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FLUORESCENT ANTIBODY TEST IN VISCERAL LEISHMANIASIS

I — Sensitivity of the test

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SUMMARY

Eighteen sera from patients proved to be parasitized by *Leishmania donovani*, were tested by the indirect fluorescent antibody test using a leptomonad antigen. In 14 sera (77.7 per cent) the F.A. test titers were found to be higher than the C'.F. test titers, which leads us to think that the former test is the most sensitive.

The normal controls have never shown fluorescence.

INTRODUCTION

The serology of visceral leishmaniasis is based mainly on complement fixation test using a heterologous antigen [2, 5].

Several kinds of antigens have been proposed for the fluorescent antibody techniques (F.A. test) in the diagnosis of the American Kala-Azar. The leptomonad forms from *Leishmania donovani* cultures are more frequently used as antigen [3, 6] and macrophage containing intracellular forms of the parasite have also been reported as antigens for the F.A. test in the detection of antibodies to visceral leishmaniasis [4].

The results reported by the Authors vary from non-specific staining [6] to good specificity of the test [3].

On the sensitivity of the reaction we were unable to find detailed reports in literature. Thus we decided to conduct these experiments comparing the results of the F.A. test against other serological and direct evidences of parasitism by *Leishmania donovani*.

MATERIAL AND METHODS

Sera — Eighteen sera from patients known to be parasitized by *L. donovani* through complement fixation (C'.F) test, examination and culture of aspirates of sternal bone marrow, were tested by the indirect fluorescent antibody test for visceral leishmaniasis. The sera were kept at —20°C and were inactivated at 56°C for 30 minutes just before testing. Each sample was diluted from 1:10 to 1:12,800 in normal saline. Normal control sera were obtained from people known not to have undergone actual parasitism or previous exposition to *L. donovani* infection.

Antigen — Leptomonad forms were obtained from *L. donovani* cultures grown in semi-solid medium at 22°C temperature. They were washed 3 times in phosphate buffered saline (PBS) pH 7.2, by centrifugation at 1,500 rpm, 10 minutes.

A small drop of the final sediment was put on each microscope slide and a smear

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was prepared so as to show 100 leptomonad forms for each 10 X microscopic field, approximately. The smears were then dried at room temperature with help of a small fan.

On each slide, 4 circles, 8 mm in diameter, were drawn with a red enamel paint. This was made in order to allow the proper individualization and the easy identification of the different serum dilutions tested. The smears were then stored at 4°C, for no longer than 15 days. We did not use any fixative on the smears.

A heterologous antigen [2] was used for the complement fixation tests, which were performed according to Kolmer's technique.

The cultures were made from aspirates of sternal bone marrow in semi-solid medium and at 22°C of temperature.

Direct examination was also performed using Giemsa stained samples of the material collected for cultures.

Technical procedures — In general we followed the steps listed below, as proposed by some Authors [1].

1) A drop of each serum dilution is put into one of the enamel-limited portions of the smear, and the preparation kept at 37°C for 30 minutes in a humid chamber.

2) The smears are submitted to two 5 minutes baths in phosphate buffered saline pH 7.2, and subsequently dried up at room temperature.

3) Labelled anti-human globulin diluted in bovine serum albumin-saline, pH 7.1, is then applied following the same techniques described in step 1. The anti-human globulin employed was labelled with fluorescein-isothiocyanate in our laboratory, and when titrated, it was found to be effective at a dilution of 1:10.

4) Washing and drying anew, as in step 2.

5) Mounting with buffered saline plus glycerin pH 7.1.

Microscopical examination — Fluorescent microscopy was accomplished with a ZEISS GF 425 microscope assembled with an OSRAM HBO 200 air-cooled ultraviolet lamp. As exciter filters we used BG 12 and UG 5 and, as barrier filters, numbers 53 and 44 ZEISS.

RESULTS

The fluorescent antibody was found to deposit mainly around the leptomonads and on the flagellum. Many times the blepharoplast was also stained but the nucleus was very seldom stained. At the highest dilutions we could observe only small fluorescent spots on the cellular membrane. This was considered as an indication of positive reaction, since the normal controls never showed any fluorescence at all.

The results of the direct examination and culturing of bone marrow samples are shown in Table I as well as the titers of the C'.F. and F.A. tests.

Two cultures were contaminated but the corresponding bone marrow sample was positive for *Leishmania donovani*. Two sera were negative by the C'.F. test and positive by F.A. test, one of them showing a very high titer.

COMMENTS

Fluorescent antibody titers were generally higher than those observed with the complement fixation test. This second test is generally performed using a heterologous antigen, thus the antibodies detected are quite non-specific.

On the other hand, considering the better results attained in the present experiments in comparison to those afforded by C'.F. tests, we think that the antibodies detected by F.A. test are highly specific and of a different nature.

Only four sera have shown a lower F.A. test titer than the C'.F. test. After the C'.F. testing, these sera were stored at —20°C for different periods of time and this probably contributed to decrease the titer of the specific antibodies in these sera.

According to the results obtained, we think that the sensitivity of the F.A. test for the diagnosis of Kala-Azar is very good, and better than that of the C'.F. test.

TABLE I

Results of direct and serological examinations in 18 patients with American Kala-Azar

Patient	*Leishmania donovani* in Bone marrow	Culture	C'F test	FA test
G.A.M.	+	+	1:5,120	1:6,400
M.E.	+	+	1:80	1:800
J.A.	+	+	1:160	1:1,600
V.P.	+	+	1:80	1:800
A.A.	+	+	1:1,280	1:1,600
F.D.	+	+	1:160	1:400
S.L.G.	+	+	1:40	1:800
J.J.	+	+	1:640	1:800
A.F.J.	+	*	1:1,280	1:1,600
S.B.A.	+	+	1:40	1:200
M.J.	+	+	1:2,560	1:200
J.S.A.	+	*	—	1:1,600
S.M.J.	+	+	1:320	1:1,600
N.R.P.	+	+	1:2,560	1:800
B.A.	+	+	—	1:40
C.M.	+	+	1:1,280	1:400
G.J.	+	+	1:320	1:400
S.J.	+	+	1:2,560	1:400
6 normal controls	—	—	—	—

* = contaminated culture
— = negative
C'F = complement fixation test
FA = fluorescent antibody test

RESUMO

Teste de imuno-fluorescência na leishmaniose visceral. I — Sensibilidade do teste

Dezoito soros de indivíduos comprovadamente parasitados por *Leishmania donovani* foram testados através da reação de imuno-fluorescência, usando-se como antígeno uma suspensão de leptomonas cultivadas em meio semi-sólido. Os títulos obtidos foram geralmente mais elevados que os observados através da reação de fixação do complemento realizada com antígeno heterólogo.

Os Autores são de opinião que a reação de imuno-fluorescência é mais sensível que a de fixação do complemento no diagnóstico do Calazar americano.

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