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Leishmania infantum heat shock protein 83 for the serodiagnosis of tegumentary leishmaniasis

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Abstract

The serologic assay is an important tool in the diagnosis of leishmaniasis. One of the most commonly used tests is enzyme-linked immunosorbent assay (ELISA). Since total *Leishmania* promastigotes are used as antigen in the routine assay, false-positive reactions are frequent due to cross-reaction with sera from other diseases, mainly Chagas' disease. Therefore, an antigen that determines less cross-reactivity has been pursued for the serodiagnosis of leishmaniasis. In the present study we analyzed the use of recombinant *Leishmania infantum* heat shock protein (Hsp) 83 in ELISA for the serodiagnosis of cutaneous (N = 12) and mucocutaneous leishmaniasis (N = 14) and we observed the presence of anti-*L. infantum* Hsp 83 antibodies in all samples as well as anti-*Leishmania* total antigen antibodies. When cross-reactivity was tested, chronic Chagas' disease patients (N = 10) did not show any reactivity. Therefore, we consider this *L. infantum* Hsp 83 to be a good antigen for routine use for serodiagnosis of tegumentary leishmaniasis.

Key words
- *Leishmania infantum*
- Hsp 83
- ELISA
- Serodiagnosis
- Tegumentary leishmaniasis
- Chagas' disease

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Leishmaniasis is a disease caused by protozoa of the genus *Leishmania* which can occur in the cutaneous, mucocutaneous or visceral form. Serological tests are most commonly used for diagnosis and, in addition to immunofluorescence, enzyme-linked immunosorbent assay (ELISA) is the assay of choice because of its sensitivity and higher predictive value (1). However, since the antigen used for anti-*Leishmania* ELISA is a total promastigote antigen, false-positive reactions are frequent when sera from patients with other diseases, mainly Chagas' disease, are tested (2-4). Therefore, an antigen with less cross-reactivity has been pursued for the serodiagnosis of leishmaniasis.

Several investigators have reported heat shock proteins (Hsp) from *Leishmania* belonging to the 60, 70, 83, and 90 families that have been tested for the serodiagnosis of leishmaniasis. *L. major* Hsp 60 were tested with cutaneous leishmaniasis sera (5), *L. braziliensis* Hsp 83 and Hsp 70 with cutaneous, mucocutaneous and diffuse cutaneous leishmaniasis sera (6), and *L. donovani* Hsp 70 (7) and Hsp 83 of *L. infantum* with canine

visceral leishmaniasis sera (8). Except for *L. donovani* Hsp 70, all sera reacted with samples from different leishmaniasis patients. In the study with *L. infantum* Hsp 70, however, in addition to the expected reactivity with visceral and mucocutaneous leishmaniasis sera, cross-reactivity was observed with sera from Chagas' disease patients (9).

Table 1. Titers and median of anti-Hsp 83 and anti-*Leishmania major*-like total antigen (anti-*leishmania)* in mucocutaneous and cutaneous leishmaniasis patients.

Mucocutaneous leishmaniasis			Cutaneous leishmaniasis		
Patient	anti-Hsp 83	anti-*leishmania*	Patient	anti-Hsp 83	anti-*leishmania*
GC	320	5120	GCS	80	40
MLC	640	20240	GNS	320	2560
JM	320	640	EMR	320	320
YM	160	160	DMR	160	160
BEA	160	320	CJS	640	320
ACS	1280	1280	NSA	640	160
OL	320	320	JVS	320	40
AMM	160	640	CAA	160	1280
MNP	640	640	RDO	320	640
JFS	80	640	MDM	80	1280
FR	1280	640	AMP	80	160
AMS	160	320	ESC	320	640
EVL	160	160			
AFS	320	5120			
Median	320	640	Median	320	320

ELISA-IgG test. Positive when titers were ≥40.

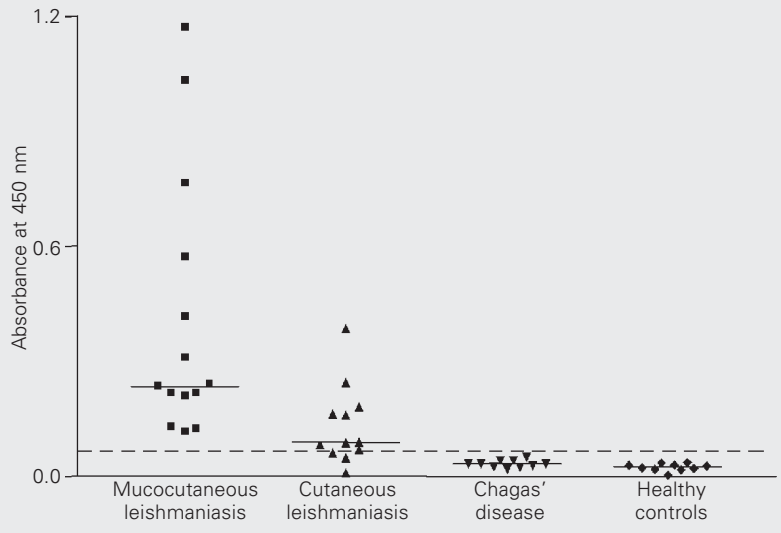

Figure 1. Reactivity of sera from cutaneous (N = 12) and mucocutaneous (N = 14) leishmaniasis patients, Chagas' disease (N = 10) patients and healthy controls (N = 10) with recombinant *L. infantum* (Hsp 83) in the ELISA-IgG test. The broken line indicates the cut-off values, corresponding to the mean absorbance value of the control sera +2 SD. The solid horizontal lines indicate the median value.

In the present study we analyzed the recombinant *L. infantum* Hsp 83 (8) using ELISA for serodiagnosis of tegumentary leishmaniasis, we compared the performance with the *L. major*-like total antigen used in routine tests (9) and we analyzed the cross reactivity with Chagas' disease samples. The recombinant protein was produced and purified by Ni-NTA affinity chromatography (QIAGEN GmbH, Hilden, Germany) according to the method of Angel et al. (8) and the anti-Hsp 83 antibody was detected by ELISA. Briefly, microplates were coated with 50 µl/well of recombinant antigen (1 mg/ml *L. infantum* Hsp 83) diluted in 60 mM sodium carbonate buffer, pH 9.6. The sera and peroxidase-conjugate anti-human IgG antibody were diluted in PBS-T (0.15 M NaCl, 10 mM sodium phosphate, pH 7.2, 0.05% Tween-20) containing 1% bovine serum albumin (Sigma, St. Louis, MO, USA). The substrate used was 5.2 mM 5-aminosalicylic acid, and 1.5 mM H_2O_2. The reaction was stopped by the addition of 25 µl of 1 M NaOH to each well and absorbance was measured at 450 nm. Cut-off of the test corresponded to mean plus 2 standard deviations.

We analyzed sera from patients with cutaneous (N = 12) and mucocutaneous (N = 14) leishmaniasis examined in the Department of Dermatology, University of São Paulo Medical School. All sera had been sent for routine serological tests for leishmaniasis. The diagnosis was confirmed in all cases by a positive Montenegro skin test (10), induration ≥5 mm, and/or by histopathology of the skin lesion (11), and by the detection of anti-*Leishmania* antibody by the immunofluorescence test. Sera from 10 normal blood bank donors were used as controls and sera from 10 Chagas' disease patients (kindly provided by M.G. Valin from the Immunology Section of the Laboratório

Central, HC-FMUSP) were also analyzed. The research protocol was analyzed and approved by the Ethics Committees of the Instituto de Medicina Tropical de São Paulo, USP, and of the Heart Institute, Hospital das Clínicas, Faculdade de Medicina, USP.

All patients with tegumentary leishmaniasis showed the presence of anti-*L. infantum* Hsp 83 antibodies (Table 1). When we compared the reactivity to Hsp 83 and *Leishmania* total antigen we found some variations but all samples were positive for both antigens (Table 1). When cross-reactivity was tested with Hsp 83 antigen, sera from Chagas' disease patients did not show any reactivity (Figure 1). When the same serum samples were tested with *Leishmania* total antigen they all showed cross-reactivity (median absorbance = 0.608, range: 0.165-0.737, cutoff = 0.158).

The absence of cross-reactivity with Chagas' disease sera is important since the endemic areas for both diseases often overlap. The carboxyl-terminal region of *L.* (*Viannia*) *braziliensis* Hsp 70 showed no cross-reactivity with Chagas' patient sera, but this antigen did not react with sera from cutaneous leishmaniasis patients infected with *L. amazonensis* (12). Thus, this antigen is not suitable for use in diagnosis in Brazil, where patients from some regions are infected with these species of *Leishmania*. The *L. infantum* Hsp 70-derived 50-mer peptide also showed no cross-reactivity with sera from Chagas' disease patients (9). However, compared with the recombinant *L. infantum* Hsp 83 used in the present study, the synthesis of a peptide is technically more complex than the production of a recombinant antigen. Therefore, we consider this *L. infantum* Hsp 83 to be a good antigen for routine use for serodiagnosis of tegumentary leishmaniasis.

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