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Circulating immune complexes in schistosomiasis

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Summary. Circulating immune complexes (CIC) were investigated by the [^{125}I]Clq binding test, the complement fixation test (CFT) and optical density measurement after redissolving 3% polyethylene glycol precipitates of serum from patients infected by *Schistosoma mansoni*. A highly significant correlation was obtained among these three techniques. More than 60% of the patients demonstrated significantly higher values than control individuals. The level of CIC was found to be higher in the mild than in the hepatosplenic form of the disease. Parasite antigen, IgG, IgM and IgE were characterized in these CIC. In experimental schistosomiasis in mice, maximum levels of CIC, evaluated by the CFT, were observed between the 40th and the 70th day of infection.

INTRODUCTION

The involvement of antigen-antibody complexes in the renal injury associated with *S. mansoni* schistosomiasis, particularly the hepatosplenic form of the disease, has long been suspected. Granular deposits on the glomerular basement membrane have been demonstrated by a number of workers (Andrade & Queiroz, 1968; Brito, Gunji, Camargo, Penna & Silva, 1970; Andrade, Andrade & Sadigursky, 1971; Silva, Brito, Camargo, Boni, Lopes & Gunji, 1970; Queiroz, Brito, Martinelli & Rocha, 1973), as well as deposits of IgG, IgM and the third component of complement (Silva *et al.*, 1970). Similar findings have been reported for experimental schistosomiasis in mice and hamsters (Natali & Cioli, 1974; Hillyer & Lewert, 1974). There are now numerous reports of the presence of specific schistosome antigens in the circulation of infected animals (Berggren & Weller, 1967; Gold, Rosen & Weller, 1969; Okabe & Akusawa, 1971; Nash, Prescott & Neva, 1974; Bawden & Weller, 1974), and in human urine (Okabe & Tanaka, 1961; Carlier, Bout, Bina, Camus, Figueiredo & Capron, 1975). All these findings suggest that circulating immune complexes (CIC) are a feature of schistosomiasis and may well contribute to the associated pathology.

Numerous methods are now available for the detection and measurement of CIC and three of these have been used in the present study. Infected and control human sera have been investigated by the [^{125}I]Clq binding test (C1q-BT) (Nydegger, Lambert, Gerber & Miescher, 1974); the complement fixation test (CFT) based on the anticomplementary activity of immune complexes (Shulman & Barker, 1969); and by optical density measurements (OD) on redissolved 3% polyethylene glycol precipitates of serum (Creighton, Lambert & Miescher, 1973). Additionally, the antigens and immunoglobulins making up these complexes have been investigated. The changes in CIC in experimentally

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infected mice have been followed by the CFT during the course of infection.

MATERIALS AND METHODS

Sera

Man. All human sera came from Bahia state, Brazil. Blood was allowed to clot at room temperature for 60 min. Serum was separated by centrifugation at 4° and used after storage at −20°. Fifty-six subjects infected by *S. mansoni* had both parasitological and serological tests (immunoelectrophoretic analysis and haemagglutination tests) positive. The patient's clinical forms were classified according to Prata and Bina (1968) into forty mild forms and sixteen hepatosplenic forms. Fourteen control subjects, without schistosomiasis, did not have eggs in the faeces and serological tests were negative.

Mouse. Sera were taken every 5 days for 95 days from 100 C57 black mice infected with 100 furcocercariae of *S. mansoni* (Puerto Rico strain) and pooled. Thirty control mice were bled likewise.

Preparation of S. mansoni *antigen*

A whole antigen extract of *S. mansoni* was prepared with adult worms obtained from experimentally infected hamsters according to Capron, Biguet, Vernes & Afchain (1968). Parasite specific antigen was obtained by affinity chromatography with IgG antibody isolated from anti-*S. mansoni* hyperimmune rabbit serum, absorbed by the serum and liver of normal hamsters, and fixed to CNBr activated sepharose (Pharmacia, Sweden). The parasite antigen was eluted by acid dissociation with glycine-HCl buffer, pH 2·8, immediately neutralized by dialysis against phosphate buffer solution pH 7·2 (PBS) and radioiodinated according to Morrison, Bayse & Webster (1971).

Preparation of antisera

Anti-*S. mansoni* hyperimmune rabbit serum was prepared against a whole extract of adult schistosomes according to the technique of Capron *et al.* (1968). The monospecific serum against antigen called '4', specific for the genus Schistosoma (Capron *et al.*, 1968) was obtained according to Bout *et al.* (in preparation). Briefly, the precipitation line (band 4) of this antigen was identified in bidimensional immunoelectrophoresis performed with *S. mansoni* whole antigen extract and rabbit anti-*S. mansoni* serum. After 48 h washing with PBS, the agarose containing the band 4 was excised, dispersed in 0·5 ml saline, and emulsified in 0·5 ml Freund's complete adjuvant. The antigen–antibody–adjuvant mixture was injected i.d. into rabbits according to the method of Vaitukaitis, Robbins, Nieschlag and Ross (1971).

Antihuman IgG, IgM and IgA sera were obtained from Hyland laboratories.

Detection of immune complexes

PEG precipitation. CIC were first investigated by precipitation of sera with polyethylene glycol (PEG) (mol. wt: 6000) according to Creighton *et al.* (1973), with minor modifications, i.e., the precipitation time was reduced to 2 h, final PEG concentration was 3% and the samples were centrifuged at 2500 *g*. The pellet was dissolved in distilled water equal in volume to the initial volume of serum. The OD (280 nm) was determined using an aliquot of the aqueous immune complexes solution diluted to 1/10 with 0·1 N NaOH.

Anti C activity. The complement fixation test (CFT) was performed according to Wasserman & Levine (1961) with another aliquot of 3% PEG precipitate of serum (PEG ppt) previously redissolved in PBS, and was used to determine the anticomplementary activity of the CIC (Shulman & Barker, 1969 and Thiry, Clinet, Toussaint & Vereerstraeten, 1973).

Clq-binding test. The radiolabelled Clq-binding test (Clq-BT) was performed according to Nydegger *et al.* (1974) The test is based on both the large molecular size and the Clq-binding property characterizing immune complexes. Briefly, 0·2 ml of undiluted serum was mixed with 0·2 ml veronal buffered saline, pH 7·2. 50 μl [^{125}I]Clq containing 1 μg/ml of Clq was added to each tube. All tests were done in duplicate. The mixtures were incubated for 60 min at 25° and for a further 60 min at 4°. Three millilitres of PEG was then added to a final concentration of 2·5% and the samples incubated at 4° for 2 h. The tubes were then centrifuged at 1000 *g* for 20 min, the supernatants discarded, and radioactivity was measured in the precipitates. Results were expressed as percent [^{125}I]Clq precipitated. They were calculated on the basis of protein-bound radioactivity (precipitable by 20% trichloroacetic acid).

Detection of Ig antibodies and parasite antigen in complexes

Immunoglobulins and parasite antigen were examined in the 3% PEG ppt washed three times with 3% PEG, and redissolved in PBS (if not otherwise specified), to give the initial volume. Investigation of IgG, IgA and IgM was performed by double diffusion as described by Ouchterlony (1962). Total IgE was measured by a radioimmunosorbent test (Pharmacia, Sweden) in sera and in the dissolved PEG ppt. Specific antigen was characterized by double diffusion (Ouchterlony, 1962) and immuno-electrophoretic analysis (Scheidegger, 1955; Capron, Biguet, Rose & Vernes, 1965) in the PEG ppt dissolved in glycine–HCl buffer 0·1 M pH 2·8. Detection of anti-*S. mansoni* antibodies was performed by dissociation–reassociation: the PEG ppt obtained from 0·2 ml serum was washed three times with 3% PEG, then dialysed against distilled water and lyophilised. Subsequently, ^{125}I-labelled parasite specific antigen was added (10,000 c.p.m. in 10 μl) followed by 1·0 ml of glycine–HCl buffer pH 2·8 and stirred continuously for 15 min. After addition of phosphate buffer 0·1 M pH 8·4 (1 ml), the solution was dialysed against borate-buffered saline, pH 8·0 for 24 h. Precipitation was finally obtained with PEG at a final concentration of 3%. After three washings, the radioactivity of the pellets was measured in a well-type scintillation counter.

Statistical analysis was performed by the correlation test, the Student's *t*-test.

RESULTS

The results of CIC levels are reported in Table 1.

(a) Correlative study of the three techniques (Clq-BT, CFT, OD) used to quantify CIC, in human schistosomiasis

A highly significant correlation ($P < 0{\cdot}001$) was found among the three techniques used. Partial correlation though always significant was higher between Clq-BT and CFT and Clq-BT and OD than between OD and CFT.

A highly significant difference was observed between CIC levels in infected patients and in control subjects (Table 1 and Fig. 1). Among the infected group, a significant difference was also found between the hepatosplenic and the mild form of the disease (Table 1 and Fig. 1).

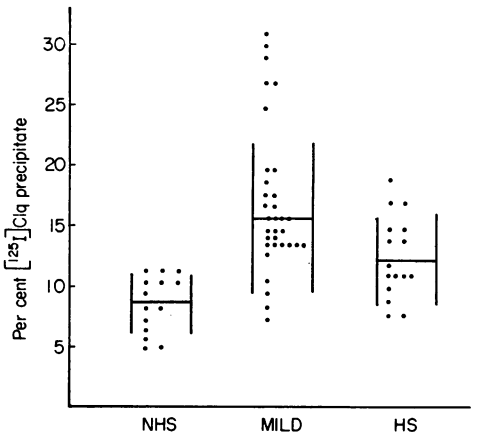

Figure 1. Immune complexes levels in control and infected patient groups. A significant increase in [^{125}I] Clq binding was observed for the mild form ($P < 0{\cdot}001$) and the hepatosplenic form ($0{\cdot}005 < P < 0{\cdot}001$).

Table 1. Study of circulating immune complexes by C1q-binding test (C1q-BT), optical density (OD) and complement fixation test (CFT)

Group	Number of cases	C1q-BT	OD	CFT
Control	14	8·3±2·3	0·13±0·04	1·5
Schistosomiasis				
Mild form	40*	15±6·1	0·45±0·19	24
Hepatosplenic form	16†	11±3·3	0·32±0·09	4

C1q-BT = percentage of [^{125}I]C1q precipitated; OD = optical density at 280 nm; CFT = geometric mean of titre.

* Comparison with controls form by Student's *t*-test: $P < 0{\cdot}001$.

† Comparison with mild form by Student's *t*-test: $0{\cdot}05 > P > 0{\cdot}025$.

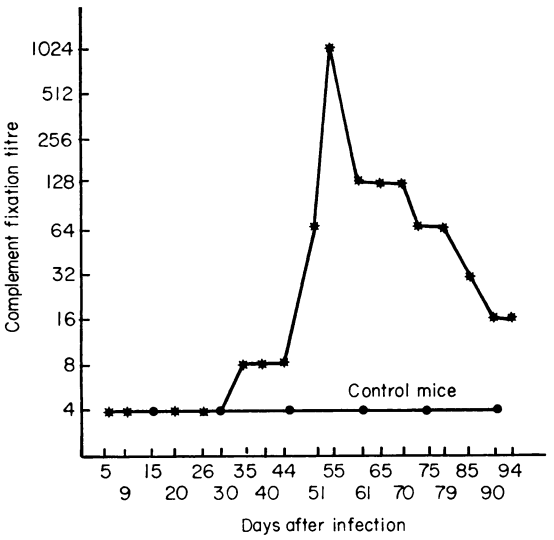

Figure 2. Anticomplementary activity in the serum of infected mice. The maximal values, expressed as titre (16–1024) were observed between the 44th and the 90th day of infection. The results for the control mice were always lower than 4 during this period.

(b) Circulating immune complexes levels evaluated by the complement-fixation test during the evolution of experimental mouse schistosomiasis

Results are reported as the reciprocal of highest positive dilution, and are shown in Fig. 2. The maximal values (16–1024) were observed between the 44th and the 90th day of infection. The titres for the control mice were always lower than 4 during the same period.

Table 2. IgE levels (iu/ml) in native sera and 3% PEG precipitated sera redissolved at initial volume

Group	Native sera	3% PEG precipitated sera
Control		
1	25	1
2	52	1
3	73	3·5
4	75	1
5	180	1·1
6	325	1
Schistosomiasis		
1	65	15
2	902	36
3	5058	36
4	6816	43

Table 3. Results of dissociation–reassociation of polyethylene glycol precipitated (PEG precipitate) components in presence of ^{125}I-labelled *S. mansoni* antigen. Percentage of radioactivity in the pellets after a second 3% PEG precipitation.

Group	Percent of radioactivity in PEG precipitate after dissociation–reassociation
Control	
1	4·4
2	6
Schistosomiasis	
1	15·5
2	10·6
3	9·2
4	8

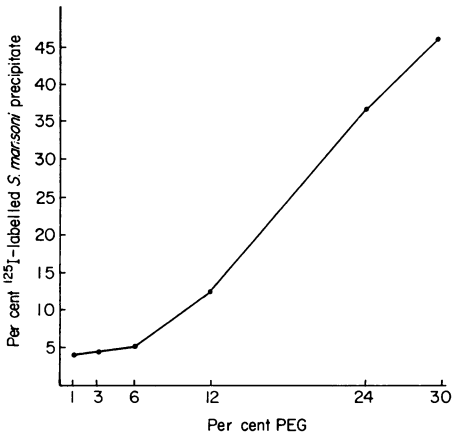

Figure 3. Solubility of specific *S. mansoni* antigen in various concentrations of polyethylene glycol and in the presence of normal human serum.

(c) Characterization of components in 3% PEG precipitates of human sera

In our experimental conditions, IgG was found in eight out of ten PEG precipitates from randomly selected infected patients, and IgM in five out of ten; IgA was not found.

Radioimmunoassay of IgE was carried out using both whole sera and 3% PEG precipitate redissolved at initial volume. A relatively high amount of IgE was found in immune complexes (Table 2).

Parasite-specific antibodies were detected and quantified by the technique of dissociation–reassociation in 3% PEG precipitates (Table 3). It was shown that, if a PEG concentration lower than

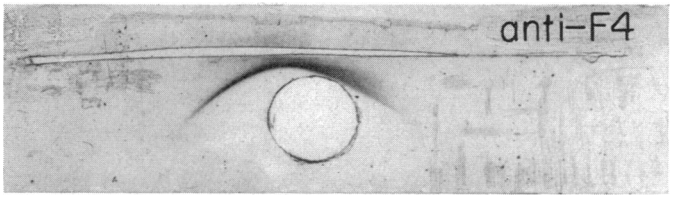

Figure 4. Characterization of *S. mansoni* antigen in 3% polyethylene glycol precipitated (PEG precipitate) serum of infected patient, by immunoelectrophoresis.

6% was used, the amount of antigen precipitated in normal human serum was low (Fig. 3).

Parasite antigen was demonstrated by immunoprecipitation. By the double diffusion test using anti-*S. mansoni* hyperimmune rabbit serum, one or two precipitation bands were obtained in two out of twenty studied PEG precipitates. By immunoelectrophoresis, in one case, a distinct precipitation line was observed using monospecific anti-antigen '4' hyperimmune rabbit serum (Fig. 4). This hyperimmune serum demonstrated no band against a lower than 8% PEG precipitate of *S. mansoni* whole extract.

DISCUSSION

Circulating immune complexes were detected in the sera of schistosomiasis patients by three different techniques. The Clq-binding test proved the most sensitive, but there was good correlation between the results of this and the much simpler CFT and OD tests. These latter tests would seem adequate for at least preliminary investigations on CIC levels in various populations of schistosomiasis patients.

In all, 60% of the infected sera contained CIC, but it is not clear why the levels were higher in the mild form of the disease than in the hepatosplenic form. The reverse was expected as it was in patients with hepatosplenic disease that Andrade *et al.* (1971) demonstrated glomerular deposits. Seemingly there is no simple relationship between CIC and schistosome-associated glomerular nephropathies, and further studies will be required to elucidate the immunopathological role of the immune complexes in man. In contrast, the maximum anticomplementary activity in the sera of infected mice was seen during the very period in which Natali *et al.* (1974) were able to elute immune complexes from the kidney.

Specific parasite antigen, IgG, IgM and IgE immunoglobulins were all detected in the CIC. They must have been present in a complexed form as free specific *S. mansoni* antigen is soluble in PEG concentrations up to at least 6 per cent (Fig. 3) and uncomplexed human immunoglobulins do not precipitate below 5% (Creighton *et al.*, 1974). Precipitation at 3% PEG therefore readily distinguishes CIC from free parasite antigens and antibodies. These preliminary studies indicated that relatively large amounts of IgE were present in the CIC.

Total IgE and parasite specific IgE levels are known to be high in schistosomiasis (Kellermeyer, Warren, Waldman, Cook & Jordan, 1973 and Dessaint, Capron, Bout and Capron, 1975) and it has been recently reported that rat IgE antibodies (Capron, Dessaint, Capron and Bazin, 1975) and possibly IgE–antigen complexes (Capron, Dessaint, Joseph, Rousseaux & Bazin, 1976) are involved in the adherence of normal rat macrophages to *S. mansoni* schistosomules, a step followed by the *in vitro* killing of these juvenile parasites. It may be that the IgE-containing CIC detected in human sera have a protective role.

Alternatively, it is known from a number of experimental systems that CIC may have an immunosuppressive function (Gorczynski, Kilburn, Knight, Norbury, Parker & Smith, 1975). Schistosomes survive in the blood of their hosts for long periods and it may be that immunosuppression mediated by CIC aids this survival (Wilson, 1974). Further investigations on the part played by immune complexes in immunity against schistosomes are underway.

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