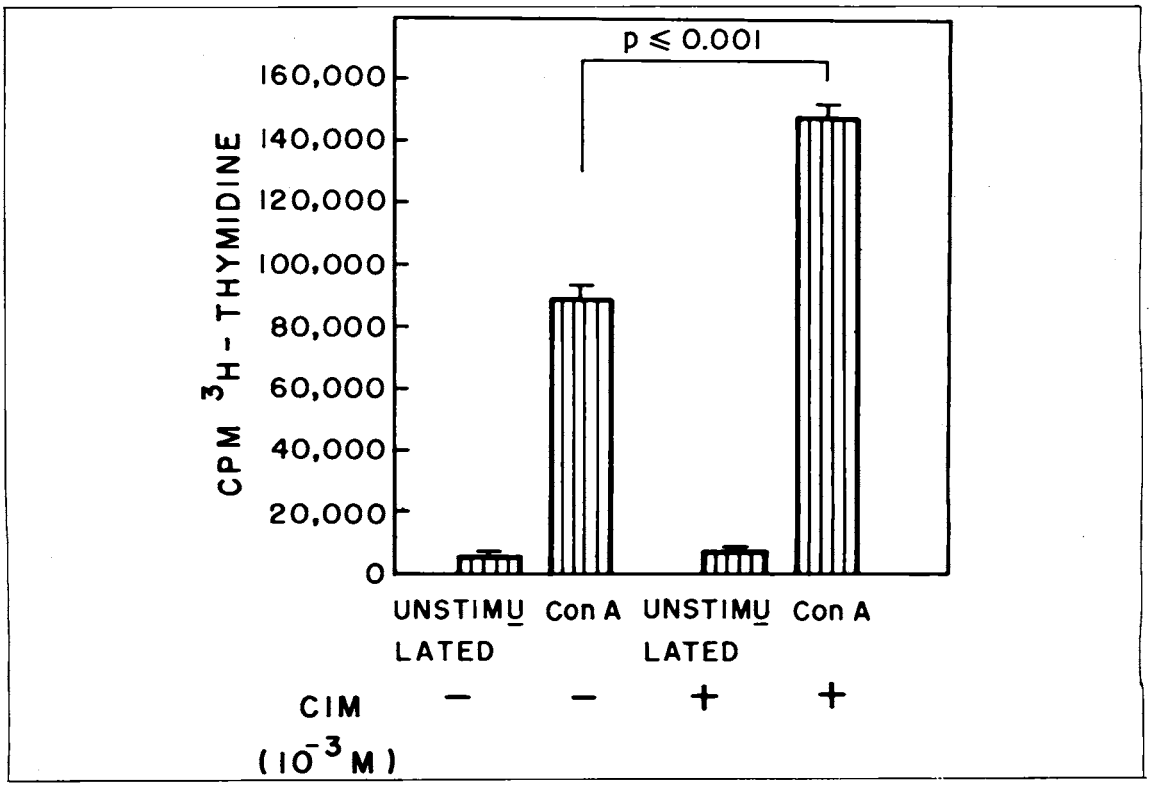
In order to evaluate the action of CIM on co-cultured cells, 5 separate assays were done, with the $2,5x10^5/2,5x10^5$ normal/infected cell ratio. The addition of 10^{-3}M of the drug enhanced significantly the ^{3}H-Tdr incorporation by co-cultured cells (89106±3988 to 148636±1702; $p<0,001$) (Fig. 3). These results show that CIM restored the proliferative response of the co-cultures to Con A, but did not define which subpopulation was stimulated, since at 10^{-3}M, CIM could have enhanced the proliferation of either normal or infected cells or of both.

DISCUSSION

The results presented here show that the suppressed mitogenic response of splenocytes from **T. cruzi**-infected rats to Con A be restored in the presence of Cimetidine.

Immunosuppression of the immune response can be mediated by several mechanisms[12,6,17]. Interaction between histamine and H2 receptors results in the activation of suppressor cells and in the production of histamine-induced suppressor factors[3]. As a consequence of these interactions, histamine can supress specific[14] or nonspecific[18,10] immune responses. Cimetidine, a histamine H2 receptor antagonist prevents histamine binding and therefore inhibits the "in vitro" release of histamine-induced

Fig. 3. Cimetidine-induced augmentation of the blastogenic response to Con A of co-cultured cells. Results represent the mean ± S.E. of five assays.



suppressive factors[1]. These results led us to investigate the proliferative responses of splenocytes from **T. cruzi**-infected rats in the presence of Cimetidine (CIM).

Concentrations of CIM as low as 10^{-8} M were effective in restoring proliferation to cultures of cells from infected animals as compared to 10^{-3} M required for stimulation of normal cell cultures. These results suggest that the histamine pathway participates in the non-specific immunosuppression observed during **T.cruzi**-infection. This effect could be related to the polyclonal activation of 0X8+ cells (T cytotoxic/suppressor), as already observed in the blood an spleen of acutely infected rats[9], which can bear H2 receptor. As CIM also potentiates the blastogenic response of normal cells to Con A, histamine may also play a role in normal immune regulation. However, in order to obtain this effect, a suprapharmacological dosis of CIM is necessary, which could be related to differences in the affinity of H2-receptors among possible diverse target cells in normal and infected cell cultures.

CIM did not affect the proliferation of PWM stimuled cultures, suggesting that there is no involvement of cells bearing histamine receptors in this mitogenic response. In contrast, enhancement of proliferation by CIM was shown to occur to both mitogens in cultures of humans cells[4]. This discrepancy might be explained by the distinct lymphocyte subsets activated by PWM in these two species.

The present study demonstrates that CIM restored the mitogenic response to Con A of splenocytes from **T.cruzi**-infected rats. Therefore, it can be suggested that both histamine and cells that express H2 receptors might be involved in the suppression of the immune response during **T.cruzi**-infection.

RESUMO

Efeito imunomodulatório da cimetidina sobre a resposta blastogênica de esplenócitos de ratos infectados por T.cruzi

O efeito imunomodulatório da Cimetidine (CIM), um antagonista do receptor de histamina-tipo 2, foi avaliado na resposta blastogênica a Con A em células de ratos Wistar Furth (WF) infectados pela cepa Y de **Trypanosoma cruzi (T.cruzi)**. Foi observado que apenas na concentração de 10^{-3}M de Cimetidine houve amplificação da resposta blastogênica de esplenócitos normais a Con A. Entretanto, a capacidade mitogênica de esplenócitos de animais infectados foi restaurada na presença de molaridades da droga que variaram entre 10^{-8} a 10^{-3}. Os resultados demonstraram que a CIM tem o potencial de modular a resposta mitogênica de células de animais infectados pelo **T.cruzi**, sugerindo um papel imunoregulatório da histamina e/ou células que expressam receptores H2 nesta infecção.

ACKNOWLEDGEMENTS

We wish thank Dr. Judith Kloetzel for providing Y strain of **T.cruzi**, Maria Celia Rezende for maintenance of inbred rats. Edna de Souza Joaquim for providing us with reagents, Maria de Lourdes Moura Antonio for secretarial work, Luiz Augusto Marcondes Fonseca and Dewton Vasconcelos for reviewing this manuscript. Research supported by FINEP (PADCT, nº 43.84.0819.00), CNPq (nº 403749/82) and LIM/HC/FMUSP.

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Recebido para publicação em 19/10/1990
Aceito para publicação em 16/4/1991