PRELIMINARY COMMUNICATIONS

Circulating Immune Complexes in Schistosomiasis due to *Schistosoma mansoni*

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Summary

Immune complexes have been detected in the sera of people infected with *Schistosoma mansoni* by the technique of acidification followed by double counter-current immunoelectrophoresis in hypertonic buffered gels.

Introduction

During work to develop an improved immunodiagnostic test for schistosomiasis it became apparent that some of the anomalous results, particularly false negatives, obtained in indirect haemagglutination and complement fixation tests on sera may have been due to the presence of immune complexes. The demonstration of these in *Schistosoma mansoni* infections is described below.

Methods

Sera from people known to be infected with *S. mansoni* were obtained from the Sudan, East Africa, St. Lucia, and Britain. So far as possible all sera were kept at -20°C or below until used. Three parts of 1 M citric acid were added to one part of serum to bring the pH to 2.0 and the mixture kept at 4°C for one hour to dissociate any antigen-antibody complexes. This is a modification of techniques used to elute immune complexes from tissues.^{1,2}

Double counter-current immunoelectrophoresis (D.C.I.E.) was performed on glass microscope slides with a 2 mm thick layer of 0.9% ionagar in 0.025 M sodium barbitone/acetate buffer pH 8.6 with 5% sodium chloride additive. The well pattern was composed of a central well 5 mm in diameter, with a 1.2 mm diameter well at the anodal and cathodal sides of this well, which were set 5 mm from its periphery. Acidified serum 25 μl was then placed into the central well and 5 μl of an anti-worm serum (A.W.S.) placed on the anodal side. An antiserum made by injecting a rabbit with extracts of whole adult worms was at first used, but latterly a serum obtained from a chimpanzee early in an experimental infection with *S. mansoni*, before complexes were detectable, was used as it gave more consistent results. Both antisera were absorbed with lyophilized human serum before use to remove any non-specific agglutinins for human serum products. *S. mansoni* adult worm antigen (WAg) 5 μl was placed on the cathodal side. The WAg was purified by affinity chromatography^{3,4} using immobilized antiserum from the chimpanzee after absorption with cross reacting *S. haematobium* antigens isolated by immunoelectrophoresis.⁵ A current of 10 mA was passed for 60 minutes and the slides were then soaked in 70% ethanol for 10 minutes to increase the intensity of the precipitation lines.

Double wells 3 mm in diameter on the anodal side were used for showing lines of identity against A.W.S. by antigen dissociated from circulating complexes and by worm antigen.

Results

Fig. 1 shows a typical pattern obtained by D.C.I.E. with WAg and A.W.S. against a serum from an infected adult Sudanese living in an area where *S. mansoni* is endemic. Five precipitin lines were discernable between the serum and the WAg and five lines between the serum and the A.W.S. The specificity of these reactions was shown by their failure to appear after absorption of the serum with WAg or the A.W.S. by crude worm homogenates. Further confirmation of specificity was obtained by reacting the acidified serum and the WAg in parallel against the A.W.S. Lines of identity may be seen in fig. 2.

FIG. 1—Typical pattern obtained with acidified immune serum against worm antigen (WAg) and antiserum to worm antigen (A.W.S.). Lines between serum and A.W.S. are antigen released from serum.

FIG. 2—Multiple lines of identity between worm antigen (WAg) and antigen dissociated from acidified serum, against anti-worm serum (A.W.S.).

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TABLE I—Results of D.C.I.E. Tests for *S. mansoni* Antibody and Antigen on Sera from Sudanese living in Area where *S. mansoni* is Endemic

Age Group (Years)	No. Examined	No. (%) with Antibody Lines	No. (%) with Antigen Lines	Stool Positive for <i>S. mansoni</i> Ova		Stool without <i>S. mansoni</i> Ova	
				No.	% with Antigen	No.	% with Antigen
1-4	101	48 (48)	13 (13)	11	55	90	13
5-19	643	616 (96)	341 (53)	517	62	126	14
≥20	275	273 (99)	230 (84)	204	80	71	54

TABLE II—Results of D.C.I.E. Tests for *S. mansoni* Antibody and Antigen on Sera from Different Population Groups

Groups	Age Range (Years)	No. Examined	No. (%) with Antibody Lines	No. (%) with Antigen Line.
<i>Patients from Areas where S. mansoni is Endemic</i>				
Kenyan children from lowlands	5-17	226	221 (98)	119 (53)
St. Lucian children and adolescents	5-19	25	25 (100)	17 (68)
St. Lucian adults	≥20	46	46 (100)	29 (63)
<i>Patients with Light Infections with S. mansoni</i>				
Hospital patients in U.K. before treatment	>20	24	24 (100)	0
<i>Patients without S. mansoni</i>				
Kenyan children from highlands	5-17	194	1 (0.5)	0
Asian immigrants to U.K.	>20	100	0	0
Nurses in U.K.	>20	31	0	0
Hospital patients in U.K. with helminth infections other than schistosomiasis	>20	11	0	0
Hospital patients in U.K. with <i>S. haematobium</i> infections	>20	7	0	0

Evidence that the worm antigens are bound into complexes comes from the fact that the presence of the antigen could only be shown after acidification of the test serum.

Altogether 1019 sera from people living in the Gezira area of the Sudan were tested by D.C.I.E. after acidification (table I). *S. mansoni* infection is highly endemic there and nearly all the population is known to be infected by the age of about 10 years. The frequency and the intensity of egg excretion reach a maximum between 10 and 14 years of age, and decline thereafter throughout adolescence and adult life, possibly because some immunity has been acquired. There is also a little *S. haematobium* infection in the area. The prevalence of antibodies increased rapidly with age as may be expected in the presence of repeated infections in early years. The prevalence of circulating antigens, as detected in this system, ran at a lower level than that of antibody but remained high in adult life. There was a higher prevalence of antigens in those excreting *S. mansoni* eggs at the time blood was taken than in those whose stools were apparently negative, though many of the latter would also certainly be infected. The number of antibody lines appearing against the WAg varied from one to seven and the antigen lines appearing against the A.W.S. varied from one to five.

Table II shows the results of testing sera from a variety of other groups. Kenyan children from a lowland area where *S. mansoni* is endemic and where there is also some *S. haematobium* showed a pattern very similar to the Sudanese. In St. Lucia, where *S. mansoni* is slightly less endemic than in the Sudan and Kenya, a similar picture was seen in children but no increase of antigen prevalence was found with age. In a small group of British hospital patients with light infections no antigen was detectable, though antibody was present in all. In the groups without *S. mansoni* no antigen was detectable, but cross-reacting antibody was found in one of the group of Kenyan children from an upland area where there is no schistosomiasis but other helminth infections are frequent.

Discussion

The formation of soluble antigen-antibody complexes has been suspected of playing a part in the pathogenesis of the different lesions which may result from infections with *S. mansoni*, *S. haematobium*, and *S. japonicum*.⁶⁻⁷ The histological changes occurring in kidney lesions suggest the deposition of immune complexes, but though immunoglobulins have been shown on the glomerular basement membrane specific material derived from the worm has not been shown.⁸⁻¹⁰ Precipitable worm material has been found in the urine of some who are infected with *S. japonicum*¹¹ or *S. haematobium*;¹² but worm antigens in the circulation have been shown only in small animals with massive infections of *S. mansoni*¹³⁻¹⁵ and in a few patients with

S. mansoni and *S. haematobium*^{15 16} by using untreated sera in complement fixation or gel diffusion tests.

The technique of acidifying the test serum frees antigen which may be bound in soluble complexes so that it can subsequently be shown as precipitin lines against a specific antiserum in a strongly buffered gel; this is expedited by using counter-current immunoelectrophoresis. With this method we have shown circulating antigen, some or all of it presumably bound in complexes, in a high proportion of people known to be receiving multiple challenges with *S. mansoni*. Investigations are in train to look for quantitative or qualitative correlations between these complexes and the immunopathological status of the patients. The use of highly purified antigens has aided the detection of specific antibodies, and this in itself may prove to be a useful diagnostic test.

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