

ANTI-LEISHMANIA IgA IMMUNOENZYMATIC ASSAY IN MUCOCUTANEOUS LEISHMANIASIS

(Preliminary Report)

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SUMMARY

The Authors describe an anti-Leishmania IgA-ELISA assay in mucocutaneous leishmaniasis. Increased titers were found in leishmaniasis patients, mainly in the first and second year of infection and in deep mycoses patients showing either mucosal involvement or widespread disease.

INTRODUCTION

IgA antibodies for *Leishmania* were demonstrated in the serum of patients with cutaneous and mucocutaneous leishmaniasis by SHAW & LAINSON, 1981⁵, as an evidence of amastigote mucosal invasion. As part of an effort to further a better understanding of mucocutaneous leishmaniasis serology by comparison of immunofluorescence tests (IF) and immunoenzymatic assays (ELISA) the authors devised an assay to evaluate anti-*Leishmania* IgA titers by means of an ELISA test.

MATERIAL AND METHODS

Serum samples — One hundred and twenty-two sera were assayed for IgA-ELISA and IgA-IF. Sixty-five sera were from 25 mucocutaneous leishmaniasis patients, 32 from mycoses (16 paracoccidioidomycosis, 7 chromomycosis, 5 sporotrichosis, 2 mycetoma and 2 nocardiosis). All patients had a biopsy and or parasitology diagnosis. Twenty-five sera were from normal controls. All patients were submitted to the Montenegro skin test².

IgA-ELISA — Details concerning *L. braziliensis* antigen were given elsewhere⁴. U-shaped

polypropylene plate wells (Interlab, São Paulo, Brazil) were sensitized (for 18 hours at 4°C) with an alkaline extract of *L. braziliensis* (strain 49) promastigotes cultured in LIT medium⁸. The antigen was diluted to 5 µg/ml with a 0.06 M carbonate buffer, pH 9.6 and 50 µl of dilution were added to each well. Plates were washed 3 times with PBST (NaCl 0.15 M; phosphates 0.01 M; pH 7.2; Tween-20 0.05%). Sera were diluted 1/10 and 1/100 with PBST and 50 µl of each dilution added to wells. Plates were incubated at 37°C for 60 minutes and washed 3 times in PBST. Fifty microliters of peroxidase-anti human IgA (α-chain specific) conjugate prepared according to WILSON & NAKANE⁶, diluted 1:800 for maximal activity in PBST were added to each well and incubated at 37°C for 60 minutes. Plates were washed 3 times with PBST and incubated with 200 µl of a solution containing 10 mg orthophenylenodiamine and 10 µl 30% hydrogen peroxide in 20 ml citrate-phosphate buffer pH 5.0. After 30 minutes at room temperature in a dark box the reaction was stopped by adding one drop of 4 M H₂SO₄ to each well and the contents read at 492 nm with the chromogen solution as blank.

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The optimal antigen concentration was determined by block titration of varying antigen concentrations and of positive and negative standard sera.

Precise serum titers were determined according to the equation:

$$T = \log D_1 + \frac{(\log E_1 - \log \text{of cutoff}) (\log D_2 - \log D_1)}{\log E_1 - \log E_2}$$

E_1 and E_2 being extinction values for dilutions D_1 and D_2 .

Serum titration — When positive standard sera were assayed in 2-fold dilutions starting from 1:10, straight lines were obtained by plotting logarithms of optical densities and serum dilutions between extinction values of 0.15 and 0.50. Serum titers were expressed as \log_{10} of the serum dilution corresponding to 0.200 absorbance (referred in the above equation as the cutoff value).

As mentioned, two serum dilutions were tested and titer determined according to the equation above.

To avoid the influence on titers of daily variation in test sensitivity a reactive reference serum was included in each test and serum titers corrected according to observed variations in the reference serum titers.

A thorough description of the precise serum titer assessment can be found in CAMARGO et al.¹

IgA-IF — *L. braziliensis* (strain 49) promastigote antigen was prepared according to GUIMARÃES et al.³. FITC-conjugate was prepared at the Instituto de Medicina Tropical de São Paulo from a sheep anti-human IgA (α -chain specific) serum, F/P ratio = 3.3. All sera were screened for reactivity at a 1/10 dilution. Positive and negative control sera were included in each determination³.

RESULTS AND DISCUSSION

All leishmaniasis patients had a Montenegro skin test equal to/or larger than 10 mm. Twenty-four normal control patients had negative skin tests (equal to/or smaller than 5 mm). Among the mycoses 2 paracoccidioidomycosis, 2 chromomycosis and one sporotrichosis had positive Montenegro skin tests (equal to/or larger than 10 mm).

IgA-ELISA results are shown in Fig. 1. As seen, 96.0% of values found among normal controls and 82.4% among the mycoses were below or equal to titer 3. In all, 89.2% of sera had values equal to or below titer 3. Therefore, titer 3 was considered as the cutoff between normal and abnormal values. Among the leishmaniasis sera 32/65 or 49.2% had values equal to or below titers 3. Among the remaining sera, 10 had values equal to or higher than 72.1 and 23 had titers between 3.1 and 72.

IgA-ELISA among the mycoses was not related to skin test positivity but rather to disease severity. None of the 6 false positive results had a positive skin test but there was lung or widespread mucosal involvement in 3 cases of paracoccidioidomycosis, lymph-node involvement in one of chromomycosis and one of sporotrichosis. The remaining false positive sera belonged to a chromomycosis patient. Five other patients had skin tests equal to 10 mm but normal IgA-ELISA titers.

In the normal control group the positive skin test had an IgA-ELISA titer of 1.6. The serum with an increased IgA-ELISA titer was from a negative skin test individual (Fig. 1).

IgA-ELISA showed a sensitivity of 0.5625 (among the first bleedings), specificity of 0.8703 and a positive predictive values of 0.72.

Table I shows IgA-ELISA titers in 13 patients bled 2 or more times. IgA-ELISA titers in untreated patients reached a maximum during the first and second year of infection. Following treatment for 6 of 13 patients there was a rise in IgA titers.

IgA-IF — Seven sera gave positive IgA-IF tests at a 1/10 dilution. Two sera were from mycoses which had normal IgA-ELISA titers and one an IgA-ELISA titer of 15. None were skin test positives. Among the positive leishmaniasis sera, 3 were from serial bleedings (patients MRS, AFS, VZ in Table I, first column). The fourth positive had an ELISA titer of 5. IgA-IF showed a specificity of 0.94.

The IgA-IF positive sera were from patients with length of infection ranging from 7 months to 1 year.

IgA-ELISA — PRECISE SERUM TITER ASSESSMENT

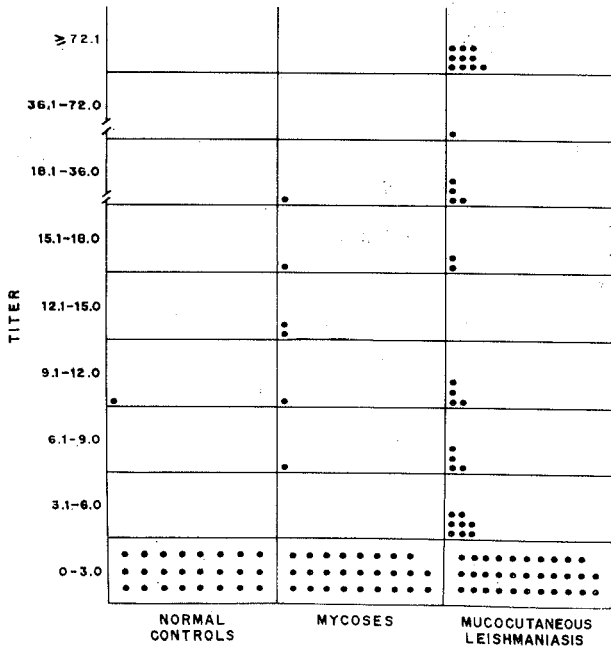


Fig. 1 — IgA-ELISA precise serum titers in 25 normal control sera, 32 mycoses and 65 mucocutaneous leishmaniasis

T A B L E I
 IgA-ELISA titer in mucocutaneous leishmaniasis patients bled 2 or more times

Initials	Infection length	Skin test diameter in mm	Bledings			
			1st	2nd	3rd	4th
MTZ	2 months	25	<1	<1	<1	<1
SMP	7 months	10	3	<1	<1	n.d.
VZ	7 months	15	3	8	2	n.d.
AFS	1 year	10	>2x10 ⁶	>2x10 ⁶	699	n.d.
MRS	1 year	10	151	97	64	47x10 ³
JFS	2 years	13	4.9	39x10 ³	n.d.	n.d.
RG	2 years	20	17	<1.6	20	n.d.
TOO	3 years	20	12	5	10	22
BCS	4 years	20	16	9.8	n.d.	n.d.
			<1.6(*)	12(*)	36(*)	<1.6(*)
IS	10 years	10	<1	<1	<1	<1
JOM	13 years	20	145	223	<1.6	n.d.
			nd	<1.6(*)	<1.6(*)	n.d.
FAM	15 years	20	1.6	2.7	6.5	1
MJN	20 years	20	3.25	3.25	3.25	6.5

n.d. = not done

(*) — second therapeutic series

RESUMO

Ensaio Imunoenzimático de IgA Anti-Leishmãnia na Leishmaniose mucocutânea

(Nota Prévia)

Os Autores descrevem a reação de IgA ELISA na leishmaniose mucocutânea. Títulos acima do normal foram encontrados no primeiro e segundo anos de infecção e em soros de micoses profundas acompanhados por envolvimento mucoso ou doença disseminada.

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Recebido para publicação em 2/7/1984.

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