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PATTERN OF CLASS-SPECIFIC FLUORESCENT ANTIBODIES ACCORDING TO THE STAGE OF THE INFECTION IN HUMAN SCHISTOSOMIASIS MANSONI

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SUMMARY

The possible association of class specific antibodies to **S. mansoni** and stage of infection was studied by indirect fluorescent antibody test (IFT) in twenty-five selected sera from patients with acute and chronic schistosomiasis mansoni. IgA antibodies were detected only in sera from acute cases, but IgG, IgM and IgE antibodies were found in acute and chronic cases. Rheumatoid factor eventually present in the sera was removed by adsorbing the sera with immunoadsorbent of IgG, before the IFT was performed. In the acute stage the hemagglutination test (HAT) showed to be less sensitive than IFT.

INTRODUCTION

Immunofluorescence, hemagglutination, complement fixation and immunoprecipitation tests can detect anti-**S. mansoni** antibodies and are a very useful tool for the diagnosis of the disease. However, the correlation between serological findings and the stage of the infection has not definitely been stablished.

The levels of different immunoglobulin classes in the sera of patients with schistosomiasis mansoni were studied by single radial immunodiffusion test [1,3,5,7,10,18]. However, as far as the clinical forms of the disease are concerned the results obtained by those Authors do not agree. Therefore, it might be more useful to determine the class-specific antibodies to **S. mansoni** instead of studying the total amount of immunoglobulin classes by radial immunodiffusion.

Recently, the immunofluorescence test carried out with class-specific conjugates to immunoglobulins detected IgG, IgM, IgA and IgE antibodies to **S. mansoni** in sera of infected patients [9,15,16], but these findings were not related to the different stages of the disease.

This report shows the results of a study based on class-specific fluorescent anti-**S. mansoni** antibodies in patients with different stages of the infection, clinically characterized as acute and chronic (intestinal, hepatointestinal and hepatosplenic) forms. Indirect hemagglutination test was also performed.

MATERIAL AND METHODS

Sera — They were collected from 25 schistosomotic patients, with the following clinical diagnosis [19]:

Acute form: 5 patients

Chronic form: 20 patients (intestinal: 6, hepatointestinal: 5, hepatosplenic: 9 patients).

Conjugates — Commercially available conjugates to human IgG, IgM, IgA and IgE (Hyland Div. Travenol Lab. U.S.A.) were used after testing their specificity by immunoelectrophoresis against selected sera with high level of immunoglobulins as determined by radial immunodiffusion test. In order to test the specificity of anti-IgE conjugate, one serum was lyophilized and concentrated 3 times.

Indirect fluorescent antibody test (IFT) — Two **S. mansoni** antigens were prepared for cryostat sectioning as follows:

1) adult worms were embedded in Tissue-Tek O.C.T. medium (*) and frozen in liquid nitrogen according to WILSON et al. [21].

(*) Ames Co., Miles Lab., U.S.A.

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2) liver specimen with eggs and granulomata obtained from infected hamster was also frozen as described by COUDERT et al. [6].

Sections were cut at 4 μ thickness, fixed on microscope slides and stored at -20°C until used. The slides were then fixed in cold acetone for 5 minutes before the immunofluorescent technique. Serum dilutions from 1/10 to 1/5,120 were added on tissue sections and, after incubating for 30 minutes at 37°C, these were washed for 20 minutes with two changes of PBS (0.15M NaCl; 0.01M phosphates; pH 7.2). An appropriate dilution of conjugate was placed on the specimens, and the slides were again incubated for 30 minutes at 37°C, twice washed in PBS and mounted with glicerol (pH 8.5) and a coverslip. All immunofluorescence tests included both positive and negative controls.

The slides were examined under a Zeiss fluorescence-microscope provided with a HBO-200 bulb, KP-500 exciter filter, Zeiss 50 barrier filter and dark field condenser.

Passive hemagglutination test — For the hemagglutination test a preserved reagent was used according to HOSHINO et al. [13]. This was prepared by lyophilizing formalin treated cells which had been preserved by aldehyde fixation after tannic acid treatment and sensitization with **S. mansoni** extracts. Sera were serially diluted from 1/20 to 1/5,120 in 0.85% NaCl and plastic microtitration plates used for this test.

Rheumatoid factor — A search for rheumatoid factor was performed in all sera by latex test (Behringwerke AG, Germany).

Ten selected sera, in which IgM was detected, were also adsorbed with immunoadsorbent of human IgG[2] and retested by immunofluorescence technique, in order to identify the immunological specificity of IgM.

RESULTS

Monospecific anti-human globulins immunofluorescence tests and hemagglutination test titers obtained in 25 sera from patients with acute and chronic schistosomiasis are presented in Table I. Figure 1 shows the fin-

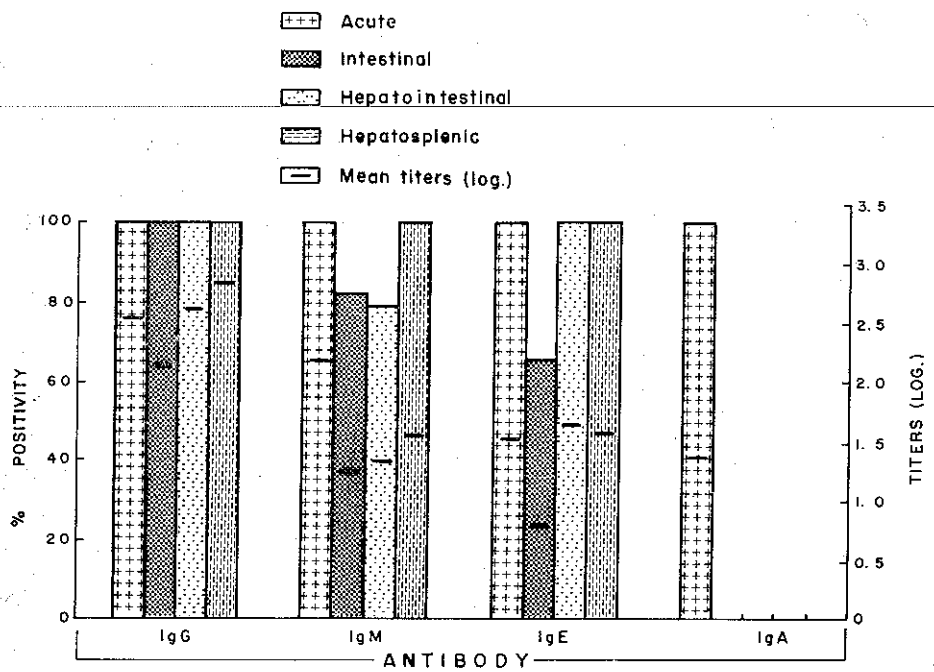

Fig. 1 – Distribution and mean titers of class specific antibodies found in sera from schistosomotic patients according to the clinical form of the disease.

T A B L E I

Titers of 25 sera from patients with acute and chronic schistosomiasis mansoni obtained by immunofluorescence test (IFT), using monospecific conjugates, and by passive hemagglutination test (HAT)

Patient		Clinical forms	I F T								HAT
			IgG Ab		IgM Ab		IgE Ab		IgA Ab		
			Liver (**)	Worm	Liver	Worm	Liver	Worm	Liver	Worm	
1.	CSS	ACUTE	320	320	80	160	20	20	40	20	40
2.	IGO		320	80	40	80	40	(***)	20	—	80
3.	JAL		640	320	320	320	80	80	40	20	80
4.	JRA		640	640	320	640	80	80	40	80	160
5.	WSS		640	320	160	160	80	40	80	40	80
6.	AFN	CHRONIC (*)	320	320	160	80	40	10	—	—	640
7.	ARL		40	80	20	20	—	10	—	—	320
8.	ATS		20	40	10	10	—	—	—	—	80
9.	ESN		640	640	40	40	80	40	—	—	40
10.	FAM		640	320	40	80	20	40	—	—	320
11.	VAS		80	80	—	—	—	—	—	—	40
12.	CRX		640	320	20	40	40	40	—	—	320
13.	DDS		640	320	40	40	40	40	—	—	320
14.	DMJ		320	320	40	40	20	20	—	—	320
15.	MCN		2560	320	80	160	320	80	—	—	640
16.	ZLS		640	80	—	—	40	40	—	—	320
17.	AMD		640	640	20	40	80	40	—	—	160
18.	ASS		320	160	40	20	20	—	—	—	320
19.	EAV		1280	320	20	80	80	40	—	—	320
20.	HVS		2560	1280	80	160	320	80	—	—	2560
21.	JBA		2560	640	20	80	160	160	—	—	640
22.	JJS		640	640	80	160	80	80	—		320
23.	LVS		1280	320	80	40	40	40	—		640
24.	MAS		640	320	20	—	40	20	—	—	1280
25.	MDX		640	640	40	80	20	10	—	—	160

(*) Chronic forms: intestinal (No. 6 to 11)
hepatointestinal (No. 12 to 16)
hepatosplenic (No. 17 to 25)

(**) Liver = liver sections with egg and granulomata
worm = adult worm sections

(***) = negative result

dings of IgG, IgM, IgA and IgE antibodies to **S. mansoni**, according to clinical form of disease and estimated in percentages and mean titers.

Fluorescent antibody to S. mansoni

IgG — All sera gave a positive reaction to adult worm and to egg (liver section) antigens, respectivelly with mean titers (*) 280 and 480 in acute cases, and 290 and 500 in chronic cases.

IgM — 17 Out of 20 sera from chronic cases gave a positive reaction to adult worm and egg antigens, and both mean titers were 30. Two sera gave negative results to worm and egg antigens and one, showed positive result only to egg antigen.

All 5 sera from acute cases were positive to IgM antibodies, and the titers tended to be higher than in chronic cases. The mean titers 210 and 140 were respectively obtained with worm and egg antigens.

IgE — Most sera (23) gave a positive anti-IgE fluorescent reaction. However, in 3 sera,

(*) mean titers = geometric mean titers

2 from chronic and one from acute schistosomiasis, positive reactions were observed with one antigen, either worm or egg. Moreover, there were 2 chronic cases in which IgE antibodies could not be detected. In acute cases, mean titers were 20 for worm and 50 for egg antigen. In chronic cases, mean titers were respectively 20 and 30. Negative and lower titers were mostly found in intestinal forms.

IgA — IgA antibodies were detected only in the acute form of the disease, with worm and egg antigens (mean titers 20 and 40, respectively).

Hemagglutination test (HAT) — Mean titers were lower in acute (80) than in chronic schistosomiasis (320).

Rheumatoid factor — The latex test revealed antibodies to IgG in two sera (AFN e LVS).

The adsorption of 10 selected sera (IGO, JAL, AFN, FAM, MCN, ZLS, HVS, JJS, LVS and MAS) with immunoadsorbent of IgG indicated that IgM antibodies first detected by immunofluorescent test were specific to **S. mansoni.** The rheumatoid factor detected in those two sera did not interfere with the titration of specific antibody.

DISCUSSION

Although some Authors [8,9,15,16] have already studied immunoglobulin classes of anti-**S. mansoni** antibodies in infected patients and animals, to our knowledge there is no investigation about the significance of these antibodies in different stages of the disease in humans.

The fluorescent antibody test showed **IgG, IgM and IgE anti-S. mansoni** antibodies in sera from acute and chronic cases. The finding of IgA antibodies only in acute cases differed strikingly from the chronic cases in which these antibodies were absent.

The presence of IgA antibodies in schistosomiasis seems to be significant since in other parasitic infections such as toxoplasmosis, trichinosis and hydatidosis IgA antibodies were found to be an indication of recent antigenic stimuli [15,17].

In **S. mansoni** infection, HULDT et al. [15] have found IgA antibodies in 6 out of 103 patients and DEELDER et al.[9] in every case from 19 selected patients. Also in **S. haematobium** infection IgA antibodies were detected in all 24 patients studied by KANE et al. [16]. However, the stage of the infection was not mentioned by those Authors.

Unlike virus or protozoan infection, IgM response in schistosomiasis does not necessarily reflect a recent infection. However, it was observed that in acute cases IgM levels were slightly higher than in chronic cases.

A continuous stimulation with different antigenic determinants might elicit IgM antibody response in the host even at a late stage of the disease. IgM antibodies to **S. mansoni** in chronic cases of human schistosomiasis were also described by SILVA & FERRI [20] and HILLYER [12].

Since rheumatoid factors are referred as usually occurring in many parasitic diseases [4,11,14], we considered the possibility of IgM reactions seen in the IFT being due to this factor. However, the tests showed that IgM detected in the sera of acute and chronic cases were specific to **S. mansoni.** For the latex test, only two revealed the presence of rheumatoid factor. Besides, after complete removal of rheumatoid factor from these two sera, both from patients with chronic infection, IgM antibodies to **S. mansoni** could still be detected. The other sera also showed no change in IgM antibody titers after treatment with insoluble IgG.

IgE antibodies to **S. mansoni** were demonstrated in 23 out of 25 sera. Some Authors [9,10] have found IgE in about 50% to 70% of infected patients. In our hands IgE as well as IgM and IgG antibodies did not differentiate acute from chronic infections.

Our results suggest distribution of class specific antibodies to vary in the course of the infection. The observed modifications permit, thus, to select more appropriate tests to be applied either for diagnostic purposes or to follow the course of the infection. More detailed studies are under way in a larger group of patients from an endemic area.

As far as HAT is concerned, it showed to be a less sensitive test than IFT in the acute stage of the disease.

RESUMO

Comportamento das imuneglobulinas específicas nas diferentes formas clínicas da esquistossomose mansônica

A possível associação de anticorpos anti-**S. mansoni** de diferentes classes de imuneglobulinas com o estágio de infecção foi estudada por técnica de imunofluorescência (IF), em 25 soros selecionados de pacientes com esquistossomose aguda e crônica. Anticorpos IgA foram detectados somente em soro de casos agudos, tendo-se encontrado anticorpos IgG, IgM e IgE, tanto em casos agudos como crônicos. O fator reumatóide, eventualmente presente nos soros, foi removido por adsorção com imunoadsorvente de IgG, antes de se efetuar o teste de IF. A reação de hemaglutinação passiva mostrou-se menos sensível do que a IF para detecção de anticorpos em casos agudos da doença.

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