JCI The Journal of Clinical Investigation

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J Clin Invest. 1978;62(6):1132-1141. https://doi.org/10.1172/JCI109232.

Research Article

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ABSTRACT In this study two groups of patients with acute Chagas' disease were identified. Group one consisted of five patients with apparent acute Chagas' disease. These patients showed symptoms and signals of an acute illness, such as high fever and enlarged spleen. One of these patients developed severe myocarditis and heart failure. Group two consisted of seven patients with inapparent acute Chagas' disease. This was a nonclinical entity, not perceived by the patient who did not seek medical care. The diagnosis was made by the shift of a serologic test which indicates the presence of immunoglobulin M antibodies to *Trypanosoma cruzi*.

The patients with apparent acute Chagas' disease showed positive delayed-type skin response to *T. cruzi* antigen. Also, their leukocytes showed significant inhibition of migration in the presence of this antigen. By contrast, the patients with the inapparent acute Chagas' disease did not show positive delayed-type skin response to *T. cruzi* antigen and no significant inhibition was observed when their cells migrated in the presence of this antigen. Of interest, none of these patients was capable of developing contact sensitivity to 2,4-dinitrochlorobenzene. However, three out of five patients with the apparent acute disease and all the normal control subjects showed positive contact reaction after sensitization to this drug.

The results of these experiments would suggest that the thymus-derived (T)-lymphocyte function is depressed in patients with the clinically inapparent acute Chagas' disease. This immunodepression seems to be acquired in the course of the *T. cruzi* infection because all patients showed positive delayed-type skin response to at least one ubiquitous microbial extract, thus indicating previously normal T-cell function. We hypothesize that *T. cruzi* antigens may directly stimulate T cells with the concomitant release of factors that might become supressive for T-cell responses. Furthermore, the suppressive effect might interfere with the T-cell response to other antigens, such as to 2,4-dinitrochlorobenzene.

INTRODUCTION

Clinical and epidemiologic studies have shown low prevalence of acute cases of Chagas' disease in endemic areas where the prevalence of the chronic Chagas infection is high (1–10). According to Chagas (2, 3) that discrepancy could be explained either by the dificulties that the doctors in the rural areas have in establishing the diagnosis of acute Chagas' disease or because physicians are not available in endemic areas where those patients should be found. Soon it became evident that the overt acute *Trypanosoma cruzi* infections usually occurred in infancy and childhood (1, 2). However, sixty years later we still do not know how to explain that discrepancy, despite the fact that many cases of flagrant acute Chagas' disease were diagnosed by the physicians in several regions of Brazil and other countries of Central and South America (11–19).

The possibility exists that an acute *T. cruzi* infection was a nonclinical entity, often unaccompanied by symptoms and, therefore, inapparent to the physician and the patient. We examined this possibility in an area of northeast Brazil where the chronic Chagas' disease is highly prevalent (20). This paper reports on a method that was designed to diagnose the inapparent acute cases of Chagas' disease. This method allowed us to demonstrate that in an endemic area for Chagas' disease the occurrence of inapparent *T. cruzi* infection is more frequent than the flagrant, apparent acute disease.

Of great interest, however, is the demonstration that patients with the inapparent acute Chagas' disease did not show delayed-type hypersensitivity to the parasite. In contrast, patients with the clinically overt acute

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The greater portion of the clinical data from this manuscript was submitted by Dr. Glória Teixeira in partial fulfillment of the requirements of the degree of Master of Tropical Medicine, Universidade Federal do Rio de Janeiro.

Received for publication 18 April 1978 and in revised form 12 July 1978.

J. Clin. Invest. © *The American Society for Clinical Investigation, Inc.*, 0021-9738/78/1201-1132 $1.00
Volume 62 December 1978 1132–1141

Chagas' disease showed strong delayed-type hypersensitivity reaction to *T. cruzi*. This demonstration indicates that the impending severity of the disease is related to the host thymus-dependent immune response to the parasite.

METHODS

Strain of the parasite. The Ernestina strain of *T. cruzi* isolated from a child with acute Chagas' disease in São Felipe, Bahia, was used throughout these studies for obtaining the antigen.

Selection of subjects. The subjects included in this study live in São Felipe, Bahia, where Chagas' disease is endemic. São Felipe is a county 200 km from the city of Salvador, the capital of the state of Bahia, Brazil. The population of about 20,000 is predominantly rural and its main occupation is agriculture. These people live in huts that can be infested with the blood-feeding reduviid bugs (*Panstrongylus megistus*) that transmit *T. cruzi*, the causative agent of Chagas' disease. 544 individuals who had shown negative serologic tests for Chagas' disease at the begining of these studies were selected among the inhabitants of this area (20). This group was, thereafter, submitted bi-monthly to clinical and serologic evaluations over a period of 18 mo to determine the onset of acute *T. cruzi* infection.

Serologic test for acute Chagas' disease. The Chagas-latex test (Behringwerke AG., Marburg-Lann, West Germany) was used for the field studies. Two drops of blood from a digital puncture were mixed with the Chagas-latex reagent. The appearance of coarse whitish precipitates, instantaneously or within 2 min, indicated the presence of immunoglobulin (Ig)M antibodies to the *T. cruzi* antigen-coated latex particles. Thus, the presence of a positive reaction was an indirect indication of a recent *T. cruzi* infection.

Demonstration of T. cruzi in the peripheral blood of patients with positive Chagas-latex test. The presence of trypomastigote forms of *T. cruzi* was thoroughly investigated in the peripheral blood of the patients with a positive Chagas-latex test. A search for the parasite was carried out on wet preparations of the buffy coat of the whole blood. However, when the search was negative, a method for concentration of the parasites was used. In brief, the method consisted in allowing venous blood to clot. After removal of the clot the supernate was centrifuged at 1,000 *g* for 20 min. The supernate was discarded and a drop of the parasite-enriched sediment was examined under coverslip at ×60 magnification.

In addition, the presence of the parasite in the peripheral blood of every Chagas-latex test positive case was demonstrated by xenodiagnosis. This consisted of allowing non-infected fourth and fifth instar nymphs of laboratory-reared *Triatoma infestans* to acquire a blood meal from subjects with suspicion of a recent *T. cruzi* infection. 12 days later the feces of the insects were examined microscopically for identification of epimastigote and metacyclic trypomastigote forms of *T. cruzi*.

Collection of antisera. 10 ml of venous blood were drawn bi-monthly and at various intervals after the diagnosis of acute Chagas' disease was made. Later collections of blood were obtained at other times after treatment of the infection with trypanocidal drugs. Venous blood was also drawn from normal, non-Chagas donors. The blood samples were allowed to clot and the sera were collected and stored at −20°C until used.

Studies of humoral immunity. Humoral antibodies to *T. cruzi* were detected in the sera from Chagas patients by the direct agglutination of enzyme-treated epimastigote forms of *T. cruzi* grown in LIT medium. This test was used to detect the antibodies to *T. cruzi* that belong to the class of the IgM, which is sensitive to treatment with 2-mercaptoethanol (21). Hemagglutinating antibodies were detected by the technique of Cerisola et al. (22). An intracellular antigen obtained by repeated freezing and thawing of the epimastigote forms was used to sensitize the blood group O, Rh negative, human erythrocytes. The capability of this antigen to sensitize the erythrocytes is lost after 4–6 h at room temperature.

Complement fixing antibodies were detected by the quantitative guinea pig complement fixation test according to standard procedures (23). The test sera were inactivated at 56°C for 30 min before use. The antigen used was the soluble supernate, or citosol (100,000 *g* × 90-min fraction), derived from homogenates of epimastigote forms of *T. cruzi* (24). At the protein concentration of 80 μg/ml this antigen elicited the highest antibody titer without anticomplementary action. $5CH_{50}$/ml of guinea pig complement was used and the degree of hemolysis was read by visual observation after addition of sensitized sheep erythrocytes and incubation at 37°C for 30 min. Anti-sheep hemolysin, guinea pig complement, and sheep erythrocytes were obtained from Instituto Adolfo Lutz, São Paulo, Brazil.

Quantitation of proteins and of immunoglobulins in the serum. Total protein concentrations were determined by an automated analyzer (Autoanalyzer, Technicon Corp., Tarrytown, N. Y.). Serum samples were fractionated by electrophoresis on cellogell membrane (Chemetron, Sebia, Paris). The bands of migration were stained with Ponceau S and decolorized with 5% acetic acid. The cellogell membrane was dried at 37°C for 1 h and the bands were automatically read by a densitometer integrator (Cellomatic et Enregistreur At-3, Sebia, Paris) that synchronously registered the tracings.

Serum levels of IgG, IgA, and IgM were determined by the automated nephelometric test with a Turner fluorimeter model 10 converted to nephelometer by removing its secondary filter (Turner Laboratory Instruments, Palo Alto, Calif.). Goat anti-human IgG, IgA, and IgM were obtained from Technicon Chemicals, ORCO, Belgium. Standard human serum used was obtained from Behringwerke AG. Assays of cellular immunity were performed.

Skin tests. Cell-mediated immunity to *T. cruzi* was assayed by the skin response to a small particle (30,000 *g* × 35-min fraction) derived from trypomastigote and amastigote forms of the Ernestina strain of *T. cruzi* grown in African green monkey kidney cell (VERO-cell) culture (25). The quantity of this antigen containing 50 μg of protein was injected intradermally in the anterior surface of the arm. Control tests were made by the intradermal injection of an equal amount of protein derived from a homogenate of noninfected VERO-cells.

Further studies on cell-mediated immunity included assays of delayed-type skin responses to eight ubiquitous antigens as follows: tuberculin, purified protein derivative, 1:1,000 solution (Serviço Nacional Tuberculose, Brazil); *Schistosoma mansoni* antigen for skin testing (kindly donated by Dr. Naftale Katz, Centro de Pesquisas René Rachou, Fundação Instituto Oswaldo Cruz, Belo Horizonte, Brazil); Montenegro antigen, saline suspension of sonicated *Leishmania brasiliensis*; Trichophyton, 1:100 suspension in saline of *Microsporum canis*, *Tricophyton rubrum*, and *Epidermophyton floccosum*; and Sporotrichum, 1:10,000 saline suspension of *Sporotrichum schenkii* (Instituto Adolfo Lutz); *Candida albicans* allergenic extract, 1:100 dilution (Hollister-Allister Laboratories, Downers Grove, Ill.). Streptokinase-streptodornase, 1:500 U/ml (Lederle Laboratories, Pearl River, N. Y.); and mumps skin testing antigen, (Eli Lilly & Co., Indianapolis, Ind.). Plastic, sterile, disposable tuberculin syringes were used in the intradermal injection of 0.1 ml of each of these antigens.

In addition, the capability to produce a contact reaction

was investigated (26). This consisted of sensitization by placing six drops of a 2% solution of 2,4-dinitrochlorobenzene (DNCB)[1] in acetone on a bandaid spot which was then placed on the interscapular surface. In each case, erythema and vesiculation were visible after 25 h of exposure. Simultaneously, a 0.1% solution of DNCB applied in the same way to the contralateral region gave no reaction. Contact sensitivity was then investigated 3 wk later by applying six drops of 0.1% solution of DNCB to the same place for 24 h. Definite erythema (+), papula (++), and vesiculation (+++) constituted positive reaction.

Inhibition of leukocyte migration. Inhibition of peripheral blood leukocyte migration was assayed by the capillary tube method (27, 28). The cells were separated by simple sedimentation and the leukocyte migration was carried out in the wells of microchambers in the presence and in the absence of antigen.

T. cruzi antigen was obtained by differential centrifugation of homogenates of trypomastigotes and amastigote forms of the parasite grown in VERO-cell cultures. The partition of the homogenates of *T. cruzi* was obtained by differential centrifugation with the same speeds described in a previous paper (29). The 30,000 g × 35-min fractions derived from these homogenates were used. Our previous studies (25, 29) showed that the small particles in the 30,000 g × 35-min fraction are capable of eliciting strong delayed-type hypersensitivity reactions. The quantity of antigen containing 100 μg of protein was used in these assays. Tests were run in quadruplicate.

Statistical analysis. Group data were compared by Student's t test.

RESULTS

Clinic and laboratory findings in the acute phase of Chagas' disease. Among 12 patients diagnosed with acute Chagas' disease, five were symptomatic and came to the doctor with complaints of an acute illness. Seven patients with acute Chagas' disease were asymptomatic and did not seek medical care. In this group of children the initial *T. cruzi* infections were inapparent and, therefore, were not perceived by the patients or by their parents. The diagnoses of cases with inapparent acute Chagas' disease were made possible by the shift of the serology given by a positive Chagas-latex test that indicates the presence of IgM antibodies to *T. cruzi*. The clinic and laboratory data on patients with acute Chagas' disease are summarized in Table I.

In the group of apparent acute Chagas' disease the youngest patient was 1 yr old and the oldest was 15 yr old. Three of these patients had signs of portal of entry of the metacyclic forms of *T. cruzi*, discharged with the feces of the infected triatomid bug, either in the skin or in the conjunctiva. Patients B and E had unilateral, bipalpebral infiltration with swelling and conjunctivitis (30) accompanied by engorgement of regional pre-auricular lymph nodes (Romaña's sign). Patient E showed this lesion 1 wk after the insect bite. Patient A had an indurated skin infiltration with swelling (Chagoma) at the left maxillary region (31, 32). Two patients in this group (C and D) did not show signs of portal of entry of the infection. All the patients with the apparent acute Chagas infection presented high fever when they sought medical care. The highest temperature observed within a period of 4 wk was 39.8°C. The spleens of patients A, C, and D were enlarged and palpated below the left costal margin. In addition, patient D had muffled heart sounds and the electrocardiogram showed increased P-R interval. These findings were accompanied by edema of the lower limbs, thus indicating a severe myocarditis with heart failure. All these patients had blood parasitemias that were detected by microscopic examination of wet preparations.

In the group of inapparent acute Chagas' disease the youngest patient was 3 yr old and the oldest was 12 yr old. None of the seven patients with inapparent acute Chagas infection showed a sign of the portal of entry of the parasite. At the moment the diagnosis was made patients F, J, K, and L had a slight increase in body temperature, which reached 37.2°C. Patients G, H, and I had normal body temperature at the time the diagnosis was made. However, the observation made during a period of 1 mo revealed occasional elevation of the temperature which reached 37.8°C in patient H. None of the patients with inapparent acute Chagas' disease had a palpable spleen. Their heart sounds were normal for the age group. However, the electrocardiogram showed a variety of abnormalities, as shown in Table I. The parasite was demonstrated in wet preparations in all cases, except that of patient J. However, this patient had a positive xenodiagnosis. Leukocytosis and lymphocytosis were common findings in acute Chagas' disease.

Humoral antibody response to T. cruzi antigens. Twelve patients with acute Chagas' disease had high titers of humoral antibodies to *T. cruzi*. By the direct agglutination tests the highest antibody titer was 1:8,192 (patient D) and the lowest titer was 1:512 (Patients A, F, H, J, and K). The treatment of the sera from these patients with the reducing agent 2-mercaptoethanol resulted in a significant fall of the titers, thus indicating the presence of IgM antibodies to *T. cruzi* which characterize the acute infection (21).

However, the patients with inapparent acute Chagas' disease had higher proportions of 2-mercaptoethanol resistant antibody. This might be explained by the fact that these patients were asymptomatic and the diagnosis was delayed in comparison with the flagrant, symptomatic cases. In the control group, subjects M to R had antibody levels below 1:64, which is consistent with absence of *T. cruzi* infection. The data on humoral antibodies to *T. cruzi* antigens are shown in Table II.

Delayed-type skin responses. Cell-mediated im-

[1] *Abbreviation used in this paper*: DNCB, 2,4-dinitrochlorobenzene.

TABLE I

Clinic and Laboratory Findings in the Acute Phase of Chagas' Disease

Subjects	Age	Sex	Color*	Chagas-latex‡ test	Sign of portal of entry	Temp	Abdominal liver	Palpation§ spleen	Heart		Leukocytes		Electrocardiogram	Parasitemia	
									Frequency	Auscultation	Total	Lymphs		Wet prepn	Xenodiagnosis
	yr					°C¶						%			
Apparent ACD															
A	1	M	M	+	Chagoma, face	39.0	++	+	140	3rd Sound	13,000	63	Normal	+	+
B	3	M	M	++	Romaña	38.5	+	—	136	3rd Sound	18,000	70	1st Degree A-V-block	+	+
C	4	M	W	++	—	39.5	++	++	130	3rd Sound	9,500	68	ND	+	+
D	7	F	M	+	—	39.4	++	++	120	Muffled Sounds	9,850	46	Increased P-R interval 1st Degree A-V-block	+	+
E	15	F	M	++	Romaña	39.8	+	—	112	Normal	21,150	67	Normal	+	+
Inapparent ACD															
F	3	F	N	+	—	37.5	—	—	146	3rd Sound	16,000	51	1st Degree A-V-block	+	+
G	4	M	M	++	—	37.5	+	—	142	3rd Sound	8,000	41	Normal	+	+
H	4	M	W	++	—	37.8	+	—	140	3rd Sound	11,250	49	Repolarization abnormality; ischemia	+	+
I	5	M	W	++	—	37.0	+	—	140	3rd Sound	10,350	63	ND	+	+
J	5	M	M	+	—	37.2	—	—	128	3rd Sound	10,850	55	Normal	—	+
K	5	M	M	++	—	37.2	+	—	110	3rd Sound	18,250	65	1st Degree A-V-block Hemi-LBBB	+	+
L	12	F	W	++	—	37.1	+	—	100	Normal	16,200	59	Normal	+	+

ND = Not done; ACD = acute Chagas' disease.

* W = white, M = mulatto, N = negro.

‡ ++ = Positive reaction, instantaneously. + = Positive reaction within 2 min after mixture of reagents.

§ + = Palpation of liver and spleen 2 cm below the costal margin; ++ = palpation of these organs 3–6 cm below the costal margin; − = impalpable.

¶ Highest temperature registered for a period of 1 mo after the diagnosis of acute Chagas' disease was made.

TABLE II

Humoral Antibody Response to T. Cruzi Antigens

Sera	Age	Sex	Color*	Direct agglutination‡	Direct agglutination‡ (2-ME-treated serum)	Complement fixation§	Indirect¶ hemagglutination
	yr						
Apparent ACD							
A	1	M	M	1:512	1:64	1:128	1:128
B	3	M	M	1:4,096	1:32	1:1,024	1:1,024
C	4	M	W	1:2,048	1:128	1:256	1:256
D	7	F	M	1:8,192	1:64	1:2,048	1:2,048
E	15	F	M	1:2,048	1:32	1:128	1:32
Inapparent ACD							
F	3	F	N	1:512	1:128	1:512	1:64
G	4	M	M	1:4,096	1:1,024	1:512	1:256
H	4	M	W	1:512	1:256	1:256	1:64
I	5	M	W	1:2,048	1:128	1:256	1:256
J	5	M	M	1:512	1:256	1:512	1:128
K	5	M	M	1:512	1:32	1:128	1:32
L	12	F	W	1:4,096	1:512	1:1,024	1:64
Controls							
M	3	M	W	1:16	—	—	—
N	4	F	M	1:64	—	—	—
O	5	F	M	1:32	—	—	—
P	5	M	M	1:16	—	—	—
Q	6	M	W	1:8	1:2	—	—
R	9	F	W	1:4	1:2	—	—

2-ME = 2-mercaptoethanol; ACD = acute Chagas' disease.

* W = white, M = mulatto, N = negro.

‡ Dilution of sera vs. enzyme-treated epimastigote forms of *T. cruzi*.

§ Dilution of sera vs. *T. cruzi* soluble antigen.

¶Dilution of sera vs. human type O Rh negative human blood cells sensitized with *T. cruzi* lysate (see text). Titers are reciprocal of serum dilutions.

munity was assessed in patients with acute Chagas' disease and in normal, control subjects by the delayed skin response to the small particle, microsomal antigen of *T. cruzi* (Table III). In the group of patients with apparent acute Chagas' disease a positive skin reaction to the *T. cruzi* antigen was observed in each case, as indicated by induration of the skin persisting for 48–72 h of injection. Patient A had a mild reaction (+) and patients D and E had a strong reaction (+++) accompanied by engorgement of lymph nodes at the epitrochlear region. In the group with inapparent Chagas' disease a mild positive reaction (+) was observed in patient F. The other patients with inapparent acute Chagas' disease (G to L) did not show delayed-type skin reactivity to the *T. cruzi* antigen. This skin test remained negative when repeated 6 mo later in patients G to L. Control, non-Chagas patients M to R showed negative skin response to *T. cruzi* antigen.

Cell-mediated immunity to a panel of eight microbial antigenic extracts was assessed in normal, control subjects and in patients with acute Chagas' disease. All the Chagas patients and the control subjects showed a positive delayed-type skin reaction to at least one of the antigens tested. Nevertheless, the skin reactivity of patients with inapparent acute Chagas' disease was less intensive than that of patients with apparent acute Chagas' disease and also of control subjects (Table III). Of interest, all the individuals included in these studies showed negative skin reaction to *S. mansoni* antigen and to an extract of *L. brasiliensis* (Montenegro test).

In these investigations, positive contact sensitivity reaction to DNCB was observed in three out of five patients with apparent acute Chagas' disease, and in each of the six control, non-Chagas subjects. Yet, the patients with inapparent acute Chagas' disease did not show positive contact reaction to DNCB. In this group of patients, contact sensitivity to DNCB was still negative 6 mo after the first exposure.

Inhibition of leukocyte migration. The test of inhibition of blood leukocyte migration (25, 27) was used to assess cell-mediated immunity in patients with acute Chagas' disease. The results of these experiments are given in Table IV. In the first group of experiments, the blood leukocytes derived from patients with apparent acute Chagas' disease were allowed to migrate in the

TABLE III
*Delayed-Type Skin Responses**

Subjects	Antigens							
	T. cruzi	PPD	SPK	Mumps	*Candida*	*Tricophyton*	*Sporotrichum*	DNCB‡
Apparent ACD								
A	+	—	+	+	—	—	—	+
B	+	—	+++	—	+++	—	—	—
C	++	—	+	—	++	—	—	—
D	+++	—	+	+	+++	—	—	+
E	+++	+	+	+	+++	—	—	++
Inapparent ACD								
F	+	—	—	+	++	—	—	—
G	—	—	—	+	+	—	—	—
H	—	—	+	—	++	—	—	—
I	—	—	ND	ND	ND	—	+	—
J	—	—	—	+	++	—	—	—
K	—	—	—	+	++	+	—	—
L	—	—	++	—	—	—	—	—
Controls								
M	—	—	+++	+	++	—	—	+
N	—	—	++	+	+++	+	—	+
O	—	—	++	+	++	—	—	+
P	—	+	+++	—	+	+	—	+++
Q	—	—	++	—	+++	—	—	++
R	—	+	+++	+	++	—	+	++

PPD = purified protein derivative; SPK = streptokinase-streptodornase; and ACD = acute Chagas' disease.

* Induration of skin persisting for 48–72 h of injection. + = Area of induration of 0.5–1.5 cm; +++ = induration measuring over 2.5 cm; and ++ = intermediate values.

‡ Development of skin lesions in previously sensitized person within 24 h of contact with the DNCB. + = Erythema; ++ = papula; and +++ = vesicles.

presence of the *T. cruzi* small particle antigen. The degrees of inhibition of migration ranged from 18.1 ±1.6 to 39.8±3.7% for patients D and E.

In the second group of experiments, the blood leukocytes derived from patients with inapparent acute Chagas' disease did not show significant degrees of inhibition of migration in the presence of the *T. cruzi* antigen. The mean reached 4.2±3.6. These results contrast sharply with those obtained from patients with apparent acute Chagas' disease for whom the mean of inhibition reached 26.8±8.0. These group differences were statistically significant ($P > 0.001$). These would suggest that the in vitro response of the immune lymphocytes from patients with the inapparent acute Chagas' disease is depressed when compared with the degrees of in vitro response obtained with immune lymphocytes from patients with the apparent acute Chagas' disease. Further evidence in support of this suggestion was provided by control studies where the degrees of migration are not different from those obtained in the group of patients with the inapparent disease.

Serum proteins and immunoglobulins in control subjects and in patients with acute Chagas' disease. In view of the evidence that the cell-mediated immune reactions can be deleteriously affected by protein deficiency (33–35), we sought to determine the serum protein and immunoglobulin levels in patients with acute Chagas' disease and in control subjects included in these studies. The reults of these experiments are given in Table V. No difference in total serum protein levels was evident between each of the two groups of patients with acute Chagas' disease and the control group. The lowest protein concentration obtained (63 mg/ml) in these studies was above the acceptable low risk (≥60 mg/ml) in evaluating protein and calorie deficiencies (36). The levels of albumin in patients with acute Chagas' disease were not significantly different from those obtained in control subjects.

The mean concentration of gamma globulin (22.1±1.7 mg/ml) obtained in patients with apparent acute Chagas' disease was much higher than that (17.5±1.1) obtained in control subjects. This difference was highly significant ($P > 0.001$) and it did not seem to be related to age differences (37, 38). On the other hand, the means of gamma globulin (18.9±3.2) in patients with inapparent acute Chagas' disease were not significantly dif-

TABLE IV

*Cell-Mediated Immunity: Inhibition of Migration of Blood Leukocytes in the Presence of T. Cruzi Antigen**

Leukocytes	Migration‡	Inhibition§
	%	%
Apparent ACD		
A	75.3	24.7±3.6
B	71.9	28.1±4.1
C	75.8	24.2±4.9
D	81.9	18.1±1.6
E	60.2	39.8±3.7
Mean	73.2	26.8±8.0(*a*)
Inapparent ACD		
F	91.8	8.2±3.5
G	91.3	8.7±3.0
H	99.4	0.6±2.4
I	99.4	0.6±1.6
J	97.2	2.8±1.1
K	95.6	4.3±2.8
L	ND	ND
Mean	95.8	4.2±3.6(*b*)
Controls		
M	97.5	2.5±0.5
N	87.9	12.1±2.9
O	90.7	9.3±6.8
P	89.2	10.8±5.2
Mean	91.4	8.6±4.2(*c*)

a vs. *b*, $P > 0.001$; *a* vs. *c*, $P > 0.002$; ACD = acute Chagas' disease; ND = not done.

* Blood leukocytes were exposed to 100 μg of the small particles (30,000 *g* for 35-min fraction) derived from *T. cruzi* homogenates.

‡ Percent migration = 100 × area of migration in presence of antigen/area of migration in absence of antigen.

§ Percent inhibition = 100 − percent migration.

ferent from those obtained in control subjects. Nevertheless, there appears to be a significant difference between the control group and the group of patients with apparent acute Chagas' disease, the latter having more IgG (Table V). However, the serum levels of IgM and IgA were not different.

DISCUSSION

We report here clinical and immunologic data on 12 children diagnosed with acute Chagas' disease. Among these patients five were symptomatic and showed an apparent disease with complaints of an acute illness. Two patients had Romaña's sign (30) and another one had a Chagoma (31, 32). All the patients with the apparent acute Chagas infection had high fever and three of them showed enlargement of the spleen when they sought medical care. In addition, one of these patients showed clinical and electrocardiographic signs of severe myocarditis with heart failure.

In marked contrast to the patients with apparent acute Chagas' disease discussed above, seven cases diagnosed with the initial *T. cruzi* infection were asymptomatic. These children with inapparent acute Chagas' disease did not seek medical care because the disease was not perceived either by the patient or by his parents. In these seven cases the diagnosis of inapparent acute Chagas' disease was suspected only by the shift of the serologic test. The parasite was then demonstrated in wet preparations. Usually these asymptomatic patients did not have fever. Furthermore, their spleens were not enlarged. However, the electrocardiographic tracings revealed a variety of abnormalities that may indicate some degree of heart cell injury.

Humoral antibodies to the parasite membrane-bound antigenic determinants and to other intracellular antigenic components were demonstrated in the sera of all patients with acute Chagas infection. The antibody titers in the group of patients with the apparent acute Chagas' disease, detected by direct agglutination, complement fixation and passive hemagglutination tests, were not significantly different from those observed in patients with inapparent acute Chagas' disease. The

TABLE V

*Serum Proteins and Immunoglobulins in Control Subjects and in Patients with Acute Chagas' Disease**

Groups‡	Proteins						Immunoglobulins		
	Total	Albumin	Alpha 1 globulin	Alpha 2 globulin	Beta globulin	Gamma globulin	IgG	IgA	IgM
					mg/ml				
A-ACD	73.5±7.3	33.5±5.1(*a*)	2.5±0.2	8.5±1.9	6.7±1.9	22.1±1.7(*d*)	16.9±0.9(*g*)	1.4±0.5	0.6±0.2
Range	(63–69)	(26–37)	(2.3–2.9)	(5.6–9.4)	(3.7–7.9)	(21–24)	(16–18)	(0.9–1.9)	(0.3–0.8)
I-ACD	75.1±4.8	35.2±3.2(*b*)	2.6±0.3	9.4±2.4	8.9±3.8	18.9±3.2(*e*)	16.4±2.3(*h*)	1.0±0.3	0.5±0.1
Range	(71–84)	(30–39)	(2.1–2.9)	(7.1–12.6)	(6.1–16.5)	(14–23)	(13–20)	(0.7–1.5)	(0.4–0.7)
Control	74.1±4.0	37.7±4.1(*c*)	2.8±0.4	8.4±1.5	7.6±1.0	17.5±1.1(*f*)	14.3±1.3(*i*)	1.0±0.4	0.6±0.2
Range	(69–79)	(33–43)	(2.1–3.4)	(7.2–10.6)	(6.2–9.3)	(16–19)	(13–16)	(0.6–1.9)	(0.3–0.8)

a vs. *c*, $P > 0.2$; *b* vs. *c*, $P > 0.5$; *d* vs. *f*, $P > 0.001$; *e* vs. *f*, $P > 0.5$; *g* vs. *i*, $P > 0.01$; *h* vs. *i*, $P > 0.1$.

* Serum samples obtained at least 3 mo after onset of infection. Data presented as means±SD.

‡ A-ACD = apparent acute Chagas' disease, $n = 5$; I-ACD = inapparent acute Chagas' disease, $n = 7$.

parasite-induced humoral antibody production was not impaired in acute Chagas' disease either when it was an apparent infection or when it was clinically inapparent. Thus, it seems that the humoral antibodies are not involved in the clinical manifestations of acute Chagas' disease.

In the experiments that assessed for cell-mediated immunity in patients with acute Chagas' disease a panel of eight microbial extracts were used. These tests revealed positive delayed-type skin response to at least one microbial antigen, indicating normal responsiveness of the thymus-dependent lymphocyte system to these antigens. However, a dichotomic type of skin responsiveness to the microsomal *T. cruzi* antigen was observed in these patients. In general, the patients with the inapparent acute Chagas infection showed a negative skin response to the *T. cruzi* antigen. In marked contrast, all the patients with the apparent acute Chagas' disease showed a positive skin response to this antigen. In two of these patients strong skin reactions were elicited at the site of antigen injection. These persisted for 72 h as local induration and swelling of the tissues accompanied by engorgement of satellite lymph nodes. This skin lesion is similar to the lesion of the portal of entry of the parasite in the body. Therefore, we suggest here that the Chagoma and the Romaña's (Fig. 1) signs are the first evidence of delayed-type hypersensitivity in acute Chagas' disease. The interval of 1 wk between the entry of the parasite through the insect bite and the development of the local lesion seems to be the time necessary for sensitizing the T-lymphocytes that produce this delayed-type of reaction.

The capability of patients with Chagas' disease to develop a delayed-type contact reaction to DNCB is decreased when compared with control, non-Chagas subjects. None of the seven patients with the inapparent acute Chagas' disease was capable of developing contact sensitivity to DNCB. However, three out of five patients with apparent acute Chagas' disease showed a positive contact skin response to DNCB. These studies appear to indicate that the thymus-dependent immune response to sensitization to DNCB is severely altered in patients with the inapparent acute Chagas' disease. Furthermore, the alteration of the thymus-dependent immune response may also be expressed in some patients with the apparent acute Chagas' disease. That the *T. cruzi* infection might be a factor involved in the induction of a defective T-lymphocyte response to some antigens appears to be indicated by the observation that all the control, non-Chagas subjects showed strong contact sensitivity to DNCB.

Furthermore, a strong indication of cell-mediated immunodepression was observed in patients with the inapparent form of acute Chagas' disease. This was given by the absence of migration inhibition of the blood leukocytes by the *T. cruzi* microsomal antigen. In general, the degrees of migration of the cells from these patients paralleled the results obtained with normal cells from control donors. By contrast, significant degrees of migration inhibition by the *T. cruzi* microsomal antigen was observed when the blood leukocytes were derived from patients with the apparent form of acute Chagas' disease.

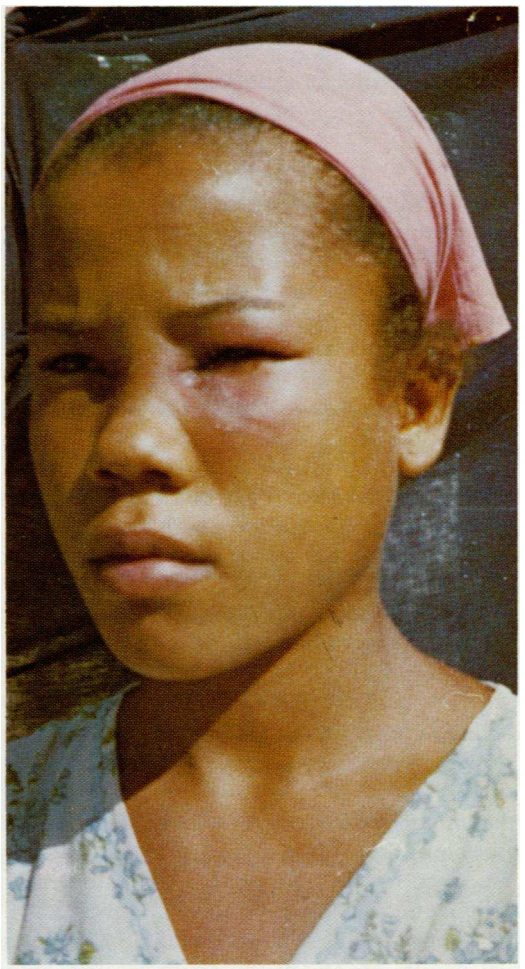

FIGURE 1 Romaña's sign (patient E). There is bipalpebral infiltration and swelling of the left eye indicating the portal of entry of *T. cruzi*. This lesion is the first evidence of delayed-type hypersensitivity in Chagas' disease.

These observations of migration of the blood leukocytes suggest that T-lymphocyte function is depressed in patients with the inapparent acute Chagas' disease but not in patients with the clinically apparent acute disease. These in vitro experiments are consistent with the delayed-type skin test that showed absence of a delayed-type response to the *T. cruzi* antigen in patients with inapparent acute Chagas' disease. Nevertheless, these patients showed various degrees of delayed-type skin responses to a panel of ubiquitous microbial antigens, thus indicating normal T-lymphocyte function to antigenic stimulation previously to the *T. cruzi* infection. Therefore, it seems that this immuno-

depression is acquired in the course of *T. cruzi* infection. Whether it is long lasting we do not know. Some tests repeated 6 mo after the acute phase of the disease were still negative for cell-mediated immunity despite the fact that the titers of humoral antibodies to *T. cruzi* remained high.

The possibility that protein-calorie malnutrition could be responsible for the immunodepression shown by the patients with inapparent acute Chagas' disease can be discarded on the basis of the serum proteins and immunoglobulin levels observed. A significant increase in the gamma-globulin fraction was seen in the patients with apparent acute disease, whereas the patients with inapparent acute disease showed levels similar to those of normal, control subjects. Furthermore, the immunoglobulin levels of individuals living in the rural area of Brazil are higher than those observed in a caucasian group that live in the United States (37, 38).

Several parasitic infections have been shown to induce suppressed humoral and cell-mediated immune responses soon after infection. Furthermore, spleen cells from *Trypanosoma brucei*-infected mice (39, 40) have shown suppressor functions, thus implicating suppressor cells in the immunologic hyporesponsiveness characteristic of this disease. We hypothesize that, as in *T. brucei* infections, *T. cruzi* antigens may directly stimulate T cells with the concomitant release of factors that might become suppressive for T-cell responses. Furthermore, this suppressive effect may alter the T-cell response to other antigens, such as to 2,4-dinitrochlorobenzene, as shown by our patients.

This paper depicts a good correlation between the clinical manifestations of the acute *T. cruzi* infection and the capacity of developing delayed-type hypersensitivity to the parasite. As a rule, the overt clinic disease is manifest in patients with strong delayed-type hypersensitivity to *T. cruzi*. These patients may become very sick and some of them may develop severe myocarditis and heart failure. These observations are in keeping with other experimental evidences (25) showing that the immune destruction of heart cells in Chagas' disease is mediated by delayed-type hypersensitivity. In a previous paper (41) we have shown that immune T-lymphocytes from Chagas patients who demonstrated strong delayed-type skin response to *T. cruzi* were capable of destroying the target human heart cells in vitro. Therefore we favor the interpretation that delayed-type hypersensitivity is responsible for the symptoms shown by the patients with apparent acute Chagas' disease.

We believe that the factors involved in the modulation of the host cell-mediated immune response to *T. cruzi* are important to determine the fate of patients with Chagas' disease. It will be most interesting to know whether the patients with the inapparent acute infection would have a better prognosis than those with the apparent acute infection in the long-run course of chronic Chagas' disease.

ACKNOWLEDGMENT

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico do Brasil, CNPq.

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