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IMMUNOLOGY OF CHAGAS' DISEASE

III — Absorption of human chagasic sera with epimastigotes of different strains of Trypanosoma cruzi

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SUMMARY

Differences in the antigenic structure of the cell surface have been shown to occur among epimastigotes of three strains of **Trypanosoma cruzi,** using the indirect immunofluorescent (IFA) test for absorbed human chagasic serum. Strain Y appears to be antigenically more complex than strains MR and F1. Strain F1 always yielded lower titers in the immunofluorescent test even when tested against unabsorbed serum. This suggests that care should be taken in choosing the adequate strain as antigen for the immunofluorescent test. The best procedure is to use a pool of several strains.

INTRODUCTION

Antigenic differences among epimastigotes or other evolutive forms of **Trypanosoma cruzi** as well, have been demonstrated by various methods [3,4,6,7,8]. The significance of these differences is not clear yet. In regard to antigenic typing, the antigenic variations noted among epimastigotes of several strains apparently do not depend on the host species or on geographic distribution. The present work was undertaken to demonstrate possible variations in titer of the immunofluorescent test for diagnosis of Chagas'disease when human chagasic serum is absorbed with epimastigotes of different strains of **T. cruzi.** These variations could be important in the proper selection of strains to be currently used in the test.

MATERIAL AND METHODS

Sera — Eight samples from humans exhibiting positive xenodiagnosis were employed. All sera had positive titers for Chagas' disease in the complement fixation, immunofluorescent and hemagglutination tests.

Epimastigotes — Strains Y, MR and Fl of **T. cruzi** were cultured in liquid medium at 28°C 1. One week cultures were employed; only epimastigotes were present in the cultures as demonstrated by microscopic examination of several fresh and stained smears.

Absorption procedure — Cultures were washed three times with phosphate buffered saline, pH 7.2 (PBS), and 0.1 ml of the packed organisms was resuspended in 0.3 ml of the serum to be absorbed. The suspension was kept for 60 minutes at room temperature and overnight at 4°C; it was then centrifuged at 10,000 g for 30 minutes in a refrigerated centrifuge. The supernatant was carefully removed and tested by indirect immunofluorescent test (IFA) for cross — and homologous reactivity. Only one absorbing procedure was performed, since this was shown to yield negative results with the homologous antigen.

IFA — The method described by CAMARGO [2] was followed. The anti-human IgG specific conjugate (Hyland Labs., Costa Mesa, Calif. USA) had a titer of 1:300. Its initial dilution to test unabsorbed serum was 1:32. Absorb-

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ed serum was tested undiluted and at two-fold dilution. The fluorescent microscope was a Zeiss Jena with a HB0200 mercury lamp and BG12 and OG1 filters.

RESULTS

The results are shown in Table I. Absorption with the homologous antigen removed all antibodies reacting in the IF test. In general, a significant drop in titer was noted in all absorbed sera. Absorption with antigen Y removed completely all antibodies against antigens MR and F1 in seven out of eight sera. Absorption with antigen F1 did not remove all antibodies reacting against antigens Y and MR in any of the sera, whereas absorption with antigen MR removed all antibodies against antigen F1 in all sera but not in those reacting against anti-antigen Y. IF titers of unabsorbed sera tested against antigen prepared with strain F1 were, in all sera, lower than the titers obtained with antigen prepared with epimastigotes of strains Y and MR.

TABLE I

Absorption of chagasic sera with culture forms of different strains of **Trypanosoma cruzi**

Serum	Absorption with strain	IFA titers (*) with strains		
		MR	F1	Y
2180	—	128	32	128
	MR	0 (i)	0	4
	F1	4	0	4
	Y	0	0	0
2193	—	256	128	256
	MR	0	0	8
	F1	8	0	4
	Y	8	8	0
2206	—	128	32	64
	MR	0	0	16
	F1	4	0	4
	Y	0	0	0
2211	—	64	32	64
	MR	0	0	2
	F1	2	0	4
	Y	0	0	0
2222	—	64	32	64
	MR	0	0	4
	F1	4	0	4
	Y	0	0	0
2202	—	128	64	128
	MR	0	0	8
	F1	4	0	16
	Y	0	0	0
2220	—	256	64	128
	MR	0	0	4
	F1	8	0	16
	Y	0	0	0
2223	—	256	64	256
	MR	0	0	8
	F1	8	0	16
	Y	0	0	0

(*) Reciprocal of titers
(i) Negative undiluted

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DISCUSSION

Incubation of blood trypomastigotes of different strains of **T. cruzi** with immune sera has suggested that antigenic variations occur in the structure of the cell membrane of these forms [5]. The results reported in this paper show that differences in the antigenic structure of the cell membrane can be demonstrated among epimastigotes of strains Y, MR and F1 of **T. cruzi** by IFA tests. Among the three strains tested, strain Y appears to be the most complex with regard to superficial antigenic composition. This is suggested by the fact that absorption of sera with strain Y antigen was able to thoroughly remove all the antibodies able to combine with superficial antigenic determinants of strains MR and F1 epimastigotes. One serum, however, showed IFA positive titers against antigen MR and F1 after absorption with "Y" epimastigotes. The explanation for this phenomenon is not clear. The fact that we used sera of naturally infected humans which may have been infected with **T. cruzi** strains other than those used to absorb the sera could perhaps explain it. Among the three strains tested, strain F1 appears to have fever common superficial antigenic determinants than strains Y and MR. Strain MR lies in between, since the MR-absorptions completely removed all antibodies against F1, but not against Y strain. The antigenic deficiency of strain F1 was also patent when unabsorbed sera were tested against its epimastigotes. In this case, IF titers were consistently lower than those obtained when the same sera were tested against Y and MR antigens. This last observation suggests that care should be taken to choose the adequate strain of **T. cruzi** when preparing antigens for the routine diagnosis of Chagas' disease by IFA. As a matter of fact, a pool of epimastigotes of several strains of **T. cruzi** should be standardized for such tests in order to overcome the variables described.

RESUMO

Imunologia da doença de Chagas. III — Absorção de soros humanos de chagásicos com epimastigotas de diferentes cepas de Trypanosoma cruzi

A absorção de soro de chagásicos com formas de cultura das cepas Y, MR e F1 de **T. cruzi** mostrou diferenças antigênicas entre as cepas, evidenciáveis através do teste de imunofluorescência indireta. A cepa antigenicamente mais complexa parece ser a Y, seguida pela MR. Das três cepas testadas, a F1 parece ser a mais pobre em antígenos de membrana passíveis de evidenciação sorológica. Em soros não absorvidos, os títulos foram sempre mais baixos quando a reação foi realizada com antígeno F1. Os resultados indicam que há necessidade de se escolher adequadamente a cepa de **T. cruzi** quando do preparo de antígeno para o teste de IF para o diagnóstico sorológico da Doença de Chagas, ou de se adotar mistura de cepas para esse fim.

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