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Suppression of Antibody Responses in Humans Infected with *Trypanosoma cruzi*

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Peripheral blood leukocytes from patients serologically positive for Chagas' disease were examined for their ability to respond to heterologous antigens in vitro. It was found that monouclear cells from chagasic patients had greatly reduced ability to respond to sheep erythrocytes (SRBC) as compared with peripheral blood mononuclear cells (PBMC) from control subjects. The reduction in anti-SRBC antibody activity was independent of antigen dose and was not a result of differences in antibody response kinetics. Depletion of plastic-adherent cells from the PBMC of patients did not affect the suppressed state of the nonadherent lymphocytes. No relationship was evident between the duration of *Trypanosoma cruzi* infection and the degree of humoral responsiveness.

A number of recent studies have shown that experimental animals infected with *Trypanosoma cruzi* have suppressed humoral and cellular immune responses against heterologous antigens (5-9, 13-18). In addition, these studies have revealed that suppressor cells and a soluble suppressor substance are generated during the course of experimental Chagas' disease (6-9, 14, 18). Little, however, is known regarding immunosuppression in chagasic patients (19).

The study reported herein examined immune responsiveness of peripheral blood mononuclear cells of chagasic patients in Colombia, South America, and determined that these people have significantly reduced capacities to develop antibody responses against sheep erythrocytes (SRBC).

MATERIALS AND METHODS

Patients. The peripheral blood from 13 patients serodiagnosed positive for Chagas' disease by immunofluorescence techniques was obtained from the Laboratorio Microbiologia y Parasitologia, Universidad de los Andes, Bogota, Colombia. Healthy donors residing in the area of study (subjects B, D, E, F, and G in Table 1) or the U.S.A. (subjects A and C in Table 1) were selected as controls. Informed consent was obtained from all participants. A brief summary of the characteristics of the normal subjects and chagasic patients is presented in Table 1. In addition to the salient clinical features of the patients as outlined below, complete medical case histories are available upon request.

Twelve patients (patients 1 through 12) presented cardiopathies characteristic of chronic Chagas' disease and were under the supervision of a physician at the time of study. The clinical diagnosis of Chagas' disease in these patients was supported by the following information: (i) the patients inhabited a zone endemic for *T. cruzi* and lived in rudimentary housing (at least during infancy and adolescence) where the insect vector of *T. cruzi* (*Rhodnius prolixus* or *Triatoma dimidiata capitata*) was known to exist; (ii) evidence existed for contact between the patients and the insect vector as chagoma scars, or there were medical reports of chagomas or edema of the eyes; (iii) patients presented severe cardiac rhythm abnormalities, including marked sinus bardycardia and atrio-ventricular block and/or intraventricular conduction disturbances (right bundle branch block plus left anterior hemiblock), and ectopic impulse formation with frequent premature ventricular beats; and (iv) patients exhibited positive serology as indicated by the indirect immunofluorescence and enzyme-linked immunosorbent assay tests (3).

The patient with a possible acute infection (patient 13) had clinical, electrocardiographic, and radiological signs of cardiac involvement, showed systemic manifestations of Chagas' disease, and was immunoglobulin M positive as determined by indirect immunofluorescence.

The overt symptoms as described by the patients included palpitation, weakness, and diarrhea. None of the patients was reported to have concurrent infections; however, most of the patients had been treated for multiple diseases before the study: amebiasis (patients 5, 8, 9, and 12); smallpox (patients 2 through 6, 8, 10, 11, and 13); rheumatic fever (patient 5); malaria (patient 6); and typhoid fever (patient 12).

Peripheral blood processing. Peripheral blood mononuclear cells (PBMC) were obtained from heparinized (20 U/ml) peripheral blood by Ficoll-Hypaque sedimentation (2). Purified PBMC were washed three times in 50 volumes of complete culture medium by centrifugation at 1,200 × *g* at room temperature for 10 min. The complete culture medium was prepared as previously described (14) with the following exceptions: RPMI 1640 replaced Hanks balanced salt solution and a 12% fetal bovine plus 2% horse serum (Reheis Chemical Co., Kankakee, Ill.) supplement was

TABLE 1. *Normal controls and chagasic patients studied*

Subject	Age	Sex	IFA titer[a]	Estimated duration of infection (yr)
Normal				
A	28	M	Negative	
B	30	M	Negative	
C	25	M	Negative	
D	30	F	Negative	
E	21	F	Negative	
F	50	F	Negative	
G	14	F	Negative	
Chagasic				
1	24	F	1/64	20
2	58	M	1/256	40
3	53	M	1/64	40
4	50	M	1/128	30
5	45	M	1/128	30
6	53	M	1/16	15
7	49	F	1/32	30
8	52	M	1/64	35
9	33	M	1/256	15
10	43	M	1/64	6
11	55	M	1/16	40
12	50	F	1/64	30
13	13	M	1/128	2

[a] Indirect-fluorescent antibody (IFA) titers were provided by the Microbiology and Parasitology Laboratory of Andes University and were determined by the method described by Camargo (3).

included. Washed PBMC were counted on a hemacytometer, resuspended in complete culture medium at a concentraiton of 5×10^6 cells per ml, and placed on ice until used (usually less than 30 min).

Culture conditions. One million PBMC (0.2 ml) were dispensed into the wells of a Micro Test II tissue culture plate (Falcon no. 3040) with fitted lid (Falcon no. 3041). Cells were then immunized with 10 to 30 μl of a 1% SRBC suspension, as indicated. Fifty microliters of a T-cell-replacing factor (see below) was added to the cultures before incubation at 37°C in a 5% CO_2-in-air humidified atmosphere. Cultures were fed with 50 μl of a nutritional cocktail (12) on the 3rd day of culture and assayed for plaque-forming cells by the technique of Jerne et al. (10) on days 6 to 8, as indicated.

T-cell-replacing factor. Twenty-five million C3H(He) (Flow Laboratories, Dublin, Va). and 25×10^6 C57BL/6 (Jackson Laboratories, Bar Harbor, Maine) spleen cells were coincubated for 24 h in 25 ml of RPMI 1640 containing 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml) in a tissue culture flask (Falcon no. 3023). The bidirectional mixed lymphocyte culture was conducted at 37°C in a 5% CO_2-in-air humidified atmosphere. After incubation the culture supernatants were collected by centrifugation at 1,500 × *g* at 4°C for 10 min, filter sterilized, and stored at −20°C until use as T-cell-replacing factor.

Removal of plastic-adherent PBMC. Purified PBMC were depleted of plastic-adherent cells by the procedure of Albright et al. (1). This technique resulted in PBMC with <1% nonspecific esterase-positive cells (11).

RESULTS

The PBMC from humans with Chagas' disease were used to study in vitro antibody responses to SRBC. It was found that the peak antibody responses of all chagasic patients were markedly below the peak antibody responses obtained with PBMC from healthy donors (Fig. 1). Although the range of responses was considerably varied for both groups studied, the median value for chagasic PBMC was approximately 3.5-fold lower than the median value for control PBMC. Furthermore, there was no clear relationship between the magnitude of the anti-SRBC antibody response and the estimated duration of infection or the anti-*T. cruzi* immunofluoroescence titer (Table 1 and Fig. 1).

Culture conditions were manipulated to ensure that the reduced responsiveness of PBMC from patients was an immunological phenomenon. By using PBMC from both control subjects and patients, it was determined that 10^6 cells stimulated with 20 μl of a 1% SRBC suspension (4×10^6 cells) yielded peak antibody responses (Fig. 1). In the small number of individuals that responded well to immunogen doses other than

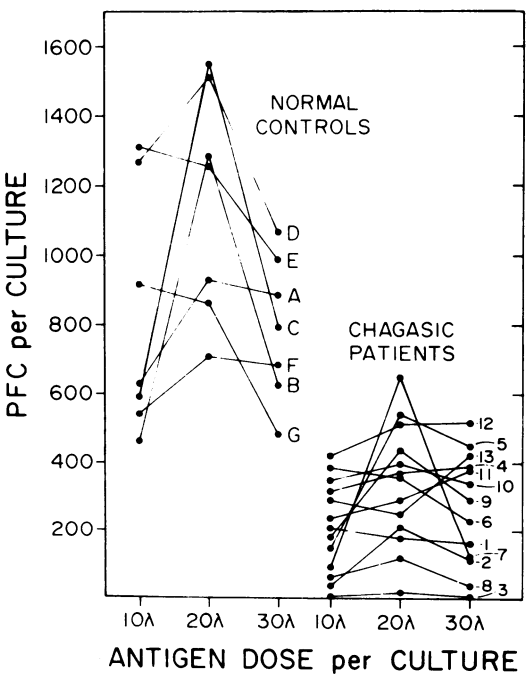

FIG. 1. *In vitro anti-SRBC antibody responses of PBMC from chagasic patients and normal controls. Each point represents the mean of five replicate cultures.*

20 μl, no statistically significant differences existed between the levels of the antibody responses obtained with the various doses of SRBC. There were no detectable plaque-forming cells in cultures with increased (2×10^6 per well) or decreased (0.5×10^6 per well) densities of PBMC (data not shown). With few exceptions, the peak antibody responses occurred in cultures of control and patient PBMC 7 days after initiation (Fig. 2).

The possible contribution of monocyte-like regulatory cells to the observed suppression, as demonstrated to occur during experimental Chagas' disease in mice (6), was examined by removing plastic adherent cells from the PBMC before culture. As shown in Table 2, however, this maneuver had no effect on the outcome of the magnitude of the antibody responses.

DISCUSSION

The findings of the present study document the existence of immunosuppression of primary antibody responses in people with Chagas' disease. The in vitro antibody responses of PBMC from chagasic patients were consistently lower than those obtained with PBMC of control subjects. The basic integrity of the reduced responses with respect to response kinetics, antigen dose independence, and responder cell density independence, however, was unaltered. Attempts to alleviate suppression of PBMC by removal of potentially suppressive adherent cells were unsuccessful, suggesting that an adherent suppressor cell is not present in the peripheral blood of chagasic patients or normal subjects. A more extensive analysis of the nonadherent cell population of patients, however, may reveal that an active immunoregulatory cell does develop during the course of the disease.

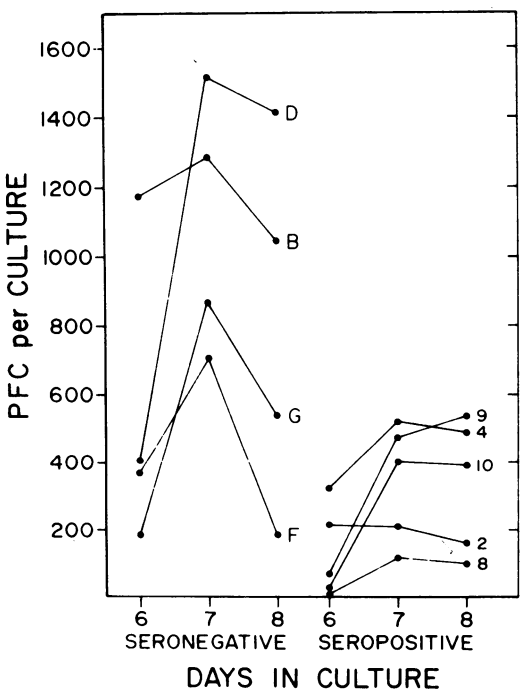

FIG. 2. *Kinetics of in vitro anti-SRBC antibody responses of PBMC of chagasic patients and normal controls. Each point represents the mean value of five replicate cultures.*

Teixeira et al. (19) reported that patients with clinically apparent acute Chagas' disease were immunoresponsive, whereas clinically inapparent patients exhibited defective T-lymphocyte functions. The patients examined in the present study were chronically infected with *T. cruzi* and presented a wide range of antibody titers to *T. cruzi* antigens. Nonetheless, in vitro anti-SRBC antibody responses of all patients clearly fell outside the range of antibody responses produced by the PBMC from control subjects. Based on the lack of a detectable influence of duration of infection or intensity of the response to *T. cruzi* on immunosuppression in chronic patients, it would be of great interest to know whether or not the clinically apparent acute patients develop immunosuppression at a later time than the clinically inapparent acute patients and, if so, what signals the onset or retards

TABLE 2. *Response of unseparated and plastic nonadherent human PBMC to SRBC in vitro*[a]

Subjects	PFC/culture	
	Unfractionated PBMC	Plastic nonadherent PBMC
Normal		
C	1,540	1,720
F	705	610
G	860	930
Chagasic		
1	210	230
6	365	410
8	120	110
9	435	380
11	290	265
12	320	345

[a] 10×10^6 purified human PBMC were obtained from seronegative and seropositive subjects and twice incubated (1 h per incubation) on 35-mm^2 plastic tissue culture dishes in 1 ml of complete culture medium in a 5% CO_2-in-air humidified atmosphere to remove plastic adherent cells, and plastic nonadherent peripheral blood mononuclear cells were then cultured in the presence of SRBC for determination of anti-SRBC antibody activity. Each point represents the average of 10 replicate cultures.

the expression of suppressed immune responses in these patients.

The possible significance of immunosuppression as it occurs in human and experimental Chagas' disease is unclear. It may be a host mechanism for focusing immune responses on the parasite or the parasite's attempt to dampen specifically directed immune responses. It is clear, though, that immunosuppression is a characteristic of the host-parasite relationship that warrants further investigation as to its biological implications.

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