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Circulating antigens, immune complexes and C3d levels in human schistosomiasis: relationship with *Schistosoma mansoni* egg output

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SUMMARY

Circulating schistosome antigens (CSA), circulating immune complexes (CIC) and C3 breakdown product – C3d – were investigated in human schistosomiasis in comparison to the *S. mansoni* egg count. A close relationship was observed between the mean number of eggs/g of stool and the detection of CSA (evaluated by the radioimmunoprecipitation–PEG assay – RIPEGA), CIC (C1q-binding test) and C3d levels (quantitated by radial immunodiffusion). All the patients with more than 500 *S. mansoni* eggs/g of stool also presented antigen '4', specific of the genus Schistosoma, in the serum. A significant correlation was noticed between levels of CSA and CIC. This suggests the involvement of several schistosome antigens in the detected CIC. No relationship was noted between CIC and C3d levels. In contrast, there was a highly significant correlation between levels of CSA and C3d. The interaction between certain schistosome antigens and the complement system is discussed.

INTRODUCTION

Circulating schistosome antigens (CSA) have been found in a variety of animals (Berggren & Weller, 1967; Houba *et al.*, 1976; Deelder *et al.*, 1976; Santoro *et al.*, 1978a & b; Carlier, Bout & Capron, 1978) and man infected with *Schistosoma mansoni* (Carlier *et al.*, 1975; Madwar & Voller, 1975; Santoro *et al.*, 1977, 1978b). These findings associated with the detection of anti-*S. mansoni* antibodies in schistosomiasis (Capron *et al.*, 1968; Camus *et al.*, 1977) provide good support for the presence of circulating immune complexes (CIC) in both human (Bout *et al.*, 1977; Smith *et al.*, 1977; Brito *et al.*, 1979; Lawley *et al.*, 1979) and experimental infections (Santoro *et al.*, 1978a, 1979; Deelder, Van Dalen & Van Egmond, 1978; Digeon *et al.*, 1979). The involvement of these CIC in the renal injury observed in human and experimental schistosomiasis has been suspected by several workers over the last few years (Andrade & Rocha, 1979; Houba, 1979). In fact, the demonstration of glomerular deposits of immunoglobulins, C3 component of complement and schistosomal antigens (Falcão & Gould, 1975; Hoshino-Shimizu *et al.*, 1976) in patients infected with *S. mansoni* suggests an immune complex mechanism which probably requires activation of complement.

In the present study, we have investigated simultaneously CSA, CIC and complement through the detection of C3 breakdown product – C3d – in human schistosomiasis in comparison to the *S. mansoni* egg output.

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MATERIALS AND METHODS

Patients. A total of 121 male Brazilian subjects with a mild form of chronic schistosomiasis were studied. All these patients, who were students at the Navy School of Recife aged between 18 and 20 years, were living in similar conditions of activity and nutrition. The *S. mansoni* infections were monitored by identification and output of viable parasite eggs in the stool according to the Kato technique adapted by Katz, Chaves & Pellegrino (1972) and by serological investigations (immunoelectrophoresis and haemagglutination). The patients were classified under three groupings according to the geometric mean egg count/g of stool of three different examinations: group I, 63 infected patients eliminating less than 100 eggs/g of stool; group II, 43 patients eliminating between 101 and 1,000 eggs; group III, 15 patients eliminating more than 1,000 eggs/g of stool. Thirty uninfected subjects living in the same conditions formed the control group (NHS). Blood was collected and was allowed to clot at room temperature for 90 min. Serum was separated by centrifugation and used after storage at −30°C. For C3d studies, blood of 53 infected patients and of the 30 control subjects was collected in EDTA at a final concentration of 20 mmol. The plasma was separated by centrifugation and stored at −70°C.

Reagents. C1q complement component was isolated from fresh normal human serum according to Yonematsu & Stroud (1971) as modified by the authors (Santoro *et al.*, 1980a) and labelled with ^{125}I. Anti-*S. mansoni* hyperimmune antiserum was prepared against a whole extract of adult schistosomes according to the technique of Capron *et al.* (1968). A monospecific rabbit serum against the antigen '4', specific for the genus Schistosoma (Capron *et al.*, 1968), was prepared as previously described (Bout *et al.*, 1978; Santoro *et al.*, 1979). The Ig fraction from these antisera was obtained by affinity chromatography with the soluble extract of *S. mansoni* antigen and radioiodinated following the method described by Morrison, Bayse & Webster (1971). Normal rabbit Ig were obtained by ammonium sulphate precipitation according to Heide & Schwick (1978).

A monospecific anti-C3d antiserum was purchased from the Netherlands Red Cross (Amsterdam, The Netherlands).

C1q-binding test. The radiolabelled C1q-binding was performed according to Zubler & Lambert (1976). Briefly, ^{125}I-C1q was mixed with serum, previously treated with 0·2 M EDTA. Free C1q was separated from C1q bound to complexes by precipitation with 3% polyethylene glycol (PEG, mol. wt 6,000). Results were expressed as percentage ^{125}I-C1q precipitated as compared with the protein-bound radioactivity precipitable with 20% trichloroacetic acid.

Radioimmunoprecipitation–PEG assay (RIPEGA). The RIPEGA was performed as described previously by the authors (Santoro *et al.*, 1978b) with minor modifications. In brief, 0·2 ml of test serum previously adsorbed with normal rabbit Ig for 2 hr at 37°C and diluted 1/5 in borate buffer (0·1 M, pH 8·4), was incubated with 0·2 ml of radioiodinated anti-*S. mansoni* antibodies (^{125}I-anti-Sm Ab) or radioiodinated anti-antigen '4' antibodies (^{125}I-anti-F4 Ab) for 1 hr at 37°C followed by 2 hr at room temperature. Free radioiodinated antibodies were separated from those bound to specific antigens by precipitation with 7% PEG. Results were expressed as percentage ^{125}I-anti-Sm Ab or ^{125}I-anti-F4 Ab precipitated as compared with the protein-bound radioactivity precipitable with 20% trichloroacetic acid.

C3d assay. The C3 breakdown product, C3d, levels were quantitated in plasma EDTA according to Perrin, Lambert & Miescher (1975). Briefly, native C3 and the high molecular weight fragments C3b and C3c were precipitated with PEG at a final concentration of 12%. In a second step, the C3d was measured in the PEG supernatant by single radial immunodiffusion with the anti-C3d antiserum. The results were expressed as per cent C3d as compared with the levels obtained with a pool of normal human serum treated with 15 mg/ml inulin.

Statistical evaluation. Results were analysed by the non-parametric correlation coefficient of Spearman (R), the parametric correlation test (r), the chi-square test, analysis of variance, or Student's t-test when required.

RESULTS

Circulating immune complexes. The results of investigation of circulating immune complexes (CIC) by the ^{125}I-C1q-binding test in sera from the three groups of infected patients (classified

according to the *S. mansoni* egg output) and in control subjects are shown in Fig. 1. A highly significant difference was observed between CIC levels in infected patients and in normal subjects ($P<0{\cdot}001$). In fact, more than 70% of the patients with schistosomiasis showed elevated ^{125}I-C1q-binding activity. Moreover, a significant difference was also noticed between IC levels in the different groups of infected patients. These data indicated a direct relationship between egg output and levels of CIC in human schistosomiasis.

Circulating schistosome antigens. Total circulating schistosome antigens (CSA) were studied by the RIPEGA with ^{125}I-labelled anti-Sm Ab in the serum from both normal and infected subjects cited above (Fig. 2). As for CIC, the mean levels of CSA in the majority of the infected patients were significantly higher than in control subjects ($P<0{\cdot}001$). Moreover, a close relationship was also observed between *S. mansoni* egg count and levels of CSA.

Correlation between CIC and CSA. The relation observed between *S. mansoni* egg output and both CIC and CSA caused us to investigate a possible relationship between these two immunological parameters of human schistosomiasis (Fig. 3). A significant correlation ($R=0{\cdot}4916$; $2P<0{\cdot}001$) was noted between CIC and CSA.

Circulating antigen '4'. Results for the detection of antigen '4' by the RIPEGA with ^{125}I-labelled anti-F4 Ab in patients infected with *S. mansoni* are shown in Fig. 4. Here also, the mean level of ^{125}I-labelled anti-F4 Ab precipitation in infected patients was significantly higher than in control subjects. No significant difference in antigen '4' levels was noticed between patients with 3–100 eggs/g of stool and those with 101–1,000 eggs. However, all the patients with more than 500 eggs of *S. mansoni*/g of stool, as indicated in Fig. 4, showed circulating antigen '4'. No correlation was observed between levels of antigen '4' and both CIC and CSA in human schistosomiasis.

C3d levels. Levels of C3d were quantitated in plasma EDTA of the 30 control subjects and 53 patients infected with *S. mansoni* (Fig. 5). A high percentage of positive results was found in infected patients (86%). Moreover, a direct relationship was also noticed between egg count and C3d levels. In fact, patients with more than 100 *S. mansoni* eggs/g of stool showed C3d levels significantly higher than those with 3–100 eggs.

Correlation between C3d and the immunological parameters. The high levels of C3d observed in schistosomiasis suggest an activation of complement by the CIC present in infected patients. Nevertheless, when C3d and CIC levels were compared, no correlation was observed ($r=0{\cdot}14$; $P>0{\cdot}1$). In addition, no relation was noticed between C3d and antigen '4' levels ($r=0{\cdot}12$; $P>0{\cdot}1$).

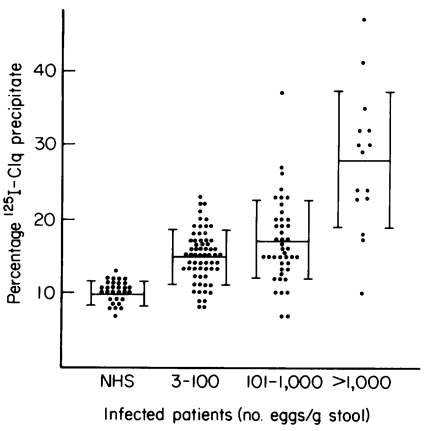

Fig. 1

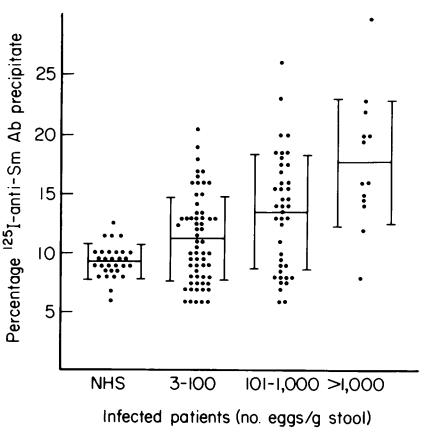

Fig. 2

Fig. 1. C1q-binding circulating immune complexes (CIC) in human schistosomiasis. Relationship to the mean of three determinations of *S. mansoni* egg count. NHS = control subjects.

Fig. 2. Circulating schistosome antigens (CSA) in patients infected with *S. mansoni* measured by the radioimmunoprecipitation–PEG assay (RIPEGA) with ^{125}I-anti-*S. mansoni* antibodies. Relationship to egg output. NHS = control subjects.

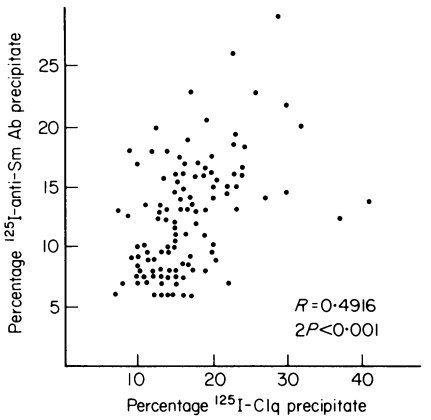

Fig. 3

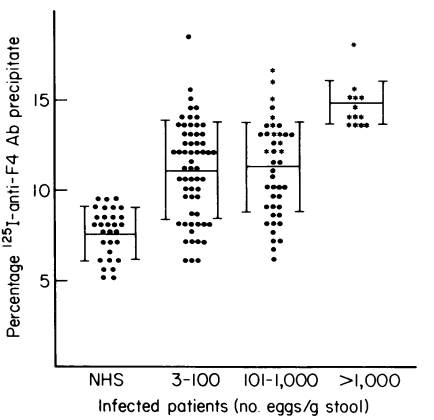

Fig. 4

Fig. 3. Correlation between circulating schistosome antigens (CSA) and circulating immune complex (CIC) levels in human schistosomiasis of *S. mansoni*.

Fig. 4. Detection of circulating antigen '4' in patients infected with *S. mansoni* by the RIPEGA with ^{125}I-anti-antigen '4' antibodies. * Patients with more than 500 eggs/g of stool. NHS = control subjects.

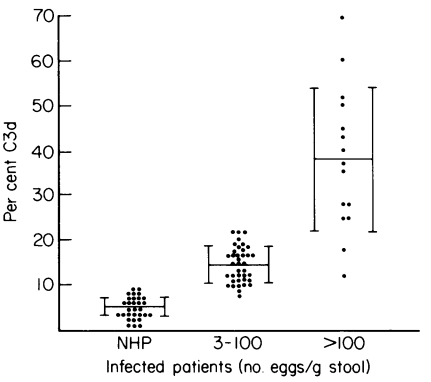

Fig. 5

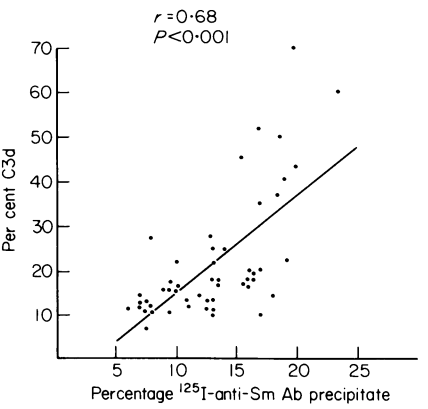

Fig. 6

Fig. 5. Levels of C3 breakdown product (C3d) in human schistosomiasis of *S. mansoni*. Relationship to the *S. mansoni* egg count. NHP = plasma of control subjects.

Fig. 6. Correlation between levels of C3d and circulating schistosome antigens (CSA) in patients infected with *S. mansoni*.

In contrast, a highly significant correlation was noted between levels of C3d and CSA in human schistosomiasis (Fig. 6).

DISCUSSION

Previous studies have established the presence of circulating antigens and immune complexes in human and experimental schistosomiasis (Bout *et al.*, 1977; Santoro *et al.*, 1978b, 1979). However, the involvement of these immunological substances in schistosomal nephropathy, although suggested by several workers, remains to be demonstrated. In fact, renal injury in schistosomiasis has been shown to be associated with even lower levels of CIC than are found in patients without renal disease (Brito *et al.*, 1979).

In the present investigation a close relationship was observed between *S. mansoni* egg output

and the detection of CSA, CIC and C3d in human schistosomiasis. In a previous study in man, Carlier *et al.* (1975) also noticed a close correlation between parasite 'M' antigen in urine from infected patients and the number of *S. mansoni* eggs in the stool. Cheever (1968) observed a direct relation between the faecal egg count and the number of worms recovered *post mortem* in human schistosomiasis. All these findings suggest strongly that CSA, CIC and C3d levels are related to the number of worms in human schistosomiasis.

Antigen '4' was also detected in serum from patients infected with *S. mansoni*. This circulating antigen, previously characterized in CIC (Bout *et al.*, 1977) and in milk from infected patients (Santoro *et al.*, 1977), was present in all the sera from patients with more than 500 eggs/g of stool. These data and the greater number of cases analysed here confirm the results of our earlier study (Santoro *et al.*, 1978b). They also suggest a possible relation between schistosome-specific antigen'4' and the number of worms in the host. The detection of schistosome antigens in serum from patients infected with *S. mansoni* and their relationship with the egg count may be very important for the diagnosis and control of this disease. In fact, the RIPEGA with specific ^{125}I-labelled anti-*S. mansoni* antibodies for the detection of CSA appears as a new method of evaluating the worm burden in the schistosomiasis of *S. mansoni*.

A close correlation was noticed between levels of CSA and CIC in human schistosomiasis of *S. mansoni*. This observation suggests the involvement of schistosome antigens in the CIC detected. Among these antigens, the circulating antigen '4' does not appear to be involved in most of the CIC detected in human schistosomiasis since no correlation was noticed between these two immunological parameters. By contrast, in murine schistosomiasis circulating antigen '4' and CIC were detected at the same time during the course of infection (Santoro *et al.*, 1979). Further studies on the schistosome antigens making up the immune complexes in human and murine schistosomiasis are necessary to clarify this discrepancy.

High amounts of C3d were observed in patients infected with *S. mansoni*. These findings should be related to the observations of Verroust *et al.* (1979) concerning the high levels of C3d in human parasitosis. All these data strongly suggest an activation of complement *in vivo* in some parasitic infections. Moreover, the presence of C3d in several connective tissue diseases has been shown to be associated with the detection of CIC (Nydegger *et al.*, 1977). However, when the levels of C3d were compared to those of the CIC quantitated in human schistosomiasis, no significant correlation was noticed. This result does not suggest an association between CIC and C3d in human schistosomiasis. In contrast, a highly significant correlation was observed between C3d and CSA. This relationship suggests strongly the involvement of certain antigens present in CSA in the appearance of high C3d levels in patients infected with *S. mansoni*. The mechanism by which these schistosome antigens are acting on C3 is not yet known. However, two possibilities can be advanced to explain this phenomenon: firstly, certain antigens released by the schistosomes in the blood of the infected patients could activate complement, probably by the alternative pathway. This would be followed by the production of C3 breakdown products in the blood circulation. There are some factors to the advantage of this first hypothesis: (1) the close relationship observed between CSA and the anti-complementary activity detected during the course of the *S. mansoni* infection in mice (Santoro *et al.*, 1979); and (2) the activation of complement by certain low molecular weight antigens purified from the antigenic extract of adult schistosomes (Santoro *et al.*, 1980b). Alternatively, the appearance of high levels of C3d in human schistosomiasis would be due to the action of the proteinases released by the schistosomes. In this case, these parasitic enzymes would react directly with C3 producing C3d. Further studies on the interaction between schistosome antigens and the complement system are in progress.

In a previous study, Brito *et al.* (1979) suggested the retention of certain schistosome antigens derived from circulating blood in the renal glomeruli of patients infected with *S. mansoni* as a possible factor in the initiation of kidney injury in human schistosomiasis. The possible activation of complement by these antigens in either the presence or absence of antibodies could be the second step in the mechanism of the schistosomal nephropathy.

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