

CROSS-REACTIVITY OF ANTIBODIES IN HUMAN INFECTIONS BY THE KINETOPLASTID PROTOZOA *TRYPANOSOMA CRUZI*, *LEISHMANIA CHAGASI* AND *LEISHMANIA (VIANNIA) BRAZILIENSIS*

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SUMMARY

We have detected antibodies, in the sera of Chagas disease, Kala-azar and Mucocutaneous leishmaniasis patients, that bind multiple antigens shared between the three causative agents. The Chagas disease sera showed 98 to 100% positive results by ELISA when the *Leishmania braziliensis* and *Leishmania chagasi* antigens were used, respectively. The Kala-azar sera showed 100% positive results with *Trypanosoma cruzi* or *L. braziliensis* antigens by immunofluorescence assays. The antibodies in the sera of Mucocutaneous leishmaniasis patients showed 100% positive results by ELISA assays with *T. cruzi* or *L. chagasi* antigens. Furthermore, the direct agglutination of *L. chagasi* promastigotes showed that 95% of Kala-azar and 35% of Mucocutaneous leishmaniasis sera agglutinated the parasite in dilutions above 1:512. In contrast, 15% of Chagas sera agglutinated the parasite in dilutions 1:16 and below.

Western blot analysis showed that the Chagas sera that formed at least 24 bands with the *T. cruzi* also formed 13 bands with the *L. chagasi* and 17 bands with the *L. braziliensis*. The Kala-azar sera that recognized at least 29 bands with the homologous antigen also formed 14 bands with the *T. cruzi* and 10 bands with the *L. braziliensis* antigens. Finally, the Mucocutaneous leishmaniasis sera that formed at least 17 bands with the homologous antigen also formed 10 bands with the *T. cruzi* and four bands with the *L. chagasi* antigens. These results indicate the presence of common antigenic determinants in several protozoal proteins and, therefore, explain the serologic cross-reactions reported here.

KEYWORDS: Kinetoplastid protozoa; *Trypanosoma cruzi*; *Leishmania chagasi*; *Leishmania (Viannia) braziliensis*; Cross-reactive antibodies; Human infections.

INTRODUCTION

The flagellate protozoa *T. cruzi*, *L. chagasi* and *L. braziliensis* are the arthropod-transmitted agents of Chagas disease, Kala-azar and Mucocutaneous leishmaniasis, respectively. These flagellates are sympatric in a number of geographic regions of South America^{1, 14}. Although these infectious agents show some common biologic features, they produce distinct pathologies with broad clinical and epidemiologic significance. It has been estimated that 18 million people are infected with *T. cruzi*²², and two million with the Leishmaniasis⁷. These endemic diseases are major

public health problems, causing physical disability and severe economic burden in the rural populations of South America.

Diagnosis of each of these protozoal infections is made by direct microscopic examination of blood samples, bone marrow aspirates or biopsies of ulcerated skin lesions. Either the blood or the macerates of solid tissues can be cultured in liquid medium or injected into susceptible laboratory animals for final demonstration of

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the parasite⁵. All these time-consuming procedures are usually effective in the overt acute phase of the infections. However, in the chronic infections several immunological assays have been used for performing diagnoses. Physicians and field-workers frequently rely on indirect immunofluorescence (IF), indirect hemagglutination (IH), enzyme-linked immunosorbent assay (ELISA) and passive agglutination of the parasite culture forms by serum antibodies, in order to obtain information on the possible causal agent of the infections. Also, skin reactions against antigens from these Trypanosomatidae have been employed^{12, 19}.

The close relationship shown by these protozoa of the order Kinetoplastida suggests that common antigens may be encountered among these species. In fact, early studies have shown some degree of serologic cross-reactivity among these distinctive clinical entities^{3, 4, 11, 13}. In this paper we examine the common antigenic determinants in whole cell protein from these flagellates, and on the extent of cross-reactivity among antisera obtained from Chagas disease, Kala-azar and Mucocutaneous leishmaniasis patients.

MATERIALS AND METHODS

Parasites:

Trypanosoma cruzi: The T12E clone derived from the Ernestina stock of the parasite was used in this study. This stock was isolated from a child with acute Chagas disease¹⁵ and has been maintained in the laboratory through serial passages in liver infusion tryptose (LIT) and, also, in blood-agar bi-phasic medium. The parasite cloning was performed in fetal mouse skeletal muscle culture infected with a single blood trypomastigote¹⁸. The protein preparation obtained from epimastigote forms grown in LIT medium supplemented with 5% fetal calf serum (FCS) was used.

Leishmania chagasi: The EO1345 stock of the parasite isolated from the vector *Lutzomyia longipalpis* at the State of Bahia, Brazil, was employed. The zymodeme and schizodeme profiles of this parasite have been characterized¹⁶. The promastigote forms grown in LIT medium with 20% FCS supplemented with gentamycin (50 µg/ml) and penicillin (100 IU/ml), were used in this study.

Leishmania (Viannia) braziliensis: The LTB300 stock of the parasite⁶ was used. The cells were grown in Schneider's *Drosophila* medium (Gibco, USA) with 20% FCS supplemented with 1 mM L-thyrosine, 5 mM L-cysteine and 7.5% sodium bicarbonate, pH 7.2.

Antigens

Whole cell protein: The exponential growth phase *T. cruzi*, *L. braziliensis* and *L. chagasi* were used for obtaining soluble antigens. Usually 1×10^8 culture forms were collected and washed three times in PBS, pH 7.2. The pellet was resuspended in 1:10 (w/v) double distilled water and frozen at -20°C . After three cycles of freezing and thawing, the ruptured cells were sedimented by centrifugation at $5000 \times g$ for 20 min. The supernatant retained the soluble proteins, which were mixed with the proteinase inhibitors (1 mM PMSF, 1 mM EDTA and 10 µM TLCK) and used in these experiments. The protein content in the soluble antigen was determined by LOWRY's¹⁰ method.

Killed parasites: The *T. cruzi* and *L. braziliensis* forms grown in liquid media were used for preparation of antigens. Usually the exponential growth phase parasites were harvested from 100 ml of culture medium. After centrifugation at $1500 \times g$ for 10 min, the sedimented parasites were washed three times in PBS, pH 7.2. The cells were then suspended in PBS and fixed in 2% formaldehyde (v/v) final concentration, overnight at 4°C . After centrifugation and washings in PBS the dead parasites were diluted to 6×10^3 cells/ml in distilled water. 5 µl of the cell suspension were dipped onto each division on the surface of glass slides, air dried, wrapped on aluminum foil and frozen at -20°C until use.

The *L. chagasi* (10^7 cells/ml) cells were collected by centrifugation and washed three times in Locke's solution, at $1500 \times g$ for 15 min. These parasites were resuspended in Locke's containing 0.4% (v/w) trypsin (Difco). After incubation at 37°C , for 45 min, the cells were washed three times. The parasite cells were resuspended overnight in the same solution containing 2% formaldehyde. After two washings in citrate-saline (0.15M NaCl, 0.05M sodium citrate) the cells were resuspended in Locke's solution containing 1% Coomassie brilliant blue. The dead parasites were kept under agitation for 90 min, washed twice with citrate-saline and resuspended in the same solution containing 0.43% formaldehyde. This antigen was stored in dark bottle, at 4°C , until use.

Serum samples:

Chagas disease: The 64 samples of Chagas patient sera used in this study were from street-sweepers at the Cleaning Department of Brasília, Brazil. All these chronically infected patients were born in poor rural areas of the States of Goiás, Minas Gerais and Bahia, where *T. cruzi* transmission through the Triatomine vector takes place. All sera used in this study had serologically tested positive for Chagas disease.

Kala-azar: Serum samples were obtained from 40 Kala-azar patients. Twenty eight samples were obtained from in-patients at several hospitals in the Federal District, Brasília. The remaining cases came from the Infectious Disease Division at the Hospital of the University of Bahia, Brazil. In every case the diagnosis was made by direct demonstration of amastigotes of *L. chagasi* in bone marrow cells and/or by sub-culture in blood agar.

Mucocutaneous leishmaniasis: Serum samples were obtained from 63 patients from the Três Braços county, Bahia, Brazil, where the disease is endemic. In every case the skin biopsy showed the amastigote forms in the phagocytic cells at the border of ulcerated leishmaniasis lesions, or by inoculation of macerated tissue in susceptible hamsters, with subsequent recovery of the parasite from resulting lesions.

Controls: Fifty-six control sera were obtained from healthy street-sweepers at the Cleaning Department of Brasília, Brazil. None of the individuals had lived in endemic areas for any of the diseases under consideration. The serum samples were stored at -20°C until use.

Immunologic assays:

ELISA: The standard ELISA assay was carried out on 96-well plates (Dynatech, USA). The optimal concentration of antigen was determined by checkerboard titration experiments carried out according to standard procedure²¹. Thereafter, the quantity of 0.1 μg of protein in each antigen diluted in carbonate buffer saline solution, pH 9.6, was used to coat the negatively charged plate wells overnight, at 4°C . The excess of antigen was discarded and the plates were washed three times with PBS/Tween 20 at 0.05% (v/v). The non-specific binding sites were blocked by a 2% suspension of non-fat milk in the same solution. The antigen-sensitized plates were washed three times with PBS-Tween, air dried at room temperature and stored at -20°C . Fifty μl of serum dilutions were placed on the wells and the plates were incubated for 1 h at room temperature. After automated washings, (Utrawash, Dynatech Laboratories) the wells received 50 μl of a 1:1000 dilution of peroxidase-labelled rabbit anti-human IgG (Cappel Laboratories, USA), and the reaction that was formed after washing and incubating for 10 minutes with a mixture of ortho-phenylenediamine and hydrogen peroxide was stopped by addition of 1 N H_2SO_4 . The optical densities were read in a Titertekplus spectrophotometer at a wavelength setting of 490 nm.

Indirect hemagglutination (IH): This procedure was performed according to a technique previously described¹⁷. A positive result was indicated by agglutinated red cells lining the bottom of the wells with serum dilutions above 1:8.

Indirect immunofluorescence (IF): This assay was run as described by CAMARGO². The fluorescein-conjugated anti-human IgG (Cappel Laboratories, USA) was used in the dilution 1:100. A positive result was indicated by apple-green staining of the parasite silhouette with serum dilutions above 1:20.

Direct agglutination: Serial dilutions of each serum sample were carried out on the wells of microtitration plates. Fifty μl of the serum in saline solution containing 1% (v/v) FCS and 0.1 M 2-mercaptoethanol were placed on the wells, which received equal volume of the formaldehyde-killed parasite suspension. The plate was covered with a plastic membrane and incubated for 24 h at room temperature in a moist chamber. Positive and negative control sera were used in these assays. Agglutination of the parasite forms by serum dilutions 1:512 and above was scored as positive⁸.

Western blot: SDS-PAGE analyses of the whole cell protein (1 mg/ml of protein) were carried out in 10% gels, according to the procedure described by LAEMMLI⁹. The proteins in the gel were transferred to nitrocellulose membrane (Multiphor II, Pharmacia, Sweden), according to TOWBIN²⁰. The 3 mm width nitrocellulose strips were incubated with borate-buffered saline solution (BBS), pH 7.2, containing 2% non-fat milk for 3 h at room temperature with gentle shaking. In these assays the *L. chagasi* antigen required 5% non-fat milk. After three washings in BBS-milk the strips were treated with a 1:1000 dilution of peroxidase-labelled rabbit anti-human IgG (Cappel Laboratories) and incubated for 2 h at room temperature. The strips were washed and then treated with 4-chloro-naphtol and hydrogen peroxide to reveal the protein bands. The M_w of each band was calculated by using Sigma molecular weight standards: Carbonic anhydrase (29,000); Ovalbumin (45,000); Bovine albumin (66,000); Phospholipase-B (97,400); β -galactosidase (116,000); and Myosin (205,000).

RESULTS

The results of serologic assays carried out with sera of Chagas disease, Kala-azar and Mucocutaneous leishmaniasis patients are shown in Table 1. In these assays each serum was screened with homologous and heterologous antigens by ELISA, IH, and IF. All serum samples from chagasic patients yielded antibody titers above the control sera by each serologic test employing *T. cruzi* antigen. However, all Kala-azar and Mucocutaneous leishmaniasis samples also showed high IF antibody titers against the same *T. cruzi* antigen. In addition, 10% of the Kala-azar and 55% of the Mucocutaneous leishmaniasis sera yielded IH antibody titers, whereas 15% and 5%, respectively, reacted in ELISA with the *T. cruzi* soluble antigen. None of the control sera yielded a positive serologic reaction when this antigen was used.

TABLE 1
Cross-reactivity among sera from Chagas disease, Kala-azar and Mucocutaneous leishmaniasis patients*.

Antisera	Total	Antigens								
		<i>T. cruzi</i>			<i>L. chagasi</i>			<i>L. braziliensis</i>		
		ELISA (%)	IH (%)	IF (%)	ELISA (%)	IH (%)	IF (%)	ELISA (%)	IH (%)	IF (%)
Chagas	64	100	100	100	98	14	35	100	3	70
Kala-azar	40	15	10	100	100	47	100	100	2	100
MCL	63	5	55	100	100	14	35	100	14	100
Controls	56	0	0	0	2	12	0	2	2	0

* Sera from Chagas disease, Kala-azar and Mucocutaneous Leishmaniasis (MCL) patients were submitted to ELISA, IH and IF assays using homologous and heterologous antigens, whose cut-offs were OD = 0.1, and 1:8 and 1:20 dilutions, respectively. See material and methods for details.

All Kala-azar patients sera yielded high antibody titers by ELISA and IF assays with the homologous antigen. However, the ELISA also showed high antibody titers in 98% of the Chagas sera and in 100% of the Mucocutaneous leishmaniasis sera tested with *L. chagasi* antigen. Also, the IF showed 35% positive results when both, the Chagas and the Mucocutaneous leishmaniasis sera were employed. The IH showed positive results in 47% of these sera tested with the homologous *L. chagasi* soluble antigen. In addition, the IH also showed 14% positive results when the Chagas and the Mucocutaneous leishmaniasis sera were tested with this antigen.

All Mucocutaneous leishmaniasis patients sera yielded high antibody titers by ELISA and IF assays with the homologous *L. braziliensis* antigen, whereas IH gave positive results with the same antigen in 14% of the cases. It was disturbing to observe that all the Chagas and Kala-azar sera yielded high antibody titers by ELISA against *L. braziliensis* soluble antigen. Similarly, the IF assay yielded cross-reacting antibody titers with 100% of the Kala-azar sera and with 70% of the Chagas sera, when this same antigen was used. Control sera also yielded a few positive serologic tests when the *L. chagasi* (12%) and the *L. braziliensis* (2%) antigens were used (Table 1).

The profiles of IH antibody titers in 64 Chagas patients sera assayed with *T. cruzi*, *L. chagasi* and *L. braziliensis* soluble antigens are shown in Figure 1. The homologous antigen revealed antibody titers above 1:64 in all cases. In 87% of the cases the titers ranged between 1:256 and 1:4096. In contrast, seven Kala-azar sera showed anti-*T. cruzi* antibody titers below 1:64, and two cases of Mucocutaneous leishmaniasis showed antibody titer 1:32. Figure 2 shows the profiles of the ELISA antibody titers in the Chagas sera. The absorbance obtained in the ELISA with the *T. cruzi* antigen peaked at 0.6 in 44% (28/64) of the cases. On the other hand, the absorbances recorded for both *L. chagasi* and *L. braziliensis* antigens peaked at 0.2 and 0.4, respectively. The

absorbances obtained in the control experiments were significantly lower (< 0.1, data not shown).

Figure 3 shows the profiles of antibodies demonstrated by ELISA in the sera of Kala-azar patients assayed with *L. chagasi*, *L. braziliensis* and *T. cruzi* antigens. The absorbances obtained with the *L. chagasi* and *L. braziliensis* antigens were similarly high and usually much above those seen when the *T. cruzi* antigen was used. All those absorbances are significantly higher (> 0.2) than those observed when 56 control sera were used (< 0.1, data not shown). Figure 4 shows results of the ELISA in 63 Mucocutaneous leishmaniasis patients sera against *L. braziliensis*, *L. chagasi* and *T. cruzi* antigens. All these sera reacted strongly with the former two antigens, whereas only two cases showed absorbances above 0.1 with the *T. cruzi* antigen.

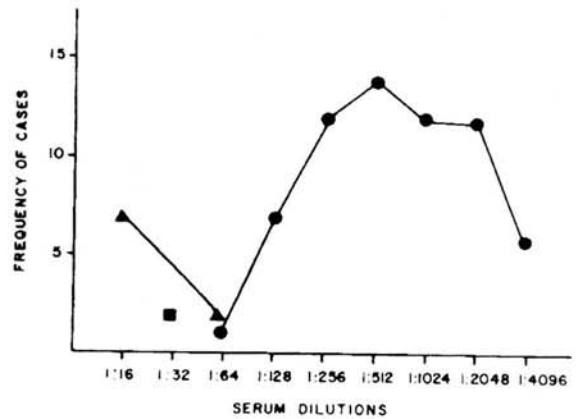


Fig. 1: Indirect hemagglutination antibody titers in 64 Chagas disease patients sera assayed with *Trypanosoma cruzi* (●—●), *Leishmania chagasi* (▲—▲) and *Leishmania braziliensis* (■) soluble antigens. High antibody titers above 1:64 was shown by the homologous antigen. Nine sera also showed antibodies (> 1:16) against *L. chagasi*, and two cases showed antibodies against *L. braziliensis*. All 56 control sera used in this study showed IH antibody titers < 1:8.

An attempt was made to distinguish *L. chagasi* from *L. braziliensis* and from *T. cruzi* infections by means of the direct agglutination of formaldehyde-killed *L. chagasi* promastigotes in suspension. It was shown that 95% (19/20) of the Kala-azar and 35% (7/20) of the Mucocutaneous leishmaniasis sera tested presented parasite agglutination in dilutions above 1:512. In contrast, 15% (3/20) of the

chagasic sera used showed parasite agglutination in dilutions 1:16 and below (Figure 5).

The Western blot assay was used to study the pattern of proteins recognized by these sera. The Chagas patients sera revealed at least 24 antigenic bands, ranging from 245 to 7 kDa, in the *T. cruzi* soluble antigen. The Kala-azar sera

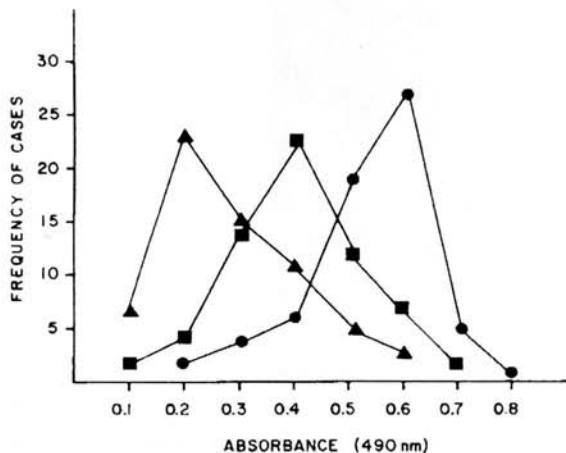


Fig. 2: Profiles of IgG antibodies shown by ELISA in 64 Chagas disease sera tested with *Trypanosoma cruzi* (●—●), *Leishmania chagasi* (▲—▲) and *Leishmania braziliensis* (■—■) soluble antigens. Notice close similarity of profiles, despite that the absorbances of the reactions of antibodies with the homologous antigens were usually stronger than those obtained with heterologous antigens. Also, cross-reactivity among these sera and *L. braziliensis* antigen was usually stronger than that obtained with

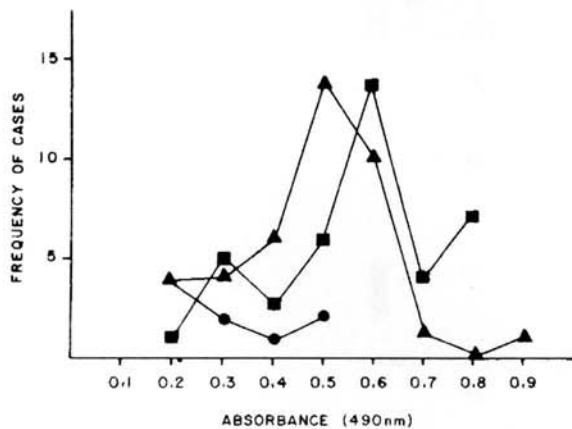


Fig. 3: Profiles of IgG antibodies shown by ELISA in 40 Kala-azar sera tested with *Leishmania chagasi* (▲—▲), *Leishmania braziliensis* (■—■) and *Trypanosoma cruzi* (●—●) antigens. Notice similarity of profiles among optical densities obtained when these sera reacted with the *Leishmania* sp. soluble antigens. Eleven of these sera also showed cross-reactive antibodies against *T. cruzi* and yielded absorbances > 0.15. All the 56 control sera yielded optical densities < 0.1.

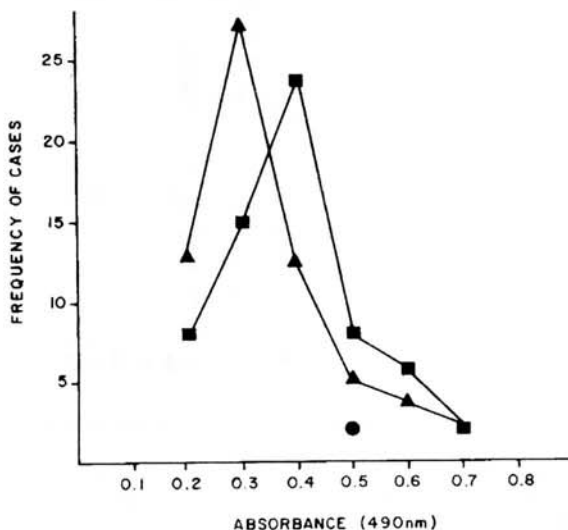


Fig. 4: Profiles of anti-IgG antibodies shown by the ELISA in 63 Mucocutaneous Leishmaniasis sera tested with *Leishmania braziliensis* (■—■), *Leishmania chagasi* (▲—▲), and *Trypanosoma cruzi* (●—●) soluble antigens. All these sera showed strong reactivity against the *Leishmania* sp. antigens, but only two showed cross-reactivity to *T. cruzi*. All 56 control sera showed negative results indicated by absorbances < 0.1.

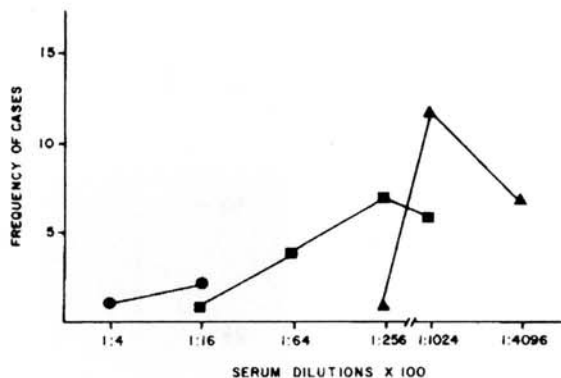


Fig. 5: Direct agglutination of formaldehyde-killed promastigotes of *L. chagasi*. Notice that 95% of the Kala-azar (▲—▲) and 35% of the Mucocutaneous leishmaniasis (■—■) sera showed agglutination of the parasite forms in dilutions > 1:512. In contrast, all the Chagas (●—●) sera showed agglutination in dilutions 1:16 and below.

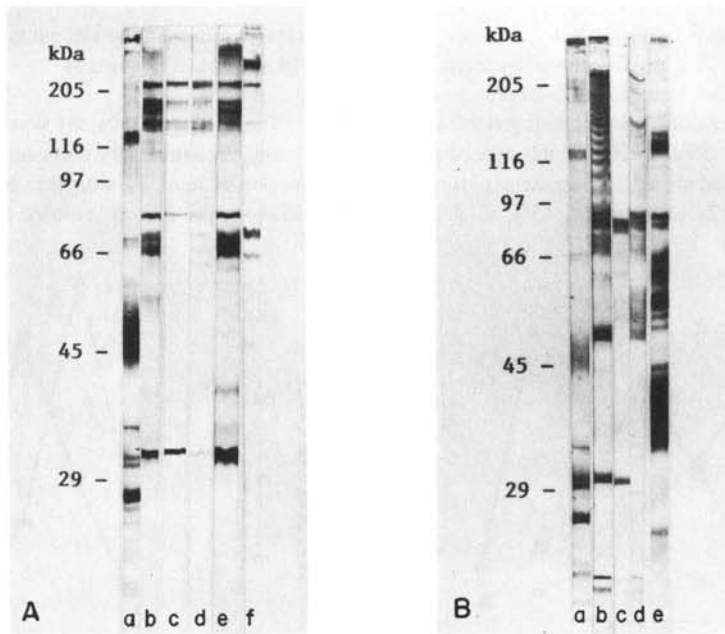


Fig. 6: Western blot analysis of Chagas sera showing cross-reactivity with *Leishmania* sp. soluble antigens. **A)** lane a, standard bands formed when the Chagas serum reacted with the *Trypanosoma cruzi* antigen; Lanes b to e, bands formed when Chagas sera cross-reacted with *Leishmania braziliensis* soluble antigen; Lane f, shows bands formed when the Mucocutaneous leishmaniasis serum reacted with the homologous antigen. **B)** lane a, standard bands formed when the Chagas serum reacted with the homologous antigen; Lanes b to d, bands formed when these sera cross-reacted with *Leishmania chagasi* antigen. Lane e, bands formed when Kala-azar serum reacted with the homologous antigen.

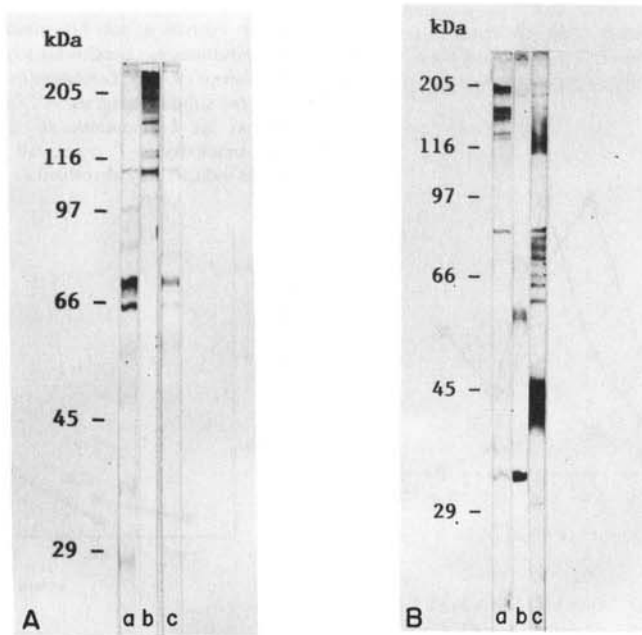


Fig. 7: Western blot analysis of *Leishmania braziliensis* sera showing cross-reactivity with *Trypanosoma cruzi* and *Leishmania chagasi*. **A)** Lane a, standard bands formed when Mucocutaneous leishmaniasis serum reacted with *L. braziliensis* antigen. Lanes b and c, bands formed when these sera cross-reacted with *T. cruzi* antigen. **B)** Lane a, standard bands formed when Mucocutaneous leishmaniasis serum reacted with the *L. braziliensis* antigen. Lane b, bands formed when the serum cross-reacted with *L. chagasi* antigen. Lane c, standard bands formed when the Kala-azar serum reacted with the *L. chagasi* antigen.

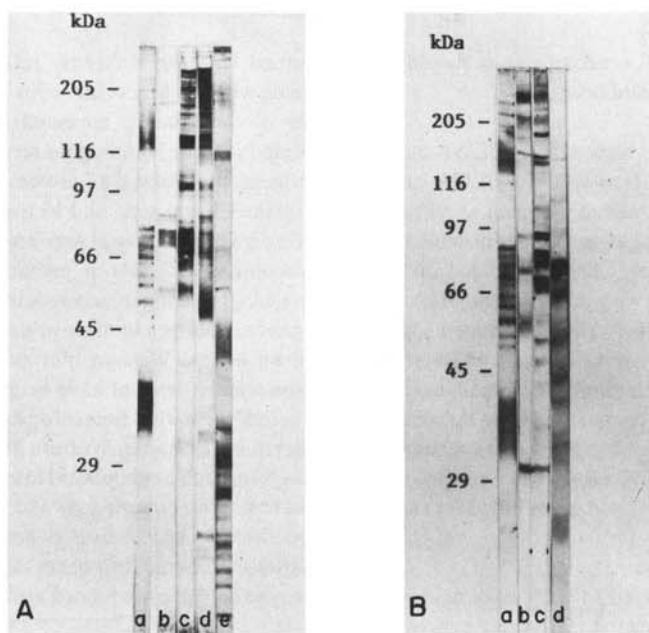


Fig. 8: Western blot analysis of Kala-azar sera showing cross-reactivity with *Trypanosoma cruzi* and *Leishmania braziliensis* soluble antigen. **A)** lane **a**, standard bands formed when Kala-azar serum reacted with the *Leishmania chagasi* antigen. Lanes **b** to **d**, bands formed when these sera cross-reacted with *T. cruzi* soluble antigen. Lane **c**, standard bands formed with Chagas sera and *T. cruzi* antigen. **B)** lane **a**, standard bands formed with the Kala-azar serum and the homologous antigen. Lanes **b** to **c**, bands formed with Kala-azar sera and *L. braziliensis*. Lane **d**, standard bands formed with Mucocutaneous leishmaniasis serum and the homologous antigen.

also revealed at least 14 bands cross-reacting antigens, whereas the Mucocutaneous leishmaniasis sera cross-reacted with 10 bands. Figure 6A shows M_r 250, 245, 210, 129, 123 and 69 kDa bands formed when the *T. cruzi* soluble antigen was treated with either the homologous sera or with the Mucocutaneous leishmaniasis sera. Figure 6B shows M_r 250, 220, 133, 129, 123, 76 and 74 kDa bands when the same antigen was analysed with the anti-Kala-azar sera.

We also assayed by Western blot the *L. chagasi* soluble antigen with homologous and heterologous sera. It was shown that at least 29 bands ranging in size from 250 to 8 kDa formed with the Kala-azar sera. The same antigen formed 13 bands with the Chagas sera and at least 4 bands with the Mucocutaneous leishmaniasis sera. Of interest, the 250, 220, 133, 129, 123, 76 and 64 kDa bands were present when both the Kala-azar and the Chagas sera were used. The 133 kDa band was also present among the several bands formed when the Mucocutaneous Leishmaniasis sera was used (Figure 7).

Finally, the *L. braziliensis* soluble antigen was analysed with homologous and heterologous sera. At least 17 bands were observed when the proteins in the antigen reacted with

Mucocutaneous Leishmaniasis and Chagas sera, whereas the Kala-azar formed 15 bands. The 250, 245, 210, 150, 129, 123 and 69 kDa bands shown by the homologous antisera were also identified by the Chagas sera. However, the Kala-azar sera showed only 250, 245 and 210 kDa bands in the same antigen. Figure 8 shows profiles of cross-reactivity of Kala-azar sera that showed positive serologic tests with *T. cruzi* and *L. braziliensis* antigens.

DISCUSSION

In this study, the assays employed in the diagnosis of Chagas disease yielded positive reactions in all the sera from *T. cruzi* infected individuals when the homologous antigen was used. Control sera did not yield positive reactions in these assays. In marked contrast, the specificity of these reactions showed broad variations according with the assay used. For instance, the IF assay showed positive reactions with all Kala-azar antisera, and, also, with 55% of the mucocutaneous leishmaniasis sera. The IH and ELISA also showed several positive reactions with Kala-azar and with the Mucocutaneous leishmaniasis sera, albeit to much lesser degrees, when the *T. cruzi* antigen was used. These observations are particularly important in those geographic regions where these diseases are sympatric. In this regard, future serologic studies to

determine the prevalence of *T. cruzi* infections should take in consideration the data reported here.

The serologic diagnosis of Kala-azar by ELISA or IF is twice as sensitive than by IH. However, the ELISA carried out with *L. chagasi* antigen yielded as much as 98% and 100% positive results with Chagas and with Mucocutaneous leishmaniasis sera, respectively. The IF yielded 35% of positive cross-reactivities with these sera. The low sensitivity of the IH assay with *L. chagasi* antigen appears to rule out its employment for serodiagnosis of Kala-azar. Since the features of Visceral leishmaniasis (Kala-azar) are quite typical and distinct from Mucocutaneous leishmaniasis, the questions arising from cross-reactivities can be resolved clinically. These cross-reactions, however, should be considered carefully in screening blood for various purposes.

It has been shown that the ELISA and IF assays are highly sensitive for accomplishing serodiagnosis of Mucocutaneous leishmaniasis. These assays, however, yielded 100% cross-reactions with Kala-azar sera. In addition, the ELISA showed 100% cross-reactions with Chagas sera, whereas the IF showed 70% of positive reactions with same sera. However, the direct agglutination of *L. chagasi*-killed promastigote assay separated *T. cruzi* from the other two kinetoplastid infections. Furthermore, this assay yielded 95% and 35% positive results (above 1:512) with Kala-azar and Mucocutaneous leishmaniasis sera, respectively. In fact, it becomes practically impossible to indicate the causal agent of clinically silent infections caused by *T. cruzi*, *L. braziliensis* or *L. chagasi* on a strictly serologic basis. Of course, the reported serologic cross-reactivities should be considered in epidemiologic inquires and in field-research studies in those regions where the diseases are endemic.

We analysed the serologic cross-reactivities by Western blot and showed several common antigenic determinants (epitopes) in the soluble antigens from each kinetoplastid protozoa employed in this study. With this respect, 14 *T. cruzi* protein bands showed epitopes that can be recognized with Kala-azar sera, whereas the epitopes in 10 bands were recognized with Mucocutaneous leishmaniasis sera. There is a possibility that at least some of these epitopes are present in conserved proteins in those phylogenetically related flagellates. In fact, six common protein bands in the *T. cruzi* soluble antigen were recognized by both Chagas and Mucocutaneous leishmaniasis sera. Also, seven common protein bands were recognized by the Kala-azar sera in the *T. cruzi* antigen.

The *L. chagasi* soluble antigen formed 13 bands with Chagas sera and six of these bands were common with those

formed with the Kala-azar sera. This antigen formed for bands with the Mucocutaneous leishmaniasis sera, but only one of these bands appeared to be common with those formed with the homologous sera. The *L. braziliensis* soluble antigens also showed 17 protein bands that were recognized with the Chagas sera, and 15 bands with the Kala-azar sera. Of interest, the Chagas sera recognized common antigenic determinants in seven protein bands formed with the heterologous antigen, whereas the Kala-azar antigen showed common epitopes in three protein bands only. These bands shown by the Western blot assays should explain strong cross-reactivities that have been observed when these sera reacted with the heterologous antigen. Furthermore, discrepancies among Western blot profiles within groups of sera from each kinetoplastid infection, that were revealed by reactions with homologous and heterologous antigens (data not shown), may reflect a broad diversity of individual humoral immune responses towards these kinetoplastid parasite antigens associated with host interactions.

RESUMO

Reatividade cruzada de anticorpos em pacientes com infecções pelos protozoários *Trypanosoma cruzi*, *Leishmania chagasi* e *Leishmania (Viannia) braziliensis*.

Foram detectados anticorpos, nos soros de pacientes com doença de Chagas, Calazar e Leishmaniose cutâneo-mucosa, que se ligam a antígenos compartilhados pelos três agentes causais. Os pacientes chagásicos mostraram 98 a 100% de soropositividade pelo ELISA quando antígenos de *Leishmania braziliensis* e de *Leishmania chagasi* foram usados, respectivamente. Os soros de Calazar mostraram resultados positivos com antígenos de *Trypanosoma cruzi* ou com *L. braziliensis* pela imunofluorescência. Ademais, os anticorpos nos soros de pacientes com Leishmaniose cutâneo-mucosa tinham 100% de resultados positivos pelo ELISA com antígenos de *T. cruzi* ou de *L. chagasi*. Ademais, a aglutinação direta de promastigotas de *L. chagasi* mostrou que 95% dos soros de Calazar e 35% de Leishmaniose cutâneo-mucosa aglutinaram o parasito em diluições acima de 1:512. Em contraste, 15% dos soros chagásicos aglutinaram o parasito na diluição de 1:16 ou abaixo.

Análises pelo Western blot mostraram que os soros chagásicos que formaram pelo menos 24 bandas com antígeno de *T. cruzi* também foram 13 bandas com antígeno de *L. chagasi* e 17 bandas com *L. braziliensis*. Os soros de Calazar que reconheceram pelo menos 29 bandas com o antígeno homólogo também formaram 14 bandas com o *T. cruzi* e 10 bandas com *L. braziliensis*. Finalmente, os soros de Leishmaniose cutâneo-mucosa que formaram pelo menos 17 bandas com o antígeno homólogo também

formaram 10 bandas com os antígenos de *T. cruzi* e quatro com *L. braziliensis*. Esses resultados indicam a presença de determinantes antigênicos comuns em várias proteínas desses protozoários e, portanto, explicam as reações sorológicas cruzadas descritas aqui.

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