Rev. Inst. Med. trop. São Paulo
15(6):371-376, novembro-dezembro, 1973

UDC 616.995.122.22

IDENTIFICATION OF HEMAGGLUTINATING ANTIBODIES IN CHRONIC SCHISTOSOMIASIS

Adhemar M. FIORILLO (1), João Carlos da COSTA (2) and Joselina PASSOS (3)

SUMMARY

The hemagglutinating antibodies in sera of 8 patients with chronic *Schistosoma mansoni* infection were studied. The serum samples were fractionated in 7S and 19S immunoglobulins on Sephadex G-200. The indirect hemagglutination test was employed to detect the hemagglutinating antibodies in 7S and 19S fractions, using tannic acid-treated red blood cells sensitized with schistosome worm extract. It was concluded that hemagglutinating antibodies in chronic *Schistosoma mansoni* infection were of the type 7S, probably IgG.

INTRODUCTION

KAGAN [5] was the first to obtain results in a passive hemagglutination test for schistosomiasis using sera of rabbits immunized with schistosome cercaria, adult schistosome worms, and tannic acid-treated sheep red blood cells sensitized with the corresponding antigenic extracts. KAGAN & OLIVER-GONZALES [6] employed the hemagglutination test in sera of patients with chronic schistosomiasis but the antibody titers measured were not reproducible. Nevertheless they found higher titers when schistosome cercaria was employed as antigen. MADDISON et al. [9] used indirect hemagglutination test to study the antibody response in *Schistosoma mansoni* infected rats. HOSHINO et al. [3] used the immunofluorescent test for the detection of antibodies in the sera of patients with chronic schistosomiasis. They have found that the hemagglutination test was easier and more sensitive than the immunofluorescent test. They have also detected a significant increase of hemagglutinating antibodies in the sera of eight patients after nitro-thiamidazol (Ciba 32, 644-Ba) therapy.

The present study was designed to identify the hemagglutinating antibodies in schistosomiasis by the indirect hemagglutination test, using schistosome worm extract as antigen.

MATERIAL AND METHODS

Sera — Two serum samples were obtained from each of eight patients who had a diagnosis of chronic schistosomiasis at intervals of 30 or 60 days.

Diagnosis was established on clinical grounds and confirmed by demonstration of *Schistosoma mansoni* eggs in stools and through histologic alterations in liver biopsies obtained during splenectomy. All patients had portal hypertension and hypersplenism. Serum samples were obtained before splenectomy and kept frozen at −20°C until analysis.

Immunoglobulins — One hundred fifty mg of protein from each serum sample were

From the "Departamento de Clínica Médica, Secção de Moléstias Infecciosas, Faculdade de Medicina de Ribeirão Prêto da U.S.P., Ribeirão Prêto, São Paulo, Brasil"
(1) Associate Professor
(2) Doctor Assistant Professor
(3) Resident

fractionated in Sephadex (*) G-200, according to a modified method of FAHEY & TERRY [1]. The gel filtration was performed under the following conditions. Bed dimensions: 2.5 x 80.0 cm. Eluant: 0.1 M Tris-HCl buffer, pH 8.0 and 0.3 M NaCl. Flow rate: 20 ml/h. The protein distribution in the eluate was determined by measuring the optical density of individual effluent fractions at 280 nm in a Spectrophotometer(**). The fractions were designated as P_1, P_2, P_3, P_4 and P_5 pools as shown in Fig. 1. These pools were previously concentrated fourfolds at 4°C in cellulose dialysis tubing against saccharose (***).

After concentration the pools were dialyzed against buffered saline, pH 7.2, at 4°C. Protein concentration on whole sera was determined by a micromethod adapted for the estimation of nitrogen in the range of 10-100 μg (KABAT & MAYER [4]), using the Folin-Ciocalteu Phenol reagent.

The identification of G, A and M Immunoglobulins and albumin of P_1, P_2, P_3, P_4 and P_5 pools was carried out immunoelectrophorectically by SCHEIDEGER's [10] micromethod. Anti-human immunoglobulins A, G, M and whole serum were obtained from a Netherlands commercial supplier (****).

Indirect hemagglutination test — Adult schistosome worm extract (*****) was used to sensitize the tannic acid-treated red blood cells (HOSHINO et al. [3]), except sheep red blood cells were employed instead of human erythrocytes. The formalin treated red blood cells were obtained by KAGAN's method [7]. Merthiolate at a final dilution of 1:10.000 was added to the sensitized erythrocytes as a preservative. A single batch of sheep red blood cells was employed in all tests. The sensitized sheep red blood cells were stored at 4°C. Inactivated rabbit serum absorbed with sheep erythrocytes, was used as stabilizer in the reaction. The test was carried out in test tubes; the STAVITSKY [11] method being used for recording results.

RESULTS

An example of an elution curve of serum protein of a patient with chronic schistosomiasis, is given in Fig. 1. The combination

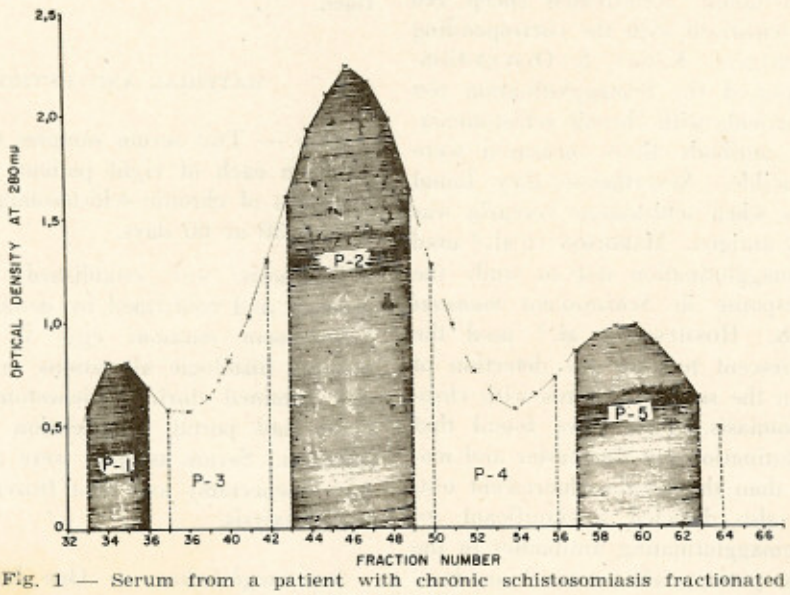

Fig. 1 — Serum from a patient with chronic schistosomiasis fractionated on Sephadex G-200.

(*) Sephadex G-200, Pharmacia-Uppsala, Sweden
(**) Gilford Spectrophotometer — Model 240
(***) Bacto-Saccharose — Difco
(****) Centraal Laboratorium Van de Bloed Transfusiedienst, Amsterdam
(*****) Lyophilized worm — Prof. J. Pellegrino, Belo Horizonte, Minas Gerais, Brasil

TABLE I

Reciprocal of hemagglutinating antibody titers in sera and fractionated sera on Sephadex G-200 columns

Patients	Whole serum (d)		7S fraction		19S fraction		Clinical findings
	1st sample	2nd sample	1st sample	2nd sample	1st sample	2nd sample	
1. M.S.M.	5120	1280	320	640	20	—	Hepatomegaly Portal pressure = 32 cm H_2O Living eggs (b) in feces
2. S.J.F.S.	2560	1280	320	80	20	20	Hepatomegaly Portal pressure = 26 cm H_2O Living eggs (b) in feces
3. J.C.J.	1280	ND (a)	160	ND	20	ND	Hepatomegaly Portal pressure = 24 cm H_2O No eggs in feces
4. I.V.C.	640	640	160	160	5	— (c)	Slight hepatomegaly Portal pressure = 10 cm H_2O Living eggs in feces
5. P.J.S.	640	320	160	40	—	—	Hepatomegaly Portal pressure = 35 cm H_2O Living eggs in feces
6. A.S.S.	160	160	80	20	5	5	Hepatomegaly Portal pressure = 26 cm H_2O Living eggs in feces
7. J.S.S.M.	80	80	80	40	10	5	Hepatomegaly Portal pressure = 26 cm H_2O Living eggs in feces
8. I.P.C.	40	40	40	10	10	10	Reduced liver Portal pressure = 34 cm H_2O Living eggs in feces

(a) ND = Not done; (b) eggs = *Schistosoma mansoni* eggs; (c) = negative
(d) 2nd sample = obtained 30 to 60 days after 1st sample

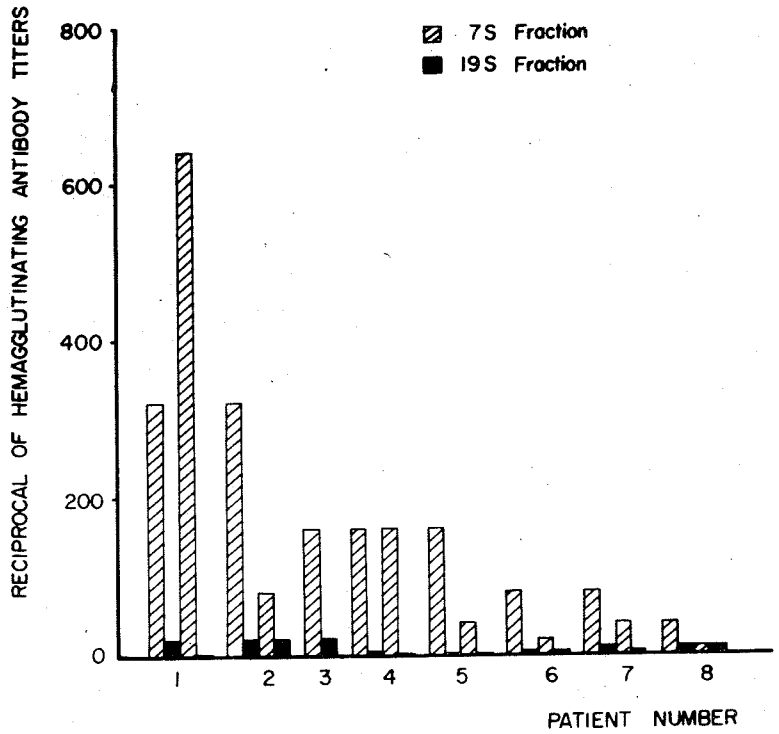

Fig. 2 — Reciprocal of hemagglutinating antibody titers in sera fractionated on Sephadex G-200.

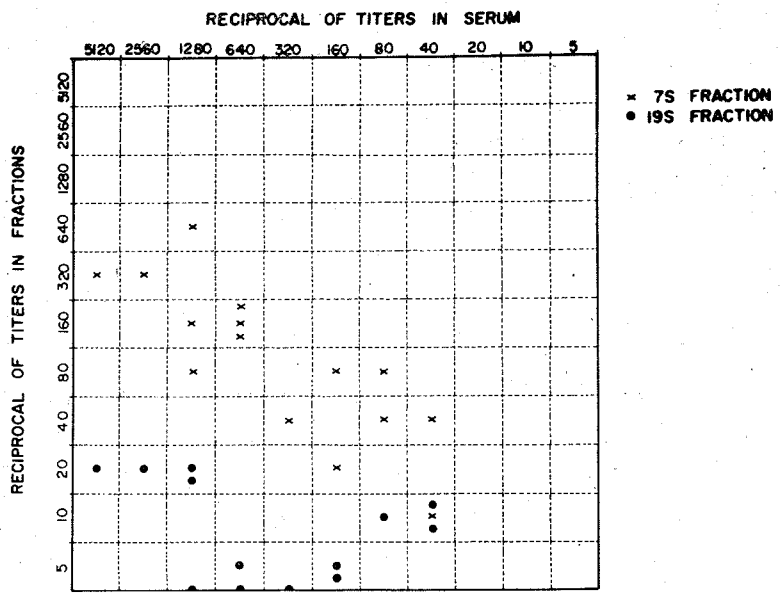

Fig. 3 — Distribution of hemagglutinating antibody titers (reciprocal) in whole sera, 7S and 19S fractions.

of fractions into P_1, P_2, P_3, P_4 and P_5 pools is also illustrated in this figure.

The immunoelectrophoresis characteristics of P_1, P_2, P_3, P_4 and P_5 pools have demonstrated the following: IgM is present in P_1 pool in addition to α 2 macroglobulin. P_2 pool consists largely of IgG; IgA, α and β globulins are also present. P_4 pool is composed of IgG and albumin. P_5 pool contains albumin, α and β globulins. It could be considered that the major immunoglobulins in P_1 and P_2 pools were IgM and IgG of the types 19S and 7S respectively. Whole sera, P_1 and P_2 pools were used for hemagglutination tests; their results are shown in Table I and Figs. 2 and 3. As can be seen in Table I the hemagglutinating antibody activity ranged from 1/40 to 1/5120 for whole sera, 1/10 to 1/640 for 7S fractions and negative to 1/20 for 19S fractions.

DISCUSSION

The patients in the present study had hepatic fibrose, portal hypertension and hypersplenism. All patients excluding one had living *Schistosoma mansoni* eggs in their stools. In two patients many worms were obtained through extracorporeal filtration during splenectomy. The histologic alterations in liver did not show typical signs of an active disease. The increased amount of immunoglobulin G in sera was demonstrated through fractionation of sera on Sephadex G-200.

The findings of hemagglutinating antibodies to *Schistosoma mansoni* in sera of patients with chronic schistosomiasis showed that high titers are not correlated with the activity grade in this phase of the disease, although HOSHINO et al. [2] demonstrated an increase in hemagglutinating antibodies in sera of patients with schistosomiasis following specific therapy probably by a liberation of antigens. As can be seen in Table I and Fig. 2 the majority of hemagglutinating antibodies is immunoglobulin 7S; 19S hemagglutinating antibody had no significant titers even in those patients with high titers in whole serum. KAGAN [8] reported in prior experience that immunoglobulin G (7S) and immunoglobulin A are the main components in the floculation test of schistosome cercaria and 2-mercaptoethanol inactivates the immunofluorescence test, suggesting that the active components in this test are immunoglobulin M. The results in the present study showed that hemagglutinating antibodies in chronic schistosomiasis are immunoglobulin 7S, probably IgG.

MADDISON et al. [9] in their longitudinal studies in rats infected with *Schistosoma mansoni* had found that the highest values in hemagglutinating antibodies were around the 8th week and persisted to be elevated until the 23rd week after infection. Further observations on longitudinal evolution of hemagglutinating antibodies as well as the behaviour of these antibodies following specific therapy are to be carried out in patients with schistosomiasis.

RESUMO

Natureza dos anticorpos hemaglutinantes na esquistossomose crônica

Os Autores estudaram os anticorpos hemaglutinantes nos soros de 8 pacientes com Esquistossomose mansoni em fase crônica. Os soros foram fracionados em componentes 7S e 19S, em coluna de Sephadex G-200. Empregaram a prova de hemaglutinação indireta para a identificação de anticorpos nas frações 7S a 19S, usando hemácias de carneiro tanizadas e sensibilizadas com extrato de vermes adultos. Concluiram que os anticorpos hemaglutinantes na fase crônica da Esquistossomose mansoni são de tipo 7S, muito provavelmente globulinas IgG.

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Recebido para publicação em 11/1/1973.