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A COMPARISON OF A NEW ANTIGEN FROM AMASTIGOTES OF *TRYPANOSOMA CRUZI* AND AN ANTIGEN FROM EPIMASTIGOTES FOR THE DIAGNOSIS OF CHAGAS' DISEASE BY THE INDIRECT IMMUNOFLUORESCENCE TEST

J. A. CERISOLA [1], M. ALVAREZ [1], M. BOCK [2] and D. WEGNER [2]

SUMMARY

A new antigen from amastigotes of *T. cruzi* was compared with the antigen from the epimastigotes. Sera of patients with and without Chagas' disease were tested by the indirect fluorescent antibody method. The antigen from amastigotes yielded titers up to 10 times higher in positive sera without showing a higher degree of nonspecificity in negative sera. Insofar as we can judge at the present time, the new amastigote antigen, therefore, seems to be superior to the antigen obtained from epimastigotes.

INTRODUCTION

Since the first description of the indirect fluorescent antibody test (IFAT) for the diagnosis of Chagas' disease by FIFE & MUSCHEL [5], numerous modifications of the method have been described. VOLLER [8] obtained good results with an antigen from *Trypanosoma cruzi* culture forms which were fixed on slides. BIAGI et al. [2] employed amastigotes from the myocardium of mice infected with *T. cruzi* as antigen. The results obtained by this technique were in a striking agreement with the results of the complement fixation reaction (CFR) in a total of 236 sera tested. GIROLA et al. [6] reported on a slide method for the IFAT. They used cultured organisms as antigen. Approximately 96% of the results agreed with the results of the CFR performed simultaneously. CAMARGO [3], in an excellent paper on the immunofluorescence test for Chagas' disease, described the slide method with the use of formalized epimastigotes. By this technique, he demonstrated complete correlation between IFAT and CFR in 494 nonreactive and 48 reactive sera. ALVAREZ et al. [1] also reported on the IFA slide test. They used cultured epimastigotes and compared the results with those obtained in 1,089 sera which were tested simultaneously by the CFR and the hemagglutination reaction (HAR). The Authors came to the conclusion that in the chronic phase of trypanosomiasis the IFAT shows a sensitivity similar to that of the HAR and somewhat higher than that of the CFR. In the acute phase, the CFR becomes positive first and, therefore, surpasses the other methods in sensitivity. CERISOLA et al. [4], who performed the IFAT with epimastigote antigen, concluded that IFAT represents the earliest immunodiagnostic evidence in acute cases of Chagas' disease. They obtained positive results in 100% of the chronic cases studied.

In the present paper, we are reporting results which were obtained in comparative studies with a new antigen prepared from

(1) Instituto de Diagnóstico e Investigación de la Enfermedad de Chagas "Dr. Mario Fatala Chaben", Buenos Aires, Argentina
(2) Pharmazeutisches Forschungszentrum, Farbenfabriken Bayer AG, Wuppertal-Elberfeld, Germany

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amastigotes (*Leishmania* forms) of *T. cruzi* and with the antigen from epimastigotes (*Crithidia* forms) employed heretofore.

MATERIAL

The studies were carried out according to a method described in a previous paper (ALVAREZ et al.[1]). The antigens were prepared as follows:

1) *Antigen from epimastigotes* — Trypanosomes cultured in blood-glucose agar were incubated for intervals of 2 to 4 days. The parasites were then removed from the culture vessels and suspended in a physiological phosphate buffer solution (phosphate content 0.01 M; pH 7.2 ± 0.1) with 1% of formalin added. The sediment was twice washed by centrifugation with buffer solution and subsequently suspended in buffer solution with 1% of formalin added.

This antigen stock suspension can be stored at 4°C for periods up to 3 months. To prepare the antigen for use, the antigen stock suspension is diluted with phosphate buffer solution until a concentration of 10 to 15 parasites per visual field (objective 40 ×; eyepiece 10 ×) is attained.

No obvious differences were seen among antigens from trypanosome strains of different origins.

2) *Antigen from amastigotes* — One-day-old HeLa cell cultures grown in 200 ml Erlenmeyer flasks are infected with about 10 million trypanosomes from tissue cultures, and on both the first and third day post-infection, the cells are washed once with phosphate buffer solution and supplied with fresh culture medium (lactalbumin hydrolyzate + vitamin mixture + addition of 15% calf serum). On the 4th day after infection, the infected cell mat is washed 3 times with phosphate buffer solution, then suspended with a sterile rubber swab, and centrifuged. The sediment in once again washed with phosphate buffer solution 3 times, after which it is suspended in culture medium (1-ml flask) and transferred into vials which are slowly cooled and preserved at − 7°C.

Subsequent processing and preparation of the amastigote antigen is similar to the procedure used with the epimastigote antigen. Measured amounts (0.03 ml) of the epimastigote and amastigote antigens are transferred to perfectly clean slides on which two circles of 10 mm diameter each have previously been drawn with a diamond pen. Each slide contains one field of each type

TABLE I

Sera of patients in chronic phase of Chagas' disease

Serum no.	Patient	Epimastigote antigen	Amastigote antigen
1	9411	1/240	1/3840
2	9412	1/480	1/3240
3	9413	1/960	1/7680
4	9414	1/480	1/7680
5	9415	1/480	1/7680
6	9416	1/3840	1/7680
7	9417	1/240	1/3840
8	9418	1/240	1/3840
9	9419	1/60	1/3840
10	9420	1/120	1/1920
11	9421	1/240	1/1920
12	9422	1/120	1/1920
13	9423	1/480	1/3840
14	9424	1/480	1/7680
15	9425	1/480	1/3840
16	9426	1/240	1/7680
17	9427	1/480	1/3840
18	9428	1/480	1/7680
19	9429	1/960	1/7680
20	9430	1/240	1/7680
21	9431	1/240	1/3840
22	9432	1/1920	1/7680
23	9433	1/960	1/7680
24	9434	1/240	1/7680

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TABLE

Sera from non-endemic

	Normal				Syphilis			
	Ag. Am. *		Ag. Ep. **		Ag. Am.		Ag. Ep.	
	no.	%	no.	%	no.	%	no.	%
Negative	112	93.3	109	90.8	18	81.4	22	100
1:5	5	4.2	11	9.2	1	4.5	0	
1:10	3	2.5	0		3	15.1	0	
1:15	0		0		0		0	
1:20	0		0		0		0	
Total	120	100	120	100	22	100	22	100

* Antigen of Amastigote of *T. cruzi*
** Antigen of Epimastigote of *T. cruzi*

of organism with the material distributed evenly over the circular areas. These slides are allowed to dry at room temperature, and evaporation is promoted by means of a mild stream of warm air produced by a fan. This is sufficient for fixation. The results are more favorable with this method than with the use of acetone or methanol as a fixative. The antigen so prepared can be stored for months at − 20°C or at 4°C in a desiccator.

PROCEDURE

The patient's serum was added simultaneously to both antigens at a dilution of 1:5. If the result was positive, the serum was further diluted until the limiting titer was determined. All reactions were run in duplicate and evaluated blind.

The following groups were tested:

A) Sera of 24 patients in the chronic phase of Chagas' disease. In the patients the presence of infection was established by the demonstration of *T. cruzi* in the peripheral blood with the aid of xenodiagnosis. This group served for evaluating the sensitivity of the two antigens;

B) Sera of 120 healthy individuals and 70 patients with various infections. Other than Chagas. These sera originated from non-endemic regions. This group was used to determine the specificity of the antigens or to determine titers in non-specific reactions.

RESULTS AND COMMENTS

The titers obtained with the sera of patients with chronic Chagas' disease are presented in Table I. As is evident from the data, the amastigote antigen proved to be significantly superior in the tests of all 24 sera of patients with Chagas' disease. The titers obtained with this antigen were several dilutions higher than those obtained with the epimastigote antigen.

II

regions

Toxoplasmosis				Amebiasis				Total			
Ag. Am.		Ag. Ep.		Ag. Am.		Ag. Ep.		Ag. Am.		Ag. Ep.	
no.	%	no.	%	no.	%	no.	%	no.	%	no.	%
20	80.0	18	72.0	15	65.2	16	49.6	165	86.8	164	86.3
1	4.0	3	12.0	4	17.4	4	17.4	11	5.8	17	8.9
2	8.0	2	8.0	2	8.7	2	8.7	10	5.3	4	2.1
2	8.0	2	8.0	1	4.3	1	4.3	3	1.6	3	1.6
0		0		1	4.3	0		1	0.5	0	
25	100	25	100	23	100	23	100	190	100	190	100

One reason for this finding could perhaps be a higher antigen content of the *Leishmania* form as compared with the *Crithidia* form. This assumption, however, would need to be substantiated by further studies.

The sera of Group B (Table II) (*) were diluted 1:5, 1:10, 1:15, 1:20, 1:25 and 1:30. Sera negative at the initial dilution of 1:5 were not tested further. Sera with fluorescence at a titer of 1:5 were diluted further to determine the titer.

In a few sera, nonspecific reactions were observed with both antigens at the lowest dilutions (1:5, 1:10). At a 1:20 dilution, only one serum tested with epimastigote antigen proved positive (0.5%).

Until further studies provide evidence to the contrary, we may infer from these results that fluorescent antibody titers higher than 1:30 with either antigen are specific and thus prove the existence of a *T. cruzi* infection,

Inasmuch as the antigen from amastigotes of *T. cruzi* reacts with high titers and does not cause nonspecific reactions more frequently than the antigen from epimastigotes, employing an amastigote antigen decidedly improves the usefulness of the immunofluorescence test in the diagnosis of Chagas' disease.

Studies aimed at determining the sensitivity of the amastigote antigen to detect antibody in acute infections are not yet concluded. The antigenic structures of the different stages of development of *T. cruzi* and the question of possible differences between different strains of *T. cruzi* remain to be investigated.

(*) The sera of Group B were made available through the courtesy of Dr. Irving G. Kagan (Center for Disease Control, Atlanta) and Mr. Earl Fife (Walter Reed Institute of Research, Washington)

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RESUMEN

Un nuevo antígeno de amastigotes de Trypanosoma cruzi *para el diagnóstico de la enfermedad de Chagas por el test de inmunofluorescencia (estudiado por comparación con antígeno de formas epimastigotes)*

Se comparó un nuevo antígeno de formas amastigotes de *Trypanosoma cruzi* obtenido de cultivo de células con un antígeno de formas epimastigotes obtenido en medio difásico. Se estudiaron por la técnica indirecta de anticuerpos fluorescentes sueros de pacientes chagásicos y nó chagásicos. El antígeno de formas amastigotes mostró títulos superiores hasta 10 veces en los sueros positivos comparados con los obtenidos con las formas epimastigotes. El antígeno de formas amastigotes mostró una inespecificidad similar en los sueros negativos. Por este estudio preliminar aparentemente las formas amastigotes son superiores a las formas epimastigotes en la reacción de inmunofluorescencia por una mayor concentración de antígenos específicos, lo que tendría que ser aclarado en posteriores estudios.

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