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FLUORESCENT ANTIBODY TEST IN VISCERAL LEISHMANIASIS

II — Studies on the specificity of the test

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SUMMARY

Six muco-cutaneous leishmaniasis, 7 Kala-Azar, 5 Chagas' disease, and 6 pulmonary tuberculosis serum samples were tested against antigens prepared from cultures of *Leishmania donovani* and *Leishmania braziliensis*, using the indirect fluorescent antibody technique. Two Chagas' disease serum samples cross-reacted with *Leishmania donovani* antigen and one with *Leishmania braziliensis* antigen. Three Kala-Azar serum samples cross-reacted with *Leishmania braziliensis* antigen and one muco-cutaneous leishmaniasis serum sample cross-reacted with *Leishmania donovani* antigen. Cross-reactions were observed only when undiluted or slightly diluted serum was used. All serum samples from patients with pulmonary tuberculosis gave negative results with both antigens. The normal control serum samples never showed any fluorescence.

INTRODUCTION

Numerous observations have been made on the significance of the Fluorescent Antibody test (F. A. test), either for diagnosing the disease caused by the parasitic flagellates or for differentiating their various species. As regards the diseases caused by *Leishmania* and *Trypanosoma* species, numerous reports [1, 2, 3, 4, 7, 9, 11, 13] are available.

WEITZ [12] was able to detect, in spite of cross-reactions, antigenic differences between *Trypanosoma brucei* and *Trypanosoma vivax* blood forms. DUXBURY et al. [3] as well as WILLIAMS et al. [13], after experiments with the indirect F. A. technique, suggested that this test might be very useful, after proper studies, for diagnosing *Leishmania* infections. They used *Leishmania donovani* culture forms as antigen and observed fluorescence when testing sera from patients with cutaneous leishmaniasis and Kala-Azar from the Mediterranean type. SHAW & VOLLER [8], also using the indirect technique, noted cross-reactions between trypanosomes and *Leishmania*. These Authors also reported that Kala-Azar serum gave strongly positive reactions when tested against antigens from leishmanial bodies and leptomonads of *Leishmania donovani*. Antigens from cultural and vertebrate forms of a Panamanian strain of *Leishmania braziliensis* were also stained with Kala-Azar serum, as were blood forms of *Trypanosoma cruzi*.

On Chagas' disease there have appeared several reports in the literature. FIFE & MUSCHEL [4] reported non-specific staining when dry antigen was used. SADUN et al. [6] described good results despite the occurrence of cross-reactions. CAMARGO [2], in an extensive sttudy, reported to have simulta-

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neously used the immunofluorescent technique and the complement fixation test, both with good results. The antigen used was cultural and blood forms of *Trypanosoma cruzi*. The same Author also reported cross-reactions with Kala-Azar and muco-cutaneous leishmaniasis sera. According to him, 10 out of 24 Kala-Azar sera yielded positive reactions, although only in low titers. Besides showing positive F.A. test, all muco-cutaneous leishmaniasis sera also afforded positive complement fixation with *T. cruzi* antigen.

MAYRINK et al. [5] employing culture forms of *Leishmania donovani* as antigen, demonstrated the good sensitivity of the F. A. test in the diagnosis of Kala-Azar, as compared with the complement fixation reaction with heterologous antigen. The F. A. test titer obtained by these Authors show that the circulating antibody level in Kala-Azar is very high. In cutaneous leishmaniasis the circulating antibody level is very low, as demonstrated by DUXBURY et al. [3] in infections caused by *Leishmania tropica* and by ODDO & CASCIO [6] when comparing the titers in both visceral and cutaneous leishmaniasis.

The purpose of the present work is to test sera from people known to suffer from muco-cutaneous leishmaniasis, Kala-Azar, and Chagas' disease, using antigens from culture forms of *Leishmania donovani* and *Leishmania braziliensis* (Brazilian strains).

As an antigen from a strain of *Mycobacterium tuberculosis* (B.C.G.), was successfully used by TORREALBA et al. [10] in complement fixation tests for the diagnosis of Kala-Azar, we thought that sera from patients with pulmonary tuberculosis might present an antibody capable of reacting in the F. A. test, to an antigen from *Leishmania donovani*. Thus we also tested sera from such patients with antigens from *L. donovani* and *L. braziliensis*.

MATERIAL AND METHODS

Sera — The sera listed below have been employed: a) Six samples from patients with Brazilian muco-cutaneous leishmaniasis presenting positive Montenegro test and characteristic lesions; b) Seven samples from patients proved to be parasitized by *Leishmania donovani* through positive bone-marrow smear and positive complement fixation; c) Five samples from Chagas' disease patients showing either positive complement fixation test or positive xenodiagnostic; d) Six samples from patients with pulmonary tuberculosis showing *Mycobacterium tuberculosis* in sputum. e) Seven control samples from people known never to have been parasitized or exposed to Kala-Azar, muco-cutaneous leishmaniasis, or Chagas' disease.

Antigens — The antigens were prepared from *Leishmania donovani* and *Leishmania braziliensis* leptomonads grown in semi-solid medium at 22°C. The smears were prepared and stored by the technique established in a previous paper [5], with some slight modifications.

Technique of the test — The indirect fluorescent antibody test was performed following the same procedure described in a previous paper [5]. The antigen was treated with the serum and with the anti-human labelled globulin for 60 minutes instead of 30 minutes as already established [5].

Microscopical examination — Fluorescent microscopy was accomplished by using a ZEISS GF45 microscope assembled with an OSRAM HBO200 ultra violet lamp. As exciter filters we used BG 12 and UG 5 and, as barrier filters 53 and 44 ZEISS.

RESULTS

Tables I, II, and III show the results from Kala-Azar, muco-cutaneous leishmaniasis, and Chagas' disease serum samples tested with *L. braziliensis* and *L. donovani* leptomonad antigens.

Sera from patients with pulmonary tuberculosis tested with the same antigens always presented negative results. Sera from normal individuals, used as controls, never afforded any fluorescence when tested with both antigens.

The fluorescent antibody was found to fix mainly on the leptomonad membrane and flagellum. The blepharoplast was also stained and sometimes showed very bright

TABLE I

Results of F.A. test in sera from patients with Kala-Azar and using leptomonad antigen from *L. donovani* and *L. braziliensis* cultures

no. patients	C'. F. test for Kala-Azar	*L. braziliensis* antigen	*L. donovani* antigen
1	1:5,120 *	1:10 *	1:6,400 *
2	1:80	—	1:800
3	1:40	und.	1:800
4	1:2,560	—	1:800
5	1:160	und.	1:400
6	1:160	—	1:1,600
7	1:2,560	—	1:200

* = Highest positive dilution
und. = Positive undiluted
— = Negative

TABLE II

Results of the F.A. test in patients with muco-cutaneous leishmaniasis using leptomonad antigens from *L. donovani* and *L. braziliensis*

no. patients	Montenegro test	*L. braziliensis* antigen	*L. donovani* antigen
1	++++	1:100 *	—
2	++++	1:20	—
3	++++	1:20	und.
4	+++	1:100	—
5	+++	1:10	—
6	++++	1:40	—

* = Highest positive dilution
und. = Positive undiluted
\+ = Positive
— = Negative

TABLE III

Results of the F.A. test in patients with Chagas' disease, using leptomonad antigen from *L. donovani* and *L. braziliensis*

no. patients	Chagas' disease C'. F. test	*L. braziliensis* antigen	*L. donovani* antigen
1	+	—	—
2	+	und.	und.
3	+	—	—
4	+	—	—
5	+	—	—
6	+	—	1:10 *

* = Highest positive dilution
und. = Positive undiluted
— = Negative
\+ = Positive

fluorescence. The nucleous did not stain, but it could be visualized. The negative reaction did not demonstrate any fluorescence, although the leptomonads could be identified as a very pale yellowish shadow. Smears treated with saline and smears not submitted to any treatment always afforded a picture simulating negative reaction.

COMMENTS

As already pointed out for infections caused by *Leishmania tropica* and *Leishmania braziliensis* (Panamanian strain), the circulating antibody level in muco-cutaneous leishmaniasis is also very low. This can be explained by the specific localization of the parasite and its antigenic capacity.

Our results are not quite in accordance with those reported by other Authors. From seven Kala-Azar samples, 3 reacted with *L. braziliensis* antigen, 2 of them when tested undiluted and the remaining one, at a very low dilution, although showing a very high titer when tested with the specific antigen. Sera from muco-cutaneous patients, when diluted, did not give cross-reactions with *L. donovani* antigen.

The amount of non-specific antibodies in undiluted serum must be such as to elicit cross-reactions, as already observed when Chagas' disease sera were tested against *L. donovani* and *L. braziliensis* antigen.

As all sera were inactivated prior to each test the complement cannot be held responsible for the fact. Sera from patients with pulmonary tuberculosis did not afford any positive reaction, even when tested undiluted. This indicates that *L. donovani* is capable of inducing an antibody which reacts with *M. tuberculosis* antigen, whereas the latter organism is not able to induce an antibody that can react with *L. donovani* and *L. braziliensis* antigens.

To account for the results obtained, we could say that *L. donovani*, causing American Kala-Azar, and *L. braziliensis*, causing muco-cutaneous lesions, must be more differentiated antigenically in order to induce more specific antibodies, thus preventing cross-reactions between themselves and other flagellates, as for instance *T. cruzi*, when diluted serum is used.

RESUMO

Teste de imunofluorescência em leishmaniose visceral

II — *Especificidade do teste*

Seis amostras de sôro de indivíduos com leishmaniose muco-cutânea, 7 com Calazar, 5 com doença de Chagas e 6 com tuberculose pulmonar foram testadas, através da reação de imunofluorescência, com antígenos preparados de culturas de *Leishmania donovani* e *Leishmania braziliensis*.

Observaram-se reações cruzadas sòmente quando o sôro era testado não diluido ou em muito baixa diluição.

Todos os soros de indivíduos com tuberculose pulmonar apresentaram teste negativo. Sete amostras provenientes de indivíduos normais e utilizadas como contrôles apresentaram sempre resultado negativo.

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