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CELL MEDIATED IMMUNITY IN CHAGAS' DISEASE. *TRYPANOSOMA CRUZI* ANTIGENS INDUCE SUPPRESSION OF THE *IN VITRO* PROLIFERATIVE REPONSE OF MONONUCLEAR CELLS

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The partial suppression of the cell-mediated immune response by Trypanosoma cruzi *antigens in patients with Chagas' disease is demonstrated in a costimulation assay with* T. cruzi *antigens and* Mycobacterium tuberculosis *purified protein derivative (PPD) or* Tetanus toxoid *(TT).*

Mononuclear cells from 13 patients with chagasic infection without evidence of heart disease, 10 patients with chagasic cardiomyopathy and 7 healthy blood bank donors were stimulated with antigen A (autoclaved epimastigotes), PPD, TT, PPD + A, PPD + TT and TT + A.

The average percentage of suppression induced by costimulation of mononuclear cells with PPD and antigen A was 47.1% in patients with chagasic infection without heart disease (INF), 38.8% in patients with chagasic cardiomyopathy (CDM) and 23.3% in healthy controls. Similar values were observed when living trypomastigotes were used. A costimulatory study with PPD and TT, PPD and A and TT and A was carried out in 8 patients with chagasic infection, in order to evaluate the possibility that this difference could be due to a nonspecific inhibitory effect. The mean suppression induced by TT + PPD was −8.9, with TT + A was 52.7 and with PPD + A was 50.1. The data reported show that T. cruzi *antigens induce a specific suppression of the proliferative response of mononuclear cells, that might be relevant to the persistence of the parasite in the host.*

Key words: *Trypanosoma cruzi* – human Chagas' disease – immunosuppression – cell mediated immunity

Chagas' disease or American trypanosomiasis, a parasitic zoonosis endemic in most of Central and South America, is an important health problem due to the apparent high prevalence of cardiac or digestive tract lesions (WHO, 1974) among the infected population.

Both humoral and cell-mediated immune responses to *Trypanosoma cruzi* antigens are present during the chronic phase of Chagas' disease in humans (Montufar et al., 1977; Mosca et al., 1979; Mosca & Plaja, 1980; DeTitto et al., 1985). However, despite a seemingly adequate immune response, the parasites persist in the blood and tissue, albeit at low levels, which require culture or xenodiagnosis for detection (WHO, 1974). This raises the possibility that the parasites might induce a down-regulation of the immune response to relevant antigens.

Immunological suppression is a complex phenomenon mediated by cells and suppressor factors. Supression may be specific involving T suppressor cells (Ts) and suppressor factors (Fs) or non-specific (Tada, 1984). The presence of suppression has been studied in experimental Chagas' disease in the acute and in the chronic phase of the infection. Cunningham & Kuhn (1980b) described in serum of infected animals the presence of a suppressor substance. Others (Kierszenbaum, 1982; Maleckar & Kierszenbaum, 1983) have reported inhibition of the lymphocyte proliferative response to mitogens by adherent spleen cells, apparently mediated by PGE-2. In addition, Scott (1981) and Ramos et al. (1979) have demonstrated the presence of specific suppressor cells in the chronic phase of the experimental infection. During the acute

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phase of the infection in humans Texeira et al. (1978) have reported, in some patients, the lack of response to *T. cruzi* antigens. We have demonstrated (Castes et al., 1986) the presence of non-specific suppression, mediated by PGE-2 in INF. This non-specific suppression, was not present in CDM, suggesting that in CDM this immunoregulatory mechanism may be abolished. Beltz & Kierszenbaum (1987), using costimulation with mitogens and trypomastigotes, reported that trypomastigotes induced a down regulation of the proliferative response to mitogens. However, stimulation of mononuclear cells with mitogens it is not a physiologic stimulus and the relevance of this observation to the interaction of the host with the parasite is difficult to assess. Usually, cell mediated immunity to an antigen is estimated, *in vitro*, by the proliferative response of lymphocytes. However, from a physiological standpoint, the relevant information is the functional consequence of the T cells activation. It has been clearly established that T lymphocytes are not a homogeneous population. They have different sbsets with different functions (Cantor, 1984). Consequently the outcome of an immune response to an antigen will be determined by which subset of T lymphocytes is activated preferentially and their interaction with the other subsets of T cells to induce a positive or negative modulation of the immune response (Russo et al., 1988; Hill et al., 1989; Mosmann & Coffman, 1989). In order to collect data related to the function induced in mononuclear cells stimulated with *T. cruzi* antigens, we have carried out a costimulatory study with *T. cruzi* antigens, PPD or TT in patients with Chagas' disease. The data reported demonstrate the presence of a specific down-regulation of the *in vitro* proliferative response of mononuclear cells by *T. cruzi* antigens.

MATERIALS AND METHODS

Patient groups – Patients were selected from the Cardiology Clinic for Chagas' disease at the Vargas Hospital in Caracas. A total of 23 patients (13 males, 10 females) with positive serology to *T. cruzi* antigens agreed to participate in this study. Patients were classified in two groups according to previous criteria (Mosca & Plaja, 1980). The 13 patients assigned to group I (INF) were seropositive with no evidence of heart disease (mean age 33.3 years old; range 29 to 55 years). The 10 patients assigned to group II (CDM) presented electrocardiographic abnormalities characteristic of either complete right bundle branch block plus anterior or posterior hemiblock of the left bundle or complete atrioventricular block with wide QRS complex (mean age 43.5 years old; range 28 to 65 years). Group III (controls) were 7 healthy blood bank donors (mean age 29.7 years old, range 24 to 47 years).

Antigen preparations – Antigen A: epimastigotes of *T. cruzi* strain Y were grown in medium 199 containing 2% fetal calf serum (Grand Island Biological Company, USA). The parasites were then separated and washed twice with Hanks' solution by centrifugation at 400 g. for 20 min. After resuspension, the parasites were adjusted to 30 x 10^6/ml and autoclaved; 20 μl of this suspension was used for every 2 x 10^5 mononuclear cells in individual well of microtiter plates. *Antigen T: T. cruzi* trypomastigotes (strain Y) were isolated from cultures of Vero cells infected a week before. The parasites were purified from supernatants of cultures using a discontinuous gradient of Percoll (Pharmacia Fine Chemicals AB, Sweden), adjusted to a concentration of 1 x 10^6/ml, and 20 μl used for 2 x 10^5 mononuclear cells. PPD: (10 μg/ml) 20 μl per well. TT: donated by the Instituto de Higiene Rafael Rangel (1 U/ml), 20 μl per well.

Lymphocyte proliferation assay – A standard microtest procedure was employed. Briefly, mononuclear cells were separated from heparinized blood by centrifugation over Ficoll-Hypaque, washed three times, and cultured in triplicate in plastic microtiter plates at a concentration of 2 x 10^5 cells/well. The medium used was RPMI 1640 (Grand Island Biological Company, USA) containing 50 μg/ml gentamycin, supplemented with 10% autologous serum. After 7 days of exposure to the antigen, 1 μCi of [^{3}H]-thymidine was added to each well, 18 h before harvesting onto fiber glass strips and β counting by liquid scintillation. Control tests with the diluent alone were also performed.

To evaluate the presence of suppression, mixtures of antigen A + PPD and antigen T + PPD were used.

The percentage of suppression (%S) induced by *T. cruzi* antigens was calculated as follow:

$$\%\,S = 100 - \frac{\text{NCPM (PPD + } \textit{T. cruzi} \text{ antigen)}}{\text{NCPM PPD + NCPM } \textit{T. cruzi} \text{ antigen}} \times 100$$

In a group of eight patients with chagasic infection a mixture of PPD + A, TT + A and TT + PPD was used to evaluated whether the suppression induced by *T. cruzi* antigens was specific or not.

Statistical evaluation – The significance of the differences of the %S among the groups was determined using the Wilcoxon rank sum test.

RESULTS

Antigens – The proliferative responses of mononuclear cells, to all the antigens, from patients and controls were compared. The only relevant difference was found between controls and INF or CDM, when *T. cruzi* antigens (autoclaved epimastigotes or living trypomastigotes) were used to stimulate mononuclear cells.

It is important to clarify that due to the objectives of this study, only patients whose mononuclear cells gave a proliferative response to PPD or TT were included in the study.

Costimulation assay with antigen A and PPD – In order to have an indirect estimate of suppression in Chagas' disease, we have evaluated the effect of stimulating mononuclear cells with *T. cruzi* antigens (A) and PPD. If each antigen induces a positive modulation of the immune response, the NCPM of the mixture should be similar, allowing for biological variation, to the sum of the NCPM induced by each antigen separately. On the other hand if one of the antigens induces a negative modulation of the immune response, the NCPM of the mixture should be lower than the sum of the NCPM to each antigen. The NCPM and the percentage of suppression of each patient of each experimental group are shown in Tables I, II and III. It is immediately evident that INF (Table I) have a higher suppression of the proliferative response than CDM (Table II), although the difference between the groups was not statistically significant.

The percentage of suppresion of INF was significantly higher ($p < 0.001$) than in blood bank controls (Table III). The difference in suppression between CDM and controls was also significant ($p < 0,05$) using a one tail test.

TABLE I

Net counts per minute to antigen A, PPD, PPD + A and percentage of suppression induced by costimulation with PPD + A in patients with chagasic infection

	Antigens			
Patients	A[a]	PPD[a]	PPD + A[a]	% Sup
1	647.3	899.2	843.0	45.5
2	3936.6	6546.4	1629.8	86.0
3	4571.9	5060.5	5933.0	38.4
4	242.0	3521.2	343.9	90.9
5	703.2	716.1	979.0	31.0
6	1025.0	1800.4	934.3	66.9
7	6536.0	4907.0	4220.0	63.1
8	2223.0	1521.0	1720.0	54.1
9	1389.0	6879.0	3000.0	63.7
10	3294.0	15672.0	9632.0	49.3
11	12243.0	9627.0	10447.0	52.3
12	6709.0	1082.0	1474.0	81.3
13	1867.0	2163.0	4550.0	−12.9

a: net counts per minute to antigens or to the mixture of antigens.
A: autoclaved epimastigotes.
PPD: *Mycobacteriun tuberculosis* protein purified derivative.
% Sup: percentage of suppression.

TABLE II

Net counts per minute to antigen A, PPD, PPD + A and percentage of suppression induced by costimulation with PPD + A in patients with chagasic cardiomyopathy

	Antigens			
Patients	A[a]	PPD[a]	PPD + A[a]	% Sup
1	1158.6	3537.1	2683.8	42.8
2	3825.0	2025.0	2950.0	49.6
3	1984.5	10357.2	3137.4	74.6
4	1552.4	1267.6	1940.5	31.2
5	613.5	22016.5	18049.5	20.2
6	1505.1	2802.6	3823.3	11.2
7	5577.0	4933.5	1959.1	81.4
8	2847.8	4050.0	7912.2	−14.7
9	869.0	1248.2	932.2	56.0
10	1571.9	5272.0	4413.2	35.5

a: net counts per minute to antigens or to the mixture of antigens.
A: autoclaved epimastigotes.
PPD: *Mycobacteriun tuberculosis* protein purified derivative.
% Sup: percentage of suppression.

Costimulation assay with T and PPD – As shown in Table IV, living trypomastigotes induced values of suppression similar to the ones observed with antigen A.

TABLE III

Net counts per minute to antigen A, PPD, PPD + A and percentage of suppression induced by costimulation with PPD + A in healthy blood bank donors

	Antigens			
Patients	A[a]	PPD[a]	PPD + A[a]	% Sup
1	168.6	10324.8	7912.8	24.6
2	403.5	8742.5	6590.5	27.9
3	163.6	747.1	566.7	37.8
4	149.2	1112.3	1140.8	9.6
5	305.8	2214.1	1886.0	25.2
6	380.6	2107.4	1760.0	29.3
7	387.0	8200.0	7747.0	9.8

a: net counts per minute to antigens or to the mixture of antigens.
A: autoclaved epimastigotes.
PPD: *Mycobacteriun tuberculosis* protein purified derivative.
% Sup: percentage of suppression.

TABLE IV

Net counts per minute to T, PPD, PPD + T and percentage of suppression induced by costimulation with PPD + T in patients with Chagas' disease

Patients	T[a]	PPD[a]	PPD + T[a]	% Sup
Chagasic cardiomyopathy				
1	880.9	3537.1	1897.6	56.3
2	675.0	2025.0	3000.0	−11.1
3	718.2	10357.2	6369.3	42.5
4	596.7	1267.6	1076.0	42.3
5	1366.8	22016.5	483.3	97.9
6	1245.6	2802.6	ND	ND
7	2002.0	4933.5	2230.8	67.8
8	182.3	4050.0	204.3	95.2
9	442.4	1248.2	1185.0	29.9
10	909.4	5272.0	4348.3	29.7
Chagasic infection				
1	1818.7	899.2	588.6	78.3
2	917.8	6546.4	183.1	97.5
3	2722.2	5060.5	3420.2	56.1
4	1964.6	3521.2	1331.3	75.7
5	1419.3	716.1	1508.7	29.3
6	1032.5	1800.4	549.7	80.6

a: net counts per minute to antigens or to the mixture of antigens.
ND = not done.
T: trypomastigotes.
PPD: *Mycobacterium tuberculosis* protein purified derivative.
% Sup: Percentage of suppression.

Costimulation assay with PPD and TT – To establish whether the suppression was specifically induced by *T. cruzi* antigens, or a non specific response due to the simultaneous stimulation of mononuclear cells, we evaluated the suppression induced by PPD + A, TT + A and PPD + TT in 8 INF. If the negative modulation is a specific process induced by *T. cruzi* antigens, the suppression induced by antigen A should be the same with either PPD or TT and be significantly different from TT + PPD.

The NCPM and the percentaje of suppression with these combinations are shown in Table V. It is evident that the mixture of *T. cruzi* antigens with either PPD or TT induces an important suppression of the proliferative response. On the other hand, the mixture of PPD + TT induced a high negative modulation in one patient and a positive modulation of the response (higher NCPM to the mixture than the sum of NCPM to PPD and to TT) in 3 patients. The statistical analysis of the data demostrated that the suppression induced by PPD + A was not different from TT + A. However, the suppression induced by PPD + A ($p < 0.01$) as well as TT + A ($p < 0.005$) was significantly higher than the suppression induced by TT + PPD, supporting the notion that the suppression was induced specifically by *T. cruzi* antigens.

DISCUSSION

A key aspect for a better comprehension of the host-parasite relationship in Chagas' disease is to understand how the parasite survives within the host in the presence of an apparently adequate immune response. An obvious possibility is the capacity of the parasite to evade the host immune response. The existence of immunosuppression in experimental Chagas' disease has been supported by data from different investigators. Ramos et al. (1978) have reported immunosuppression in *T. cruzi* infected mice and Scott (1981) has demonstrated the presence of specific suppressor cells in chronic experimental infection. Similarly, Cunningham et al. (1980a) have reported the presence of suppressor activity in serum from infected mice. Recently Revelli et al. (1986) have shown a significant reduction in the severity of adjuvant disease in *T. cruzi* infected rats. Texeira, et al. (1978) have reported specific immunosuppression in patients with an inapparent form of acute Chagas' disease.

TABLE V

Net counts per minute to antigen A, PPD, TT and percentage of suppression induced by costimulation with TT + A, TT + PPD and PPD + A in patients with chagasic infection

Patient	A[a]	PPD[a]	TT[a]	TT + A[a]	% Sup TT + A	PPD + TOX[a]	% Sup PPD + TT	% Sup PPD + A
1	6536.0	4907.0	3234.0	473.0	95.2	13552.0	−66.5	63.1
2	2223.0	1521.0	1144.0	1311.0	61.1	2352.0	11.7	54.1
3	3294.0	15672.0	1965.0	2553.0	51.5	12388.0	29.8	49.3
4	12243.0	9627.0	9544.0	9273.0	57.4	18400.0	4.0	52.3
5	7733.0	ND	6941.0	7564.0	48.5	ND	ND	ND
6	6709.0	1082.0	1023.0	7394.0	4.4	3226.0	−53.3	81.3
7	6830.0	458.0	2047.0	4321.0	51.3	2317.0	7.5	ND
8	1867.0	2163.0	728.0	1244.0	52.1	4109.0	−42.1	−12.9

a: net counts per minute to antigens or to the mixture of antigens.
ND = not done.
TT: *Tetanus toxoid.*
A: autoclaved epimastigotes.
PPD: *Mycobacterium tuberculosis* protein purified derivative.
% Sup: percentage of suppression.

However, information concerning the presence of immunosuppression in patients with chronic Chagas' disease is scarce. Morato et al. (1986) reported the presence of serum suppression in 40% of the patients studied and increment of the proliferative response upon removal of adherent cells. Castes et al. (1986) demonstrated the presence of non-specific suppression, mediated by PGE 2 in patients with chagasic infection. Beltz & Kierszenbaum (1987), using costimulation of mononuclear cells from healthy controls with mitogens and trypomastigotes, reported a down-regulation by trypomastigotes of the proliferation induced by mitogens. Because of the stimulus used to drive the *in vitro* proliferation of lymphocytes and the fact that the mononuclear cells were from controls, these results are difficult to relate to the natural infection. However, the logical conclusion is that living trypomastigotes produce or induce the production of a substance that inhibits the *in vitro* proliferation of lymphocytes. Our study, carried out in patients with Chagas' disease and healthy controls, using a well known natural antigen PPD and *T. cruzi* antigens, suggests that the proliferative response induced by *T. cruzi* antigens somehow stimulates preferentially a negative inmunoregulation, as shown by the difference between patients and controls when costimulated with PPD and *T. cruzi*. To confirm that the negative immunoregulation was caused by the *T. cruzi* antigens and was not the non-specific consequence of stimulation with two antigens, we studied the *in vitro* proliferation of mononuclear cells with TT and PPD, TT and A and PPD and A. As shown in the results, it is evident that a significant suppression of the proliferative response is observed only when *T. cruzi* antigens are present. These results clearly suggest that the suppression observed in these costimulation experiments is the consequence of the response induced by *T. cruzi* antigens.

The present study offers further support for the presence of *T. cruzi*-induced suppression of the immune response of patients with Chagas' disease.

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