Rev. Inst. Med. trop. São Paulo
7(1):7-10, janeiro-fevereiro, 1965

UDC 616.995.122

IMMUNODIFFUSION STUDIES IN HUMAN SCHISTOSOMIASIS MANSONI

II. Localization of antibodies by immunoelectrophoresis

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SUMMARY

Sera from seven patients with Schistosomiasis mansoni (four with the hepatosplenic form and three with hepatointestinal form) were submitted to electrophoresis and subsequently diffused against worm antigen and against rabbit or horse anti-human serum.

Precipitin lines corresponding to the position of 7S gamma globulin (IgG) was obtained in five sera. As to the other sera, one showed a precipitin line in the IgM (Beta-2M) position and the other showed two precipitin lines probably unrelated to the IgM.

INTRODUCTION

As far as precipitins are concerned, the nature of the human antibodies found in the sera of patients with Schistosomiasis mansoni is still to be defined.

The immunoelectrophoretic techniques are very useful for the localization of the precipitin bands, suggesting and sometimes defining their immunological and physicochemical nature.

Using double diffusion technique, to test human sera against worm antigen, BIGUET et al. [1] found precipitin lines in most cases. However submitting human serum to electrophoresis and subsequently diffusing against worm antigen they were not able to obtain precipitin lines.

According to KENT [4], better results might be obtained by dilution of the serum. Nevertheless, KENT [4] mentioned in this paper only the results of electrophoresis of worm and cercarial antigens followed by diffusion against human serum.

Submitting original or concentrated sera to immunoelectrophoresis we have been able to localize the antibodies in the serum proteins from patients with Schistosomiasis mansoni. The results of these studies are presented in this paper.

MATERIAL AND METHODS

Seven proved cases of Schistosomiasis mansoni were selected according to the intensity of the reaction of sera by OUCHTERLONY technique [5] using worm extract as antigen, as described previously [6].

Patients no. 1, 2, 3 and 4 were clinically considered as presenting the hepatosplenic

This research was supported in part by a grant from "Fundação de Amparo à Pesquisa do Estado de São Paulo".

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form and patients no. 5, 6 and 7 as presenting the hepatointestinal form. No viable eggs were found in patients no. 1 and 2 but diagnosis was confirmed by liver biopsy.

Immunoelectrophoresis of GRABAR and WILLIAMS [3] was performed as described by FERRI and COSSERMELLI [2]. Human sera were submitted to electrophoresis, diffusion being carried out against rabbit or horse serum anti-human serum * and against worm antigen prepared as described previously [6]. All human sera except no. 1 and 2 had to be concentrated 3 to 6 times in order to show satisfactory precipitin bands.

RESULTS

All of the sera showed precipitin lines belonging to the gamma-globulin system when submitted to electrophoresis followed by diffusion against adult worm antigen. Gamma-globulin system is composed of conventional or 7S gamma-globulin (IgG) beta-2A (IgA) and beta-2M (IgM).

The electrophoretic mobility of the human antibodies as revealed by diffusion against adult worm antigen is variable, most sera showing precipitin lines nearer and others more distant from the negative pole. Thus, sera from patients no. 3 and 4 (hepatosplenic form) and no. 5, 6 and 7 (hepatointestinal form) showed precipitin lines corresponding to the position of the IgG. Different results were obtained with sera from patients no. 1 and 2 (hepatosplenic form) and will now be discussed.

Original and thrice concentrated serum from patient no. 1 was studied by immunoelectrophoresis (Fig. 1). Original serum showed a precipitin line corresponding to the IgG. Concentrated serum showed the same line, but more intense and prolonged up to the application point. The double curvature which appears in such a line suggests the presence of two antibodies with different electrophoretic mobilities but related immunochemically. A faint precipitin line more distant from the antigen trough was also observed, but its correlation with any immunoglobulin remains to be determined.

Serum no. 2 was submitted to macroimmunoelectrophoresis (Fig. 2) using worm antigen in the upper trough and horse anti-human serum in the lower trough. The localization of precipitin line strongly suggests that this human antibody corresponds to a IgM.

Microimmunoelectrophoresis of the same serum and of a normal serum was performed for comparison (Fig. 3). Antibodies corresponding to IgM, precipitated as a stronger and more extended line.

Figure 4 shows serum no. 3 with precipitin corresponding to IgG. To avoid misinterpretation, both sera (no. 2 and 3) were studied simultaneously in the same agar plate, as shown in Fig. 5, where the abovementioned differences are well demonstrated.

DISCUSSION

To our knowledge, electrophoresis of human serum followed by subsequent diffusion against *Schistosoma mansoni* antigens has not been successfully applied for the antibody characterization. It is our feeling that such a lack of informations on the nature of serum precipitins in human schistosomiasis is due to the difficulties in their demonstration by immunoelectrophoresis as pointed out by BIGUET et al. [1].

As a matter of fact, we were able to obtain precipitin lines only after many trials. It must be emphasized that other schistosomotic sera were studied by us, but were not included in this paper because of the faint precipitin lines obtained. Some studies on electrophoresis of antigen followed by diffusion against the serum of infected or hyperimmunized animals and against pathologic human sera were published [1, 4].

Our results suggest that human precipitating antibodies against adult worm antigens may be mainly conventional gamma-globulin (IgG), but some sera may contain 19S type antibodies (IgM).

* Rabbit anti-human serum was prepared in the Laboratory of Immunochemistry and horse anti-human serum in the Instituto Pinheiros de São Paulo, Brazil.

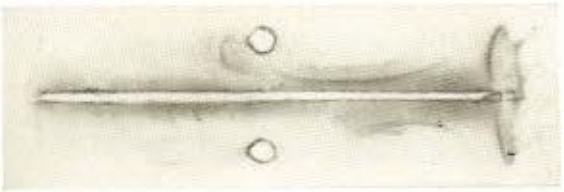


Fig. 1 — Immunoelectrophoresis of concentrated (upper well) and original serum no. 1 (lower) against worm antigen.

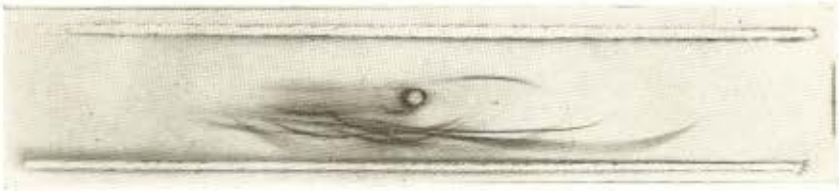


Fig. 2 — Immunoelectrophoresis of serum no. 2 against worm antigen (upper trough) and horse anti-human serum (lower).

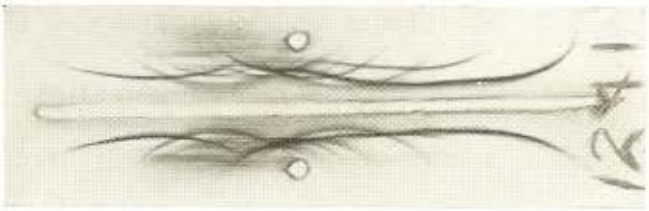
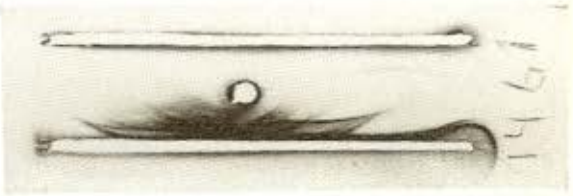


Fig. 3 — Immunoelectrophoresis of schistosomotic (upper well) and normal serum (lower) against horse anti-human serum. Differences of intensity and extension of the IgM line are well observed.

Fig. 4 — Immunoelectrophoresis of serum no. 3 against worm antigen (upper trough) and horse anti-human serum (lower). Compare the position of precipitin line, with that showed in Fig. 2.



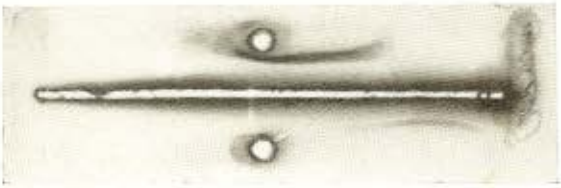


Fig. 5 — Immunoelectrophoresis of serum containing antibodies of the IgM type (upper) and of the IgG type (lower). Worm antigen is in the central trough.

Serum no. 2 deserves special mention as showed a strong precipitin line corresponding to the IgM (beta-2M), modified in shape and with a very slight double curvature when submitted to a immunoelectrophoresis against horse anti-human serum (Fig. 3). The position of that part of the curvature which is nearest to the starting point corresponds to the precipitin line formed by the same serum when submitted to electrophoresis and subsequently diffused against worm antigen (Fig. 2).

Data suggesting the presence of IgA (beta-2A) antibodies in human Schistosomiasis mansoni are lacking.

These and other problems related to the characterization of antibodies in the sera of schistosomotic patients are under investigation in our laboratory.

RESUMO

Estudos sôbre imunodifusão na esquistossomose mansônica humana. II — *Localização de anticorpos, por imuno-electroforese.*

Soros de sete pacientes com esquistossomose mansônica, dos quais quatro com a forma hepatesplênica e três com a forma hepatintestinal foram submetidos à electroforese e subseqüentemente difundidos contra antígeno de verme e contra sôro anti-humano produzido em cavalo ou em coelho.

Em cinco soros as linhas de precipitação se localizaram na posição da gamaglobulina 7S (IgG).

Quanto aos outros dois soros, ambos pertencentes a pacientes portadores da forma hepatesplênica, um mostrou a linha de precipitação na posição da gamaglobulina 19S (IgM) e o outro revelou duas linhas de precipitação, provàvelmente sem relação com a gamaglobulina IgM.

ACKNOWLEDGMENTS

Part of technical assistance was possible thanks to the "Instituto Brasileiro de Estudos e Pesquisas de Gastrenterologia" (IBEPEGE).

The writers are grateful to Dr. W. LOBATO PARAENSE and to Dr. JOSÉ PELLEGRINO (Instituto Nacional de Endemias Rurais) for supplying the infected snails and part of the lyophilized worms.

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Recebido para publicação em 3/12/1964.