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Detection and quantification of circulating antigen in schistosomiasis by monoclonal antibody II. The quantification of circulating antigens in human schistosomiasis mansoni and haematobium: relationship to intensity of infection and disease status

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SUMMARY

Circulating cathodic and circulating anodic antigens were quantified in sera of patients infected with *S. mansoni, S. haematobium* or both parasites. A monoclonal antibody and a polyclonal antiserum were applied in precipitation and solid phase immunosorbent techniques using radio- and enzyme-labelled antibody as a tracer to detect the cathodic and anodic antigen respectively. The results show that circulating cathodic antigen can frequently be detected in an immunoprecipitation or an immunoradiometric assay in serum of infected patients. The serum concentration of this antigen was found to be significantly correlated to the number of *S. mansoni* worms and to be higher in patients with the hepatosplenic form of the disease than in those without such complications. Examining paired serum samples before and after specific treatment the determination of this antigen by monoclonal antibody reliably indicated efficacy of chemotherapy in patients having received different forms of treatment.

Keywords human schistosomiasis circulating schistosome antigens effect of treatment

INTRODUCTION

For many years it has been known that adult schistosomes release antigenic material into the circulation of their mammalian hosts (Okabe & Tanaka, 1961; Nash, 1974). Such antigens have been identified in experimental as well as in human infections caused by *S. mansoni, S. japonicum* and *S. haematobium* (Deelder *et al.*, 1976; Quian & Deelder, 1983; Ismail, James & Webbe, 1984). In schistosomiasis mansoni these antigens have been detected in serum, urine, milk and glomerular deposits of infected animals and humans (Carlier *et al.*, 1975; Santoro *et al.*, 1977; Carlier, Bout &

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Capron, 1980). Recent work has demonstrated that major circulating antigens released by adult schistosomes are polysaccharides with a wide molecular weight range (Carlier *et al.*, 1980). According to their electrophoretic mobility these antigens have been classified as circulating anodic antigen (CAA) and circulating cathodic antigen (CCA), respectively (Deelder *et al.*, 1976). The former is probably identical to the gut-associated proteoglycan antigen (GASP) (Nash, Prescott & Neva, 1974), the latter to the so-called M-antigen (Carlier, Bout & Capron, 1978).

In a companion paper we reported the production of a monoclonal antibody (40 B:1) derived from a mouse-mouse hybridoma which recognizes CCA with high specificity (Nogueira-Queiroz *et al.*, 1986). Based on the promising results of an earlier investigation to quantify antigenemia by polyclonal antiserum (Santoro *et al.*, 1980), we decided to assess the value of the monoclonal antibody 40 B:1 as a diagnostic tool in various presentations of schistosomiasis mansoni and haematobium. The results show that CCA can frequently be detected in the serum of infected patients, that the concentration of CCA is significantly related to the number of *S. mansoni* worms and higher in patients with the hepatosplenic form of the disease than in those without such complications.

MATERIALS AND METHODS

Patients and controls. Three different groups of patients were investigated. The first group consisted of 143 school boys living in a hyperendemic area in the Sudan. They ranged in age from 6 to 11 years (median 8 years; 95% confidence limits 7 to 9 years). These patients were concomitantly infected with *S. mansoni* and *S. haematobium* as indicated by constant excretion of ova in stool *and* urine. They had never received treatment.

The second group consisted of 25 patients from various endemic areas of the Sudan. They all showed disease-associated pathology such as hepatosplenomegaly, collateral circulation, ascites and/or nephrotic syndrome. These patients were rather heterogeneous in age (range 7–35 years; median 13 years) as well as in the expression of pathology. Thirteen were female and 15 were male. All of them were infected by *S. mansoni*, eight showed a concomitant infection with *S. haematobium*, nine patients had received antischistosomal treatment with oxaminquine 3 to 9 months prior to admission of the study.

The third group comprised 28 patients (13 female and 15 male) from Ceara state in Northeastern Brazil. Their ages ranged from 13 to 44 years (median 27 years). None of these patients showed evidence for hepatosplenic or renal complications. Prior to admission to the study they had not received treatment.

Patients of each group were carefully matched with apparently healthy individuals from the same endemic area living under the same socioeconomic conditions. Inhabitants from a village near Khartoum were selected as controls for the first and the second group of patients. Sixteen adults from the patients' village in Ceara were used as controls for the third group. In individuals selected as controls schistosomiasis was excluded by repeated urine and stool examination as described below. Intestinal parasites (namely hookworm, *Ascaris lumbricoides, Hymenolepis nana, Entamoeba histolytica*) were present in a similar frequency as in the patient groups.

Clinical and parasitological examinations. All patients had a complete clinical examination. Hepatomegaly and splenomegaly were measured according to clinical standards and expressed in centimetres. Malaria was excluded by a thick blood film. Proteinuria, haematuria and leukocyturia were semi-quantitatively assessed by means of urine analysis reagent strips (Nephur, Boehringer, Mannheim, FRG). Egg excretion in urine was quantified by the filtration trypan blue staining technique (Feldmeier, Bienzle & Dietrich, 1979). Filtration of the whole micturition volume (up to 300 ml) was repeated on three consecutive days. Results were expressed as number of viable ova per 10 ml of urine. Ova excretion in stool was quantitively assessed by a modification of the Kato concentration technique (Blagg *et al.*, 1955). Five thick smears of 25 mg each were prepared from each of three stool samples obtained on three consecutive days. In addition, from each stool sample, one gram of stool was examined by the MIF concentration technique. Egg excretion was expressed as number of ova per gram of stool.

Treatment and follow-up of patients. The mixed infected children from the Sudan were randomly divided into four subgroups, which received praziquantel (40 mg/kg), oxamniquine (60 mg/kg) and metrifonate (3 times, 10 mg/kg), respectively. This stratification was based on the rationale that oxamniquine selectively eliminates *S. mansoni* worms, metrifonate acts on adult worms dwelling in the perivesical plexus (predominantly *S. haematobium*), and praziquantel is similarly effective against both parasite species (Doehring, Poggensee & Feldmeier, 1986). To eliminate bias due to spontaneously occuring variation of egg excretion in urine and stool, a fourth subgroup was treated with a polyvitamin preparation. Patients were rexamined 4 months after initiation of chemotherapy.

Patients still excreting ova at month 4 as well as all patients not admitted to the randomized study were treated with praziquantel. Prior to investigation informed consent was obtained in the mother language of the patients.

Preparation of antigens and antibodies. The Puerto Rico strain and the Liberia strain of *S. mansoni* were maintained in *Biomphalaria glabrata* snails and golden hamsters.

About 2,000 adult worms (Puerto Rico strain), recovered by perfusion from infected hamsters, were incubated in 5 ml of distilled water for 3 h at 37°C. The supernatant was recovered by centrifugation, sterilized by filtration through 0·22 μm membranes and lyophilized. Immunization of BALb/c mice, fusion of cells and selection of hybrids is described in the accompanying paper. Among monoclonal antibodies of these hybrids, an IgG3 produced by clone 40 B: 1 was shown to recognize a repetitive epitope of CCA, to precipitate CCA and to yield a characteristic immunofluorescence pattern on fixed sections of adult *S. mansoni* worms (Nogueira-Queiroz *et al.*, 1986). It was concentrated from ascites by means of precipitation with ammonium sulfate and purified by subsequent ion-exchange chromatography. Labelling was performed by the chloramin T method (Hunter & Greenwood, 1962).

Adult worms (Liberia strain) were lyophilized and a trichloroacetic acid fraction of the crude antigen was prepared as described by Carlier *et al.* (1980). The antigen was further fractionated by ion-exchange chromatography using DEAE-Sephacel. Fractions containing CAA were identified by immunoelectrophoresis using a rabbit hyperimmune serum against adult worm antigen. These fractions were pooled, lyophilized and then used for immunization together with complete Freund's Adjuvant. To obtain the IgG fraction of the corresponding rabbit antiserum, 1 ml aliquots were passed over a column containing 6 ml of Protein A-Sepharose. Antibodies were purified from the IgG fraction by immune affinity chromatography using trichloroacetic acid (TCA)-soluble adult worm antigen coupled to CNBr-activated Sepharose. Their $F(ab')_2$ fragments were obtained by pepsin digestion and subsequent molecular sieving on a Sephadex 200 column.

RIPEGA, IRMA and ELISA for quantification of CCA and CAA. The radioimmunoprecipitation polyethylene glycol (PEG) assay (RIPEGA) was performed as described by Santoro *et al.* (1978). PEG was used at a concentration of 8%. The specific activity of labelled 40 B: 1 was 1 μCi/μg. Results were expressed as the percentage of total radioactivity bound (%TA).

A two site-immunoradiometric assay (2S-IRMA) using polyvinyl microtitre plates (Becton-Dickinson, Oxford, UK) as a solid phase (Nogueira-Queiroz *et al.*, 1986) was used with the following modifications. Prior to coating, plates were sensitized with polymerized glutaraldehyde. For coating, the plates were filled with 100 μl of the antibody (10 μg/ml carbonate buffer pH 9·6) and kept for 48 to 72 h at 4°C. Aliquots of serum samples—diluted 1:1 to 1:5 in PBS (0·01 M, pH 7·4)—were added and incubated for 3 h at 20°C. Results were expressed as net counts per minute (ct/min).

To reduce non specific binding, 300 μl volumes of the different serum dilutions were incubated overnight with polystyrene beads coated with mouse IgG, before RIPEGA and 2S-IRMA were performed.

The ELISA was performed in round bottom, cobalt sterilized microtitre plates (Greiner, Nürtingen, FRG). The $F(ab')_2$ fragments of the immune affinity-purified antibodies (17 μg/ml carbonate buffer) were added (200 μl) to each well and incubated for 48 h at 4°C. Sera were diluted 1:1 to 1:5 in PBS and incubated for 1 h at 20°C. Immune affinity-purified rabbit IgG was labelled by the glutaraldehyde method (Engvall & Perlmann, 1972) with alkaline phosphatase (immunoassay grade, Boehringer, Mannheim, FRG). The incubation of the second antibody was done overnight at 4°C. *p*-Nitrophenylphosphate diluted in diethanolamine buffer was used as the substrate. The

optical density (O.D.) was read at 405 nm. In all assays optimal dilutions of the first and the second antibody were determined by checkerboard titration. To assess the detection limit under optimal assay conditions, crude TCA-soluble adult worm antigen was diluted in the serum of a healthy donor.

ELISA for antischistosome antibodies. IgG antibodies to crude *S. mansoni* cercarial and adult worm antigen were determined by ELISA. The assay was performed as previously described (Feldmeier & Büttner, 1983). Results were expressed as multiples of normal activity (MONA; Felgner, 1978).

Determination of circulating immune complexes. Circulating immune complexes were determined by binding to C1q. A micro-ELISA using polystyrene microtitre plates was used (Lunde, 1983). Results were expressed as units/litre (Stevens *et al.*, 1983).

Statistics. As the results of most experiments were not normally distributed and because variances varied considerably, the median and the 95% confidence limits thereof were calculated. Accordingly, statistical significance was computed by the Mann-Whitney-test and the Wilcoxon matched pairs signed rank test. Significance of regression analysis was assessed by the Spearman rank correlation coefficient test.

To determine a cut-off limit above which the immunoassays were considered to indicate specific antigen-antibody reactions, data of all controls were combined and used to calculate the upper 90th percentile as described by Reed, Henry and Mason (1971).

RESULTS

Clinical and parasitological findings

Mixed infected children from the Sudan. Physical examination was uneventful except for two cases of moderate hepatosplenomegaly. Abnormal proteinuria (> 100 mg/l), haematuria (> 5 erythrocytes/μl) and leukocyturia (> 20 leukocytes/μl) were present in 73%, 84% and 77% of the patients, respectively. Egg counts varied considerably between individuals. Egg excretion in stool ranged from 8 to 4,629 per gram (95% confidence limits 525–929), egg excretion in urine from 0·2 to 9,009 per 10 ml (95% confidence limits 40–242).

Patients with hepatosplenic schistosomiasis. Disease associated symptoms occurred in the following frequencies: hepatomegaly 100%, splenomegaly 80%, collateral circulation (as indicated by caput medusae or bleeding of oesophageal varices) 20%, ascites 55%, renal complications (generalized oedema and/or proteinuria ≥ 2 g/l) 20%. Egg excretion in stool varied from 0 to 5,154 (95% confidence limits 16–535). In eight patients a concomitant infection with *S. haematobium* was detected; however, egg excretion in urine was less than six ova per 10 ml.

Patients with S. mansoni-infection from Brazil. Physical examination did not reveal any sign of disease. The intensity of infection was low (median 20 ova per gram, range 2 to 447).

No malaria parasites were seen in the thick blood films of any of the patients. Onchocerciasis and lymphatic filariasis do not occur in the areas inhabited by patients and controls.

Detection of CCA and CAA

Figures 1a and 1b depict the individual results of the determination of CCA and CAA in the three patient and endemic control groups. Using results derived from both the dose-response curves and the 90th percentiles of the data obtained from all the controls as a cut-off limit, the RIPEGA could detect CCA in a concentration above 300 ng/ml. The 2S-IRMA and the ELISA detected CCA and CAA in a concentration of 150 and 125 ng/ml, respectively. However, the RIPEGA and the 2S-IRMA correctly identified diseased patients in similar frequencies. The positivity rate was 77 versus 74% for the mixed infected children, 60 versus 80% for patients with hepatosplenic complications and 32 versus 11% for the Brazilian patients with mild intestinal schistosomiasis. CAA was detected in the two former patient groups in considerably lower frequency (27% and 44%, respectively). Individual measurements of CCA by the RIPEGA and the 2S-IRMA significantly correlated in all patient groups examined (rho = 0·67, $P < 0{\cdot}001$). The correlation between CCA and CAA levels was lower (rho = 0·35, $P < 0{\cdot}05$).

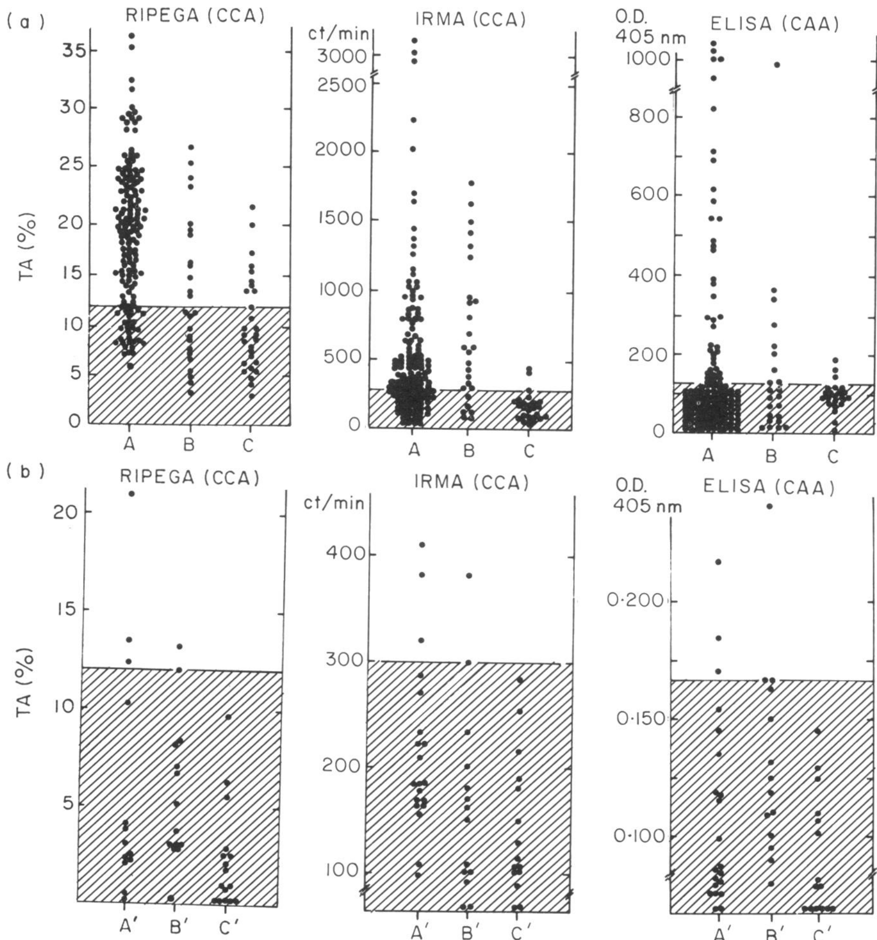


Fig. 1. (a) Quantification of CCA and CAA in the serum of patients with the various presentations of schistosomiasis. (A) Sudanese patients with a mixed *S. mansoni* – *S. haematobium* infection; (B) Sudanese patients with hepatosplenic schistosomiasis; (C) Brazilian patients with intestinal *S. mansoni* infection. The RIPEGA and the 2S-IRMA were applied to determine CCA by using the monoclonal antibody 40 B:1. For detection of CAA a polyclonal antiserum was used in an ELISA (see Materials and Methods). The shaded area represents the upper 90th percentile of the combined results of the three endemic control groups. (b) Quantification of CCA and CAA in the serum of age and sex matched controls from the same endemic areas as the patients (see Materials and Methods). (A′, B′) Healthy individuals from the Sudan; (C′) healthy individuals from Brazil. The shaded area represents the upper 90th percentile of the combined results of the three endemic control groups.

Detection of CIC and antischistosome antibodies

Results of C1q-binding tests for detection of CIC and of the quantification of anti-cercarial and anti-adult worm antibodies are summarized in Table 1. A significant higher concentration of C1q-binding CIC was observed in patients with hepatosplenic schistosomiasis than in those with an infection limited to the intestinal and urinary tract ($P < 0{\cdot}01$). No increase in CIC levels was found in the lightly infected patients from Brazil. Circulating immune complexes were present in similar concentration in the three control groups. In neither patient group the concentration of C1q-binding immune complexes was related to the amount of circulating schistosome antigen.

The levels of anti-cercarial and anti-adult worm antibodies were different in each patient group.

Table 1. CIC and antischistosome antibodies in the different patient and control groups. Data indicate the median, 95% confidence limits and % positive. The percentage of positive results was calculated by using the 90th percentile of the combined results of all controls as the cut-off value (see Material and Methods)

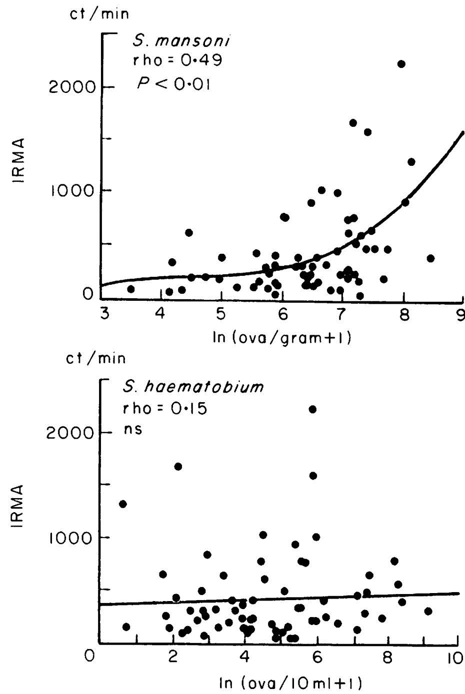
Designation	Group description	Number examined	C 1q-binding immune complexes (units/litre)	IgG-antibodies to Cercarial antigen (MONA)	IgG-antibodies to Egg antigen (MONA)
A	mixed *S. mansoni*–*S. haematobium* infection (Sudan)	143	1·18 0·95–1·25 33%	68·4 61·0–77·9 100%	51·9 46·5–57·0 100%
B	hepatosplenic schistosomiasis (Sudan)	25	1·44 1·34–1·48 88%	28·9 18·5–43·6 79%	23·0 11·7–25·6 94%
C	*S. mansoni* infection (Brazil)	28	0·85 0·75–0·98 21%	16·6 11·1–22·1 72%	23·4 13·2–29·0 100%
A′	Age-matched controls of group A (Sudan)	14	1·10 0·73–1·21 29%	2·3 0·6–5·7 17%	1·1 0·3–1·4 6%
B′	Age-matched controls of group B (Sudan)	20	1·05 0·84–1·19 25%	1·2 0·2–1·9 0%	0·9 0·1–1·2 0%
C′	Age-matched controls of group C (Brazil)	16	1·03 0·54–1·22 28%	1·5 0·1–3·4 8%	1·0 0·2–1·1 22%

Antibodies to both antigens were highest in the mixed infected patients from the Sudan and lowest in the lightly infected patients from Brazil. In hepatosplenic patients the antibody levels were comparatively low, however 79 and 94% of patients were correctly classified as positive by either method.

Relation to intensity of infection and disease status

In order to analyse whether the amount of circulating antigen was related to the parasite load, regression analysis was performed. Fig. 2 clearly indicates that, in the mixed infection children from the Sudan the concentration of CCA paralleled egg excretion in stool (rho = 0·49, $P<0·01$; upper part of the figure), but that there was no significant relationship between CCA and egg excretion in urine (rho = 0·15, $P>0·1$; lower part of the figure). Interestingly, a similar relationship could be demonstrated in the Sudanese patients with hepatosplenic schistosomiasis. Although these patients were quite heterogeneous in the expression of disease-associated pathology, the number of *S. mansoni* ova excreted in stool also correlated to the concentration of CCA (rho = 0·56, $P<0·01$). Hence, using the cut-off limit as defined above, the 2S-IRMA was calculated to detect CCA in a concentration corresponding to an egg excretion of 35 ova per gram of stool, which is the limit of sensitivity of a single Kato thick smear.

To attempt a classification of progression of disease, hepatosplenomegaly was quantified and used as an index of severity. Figure 3 shows the surprising observation that in patients with liver fibrosis, low and high concentrations of CCA appear to be reflected clinically by a moderate and a high degree of hepatosplenomegaly, respectively. However, the difference between the two subgroups is at the limit of statistical significance ($P=0·06$).



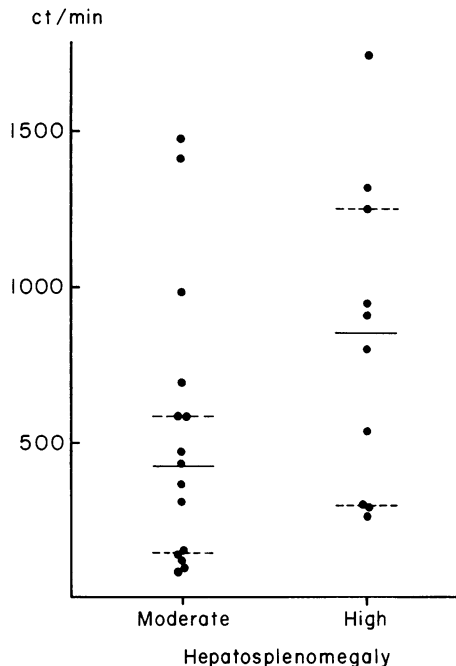


Fig. 2. Regression analysis between egg excretion in stool and the concentration of CCA (upper part of the figure) and between egg excretion in urine and the concentration of CCA (lower part of the figure). CCA was measured by 2S-IRMA.

Fig. 3. Concentration of CCA in 25 patients with hepatosplenic schistosomiasis. A combined index of liver and spleen size was used to classify patients into those with moderate and high hepatosplenomegaly. Lines indicate median and 95% confidence limits. CCA was measured by 2S-IRMA. $P = 0{\cdot}06$.

Table 2. Effect of chemotherapy on egg excretion in stool and urine and the concentration of CCA and CAA in serum

Treatment group		Before treatment				4 months after treatment			
		Egg excretion		CCA	CAA	Egg excretion		CCA	CAA
		Ova/gram	Ova/10ml	(ct/min)	(O.D.405nm)	Ova/gram	Ova/10ml	ct/min	(O.D.405nm)
Metrifonate	median	557	153·5†	433	265*	667	0·9†	345	117*
($n=34$)	95% confidence limits	497–730	91·7–318	240–585	141–541	495–890	0·3–3·6	174–474	67–435
Oxamniquine	median	700†	81	351†	256*	5†	57·4	184†	150*
($n=47$)	95% confidence limits	554–814	28·8–222	292–439	183–297	0–10	21·1–118	150–247	111–230
Praziquantel	median	709†	78·6†	298†	193†	0†	0·2†	128†	141†
($n=44$)	95% confidence limits	568–1,043	51·3–125	238–358	116–727	0–0	0·1–0·3	104–199	53–257
Placebo	median	680	157	408	220	780	86	399	174
($n=21$)	95% confidence limits	365–1,360	19·5–263	209–795	116–377	425–2,275	16·2–246	155–481	104–234

Significance of difference between pretreatment and post-treatment values: * for $P<0{\cdot}05$; † $P<0{\cdot}01$.

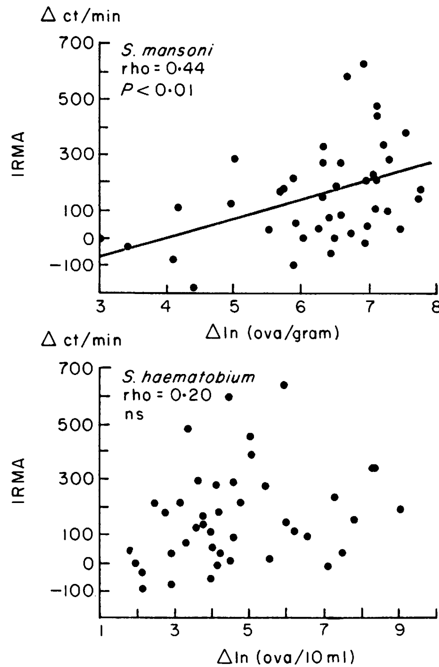


Fig. 4. Relationship between the individual decrease in parasite load (egg excretion before treatment – egg excretion after treatment) and the decrease in CCA (concentration of CCA before treatment – concentration of CCA after treatment) in 44 patients treated with praziquantel. The upper part of the figure shows a regression analysis between Δ ct/min and Δln (ova/gram). The lower part of the figure shows a regression analysis between Δct/min and Δln (ova/10ml). CCA was measured by 2S-IRMA.

Effect of chemotherapy

Based on the assumption that species-specific treatment of patients with a mixed *S. mansoni–S. haematobium* infection should be followed by corresponding changes in the concentration of circulating antigen, the Sudanese patients were randomly allotted to four different treatment groups. The results of the parasitological and immunological investigations carried out four months after chemotherapy are summarized in Table 2. The data clearly indicate that treatment of *S. haematobium* by metrifonate significantly reduced egg excretion in urine by more than 99% but left egg excretion in stool as well as the concentration of CCA unchanged. Conversely, treatment with oxaminiquine did not influence egg excretion in urine, but decreased excretion of *S. mansoni* in stool by 99%. This was followed by a significant decrease in CCA and CAA in serum ($P < 0{\cdot}01$). Treatment with praziquantel, a compound active against both parasite species, reduced egg excretion in urine and stool to about 0·1% of the initial values. Accordingly, in these patients the highest difference between the pretreatment and post treatment concentration of CCA was observed. Neither egg excretion in stool and urine nor the concentration of CCA and CAA significantly changed between the two points of observation in patients treated with a polyvitamin preparation.

As treatment with praziquantel resulted in the most obvious decrease in egg excretion in stool and urine, it was assumed that in patients treated with this compound the individual decrease of the parasite load could be quantitatively reflected by a similar decrease in the amount of antigen present in circulation. Figure 4 depicts the results obtained by regression analysis. The data clearly indicate, that in individual patients the elimination of *S. mansoni* worms, but not the elimination of *S. haematobium* worms, was significantly related to the difference in the concentration of CCA from before and after chemotherapy (rho = 0·44, $P < 0{\cdot}01$ and rho = 0·2, $P > 0{\cdot}1$, respectively). A similar observation was made in the patients with the hepatosplenic form of the disease. In those nine

patients who had received treatment 3 to 9 months prior to the investigation, the median of CCA was equivalent to 367 ct/min, whereas in those 16 patients without any previous treatment it was 811 ct/min. The difference is significant at $P=0{\cdot}01$.

DISCUSSION

It is well established that CAA and CCA are gut-associated antigens, present in the vomitus and in excretory-secretory material of adult schistosomes (Nash, 1974; Rotmanns *et al.*, 1981). Because schistosomes have to regurgitate to eliminate their residual products of digestion, such gut-associated molecules are expected to enter the circulation of the host.

Our results using monoclonal antibody 40 B:1 to quantify CCA in serum by a two site-immunoradometric assay demonstrated that antigenemia could be reliably detected in a concentration above 150 ng/ml. This contrast to reports of Abdel-Hafez, Phillipps and Zodda (1983; 1984) and our previous observation (Nogueira-Queiroz *et al.*, 1986), in which detection limits below 10 ng/ml were reported. However, detection limits based on results of dose-response curves established by diluting known amounts of antigen in serum of an 'optimal' negative reference person do not necessarily apply, if diseased individuals have to be discriminated from healthy inhabitants of the endemic area. In order to keep specificity at an acceptable level we decided to use the 90th percentile of matched controls from the endemic area as the threshold limit of sensitivity. Depending on the group of patients examined this resulted in correct identification of diseased individuals in 11 to 80%. It has to be clearly pointed out, that the antigen detection by the three methods applied in this study still yielded disappointing results as compared with data obtained by conventional ELISA for antibody detection (Table 1).

Experimental studies in schistosomiasis mansoni and schistosomiasis japonicum have shown that the amount of antigen present in the circulation was positively related to the parasite load (Gold, Rosen & Weller, 1969; Quian & Deelder, 1983). We could extend this observation to human schistosomiasis in patients from different endemic areas and could demonstrate a strong statistical correlation between egg excretion in stool and the concentration of CCA in serum. Based on the threshold determined for the 2S-IRMA, it was calculated that this assay reliably identified CCA in serum of patients with an egg excretion above 35 ova per gram of stool. This result compares favorably to those of previous reports using polyclonal antisera, in which the detection limit was calculated to be equivalent to an egg excretion between 500 and 600 ova per gram (Carlier *et al.*, 1975; Santoro *et al.*, 1980; 1981). However, it has to be stressed that 35 ova per gram of stool will also most probably be detected by a single fecal thick smear, the actual parasitological method most commonly employed in control programmes.

Data obtained from pretreatment analysis of the mixed infected patients from the Sudan as well as data resulting from species-specific treatment strongly suggest that the monoclonal antibody 40 B:1 utilized here apparently only recognized CCA released by adult *S. mansoni* worms. In contrast, the polyclonal antiserum raised against CCA seemed to detect at least part of the antigenic material released by the *S. haematobium* worms, as suggested by a significant decrease in CAA levels after selective elimination of *S. haematobium* worms by metrifonate.

Experimental and clinical observations have raised the question whether circulating schistosome antigens are aetiologically linked to disease associated immunopathology, namely liver fibrosis and/or glomerulonephritis. Indeed, antigenemia was found to closely parallel the level of circulating immune complexes (Santoro *et al.*, 1981) and deposition of such immune complexes on basal membranes probably induces glomerular disease (Digeon *et al.*, 1979; Galvao-Castro *et al.*, 1981). In addition, CAA and CCA were frequently detected, free or complexed, in glomerular deposits, Kupfer cells of the liver and macrophages of the spleen (Hoshinu-Shhimizu *et al.*, 1976; Carlier *et al.*, 1978; Deelder & Kornelis, 1980; El-Posoky, van Marck & Deelder, 1984). On the other hand the present study provided evidence that the higher the worm load of an individual, the higher will be the amount of schistosome antigen present in his circulation. Taking into account that lightly infected animals show much faster antigen clearance rates than heavily infected ones (Nash, 1982) and that in our patients the degree of hepatosplenomegaly was positively related to the

concentration of CCA, it may be speculated that persisting high levels of this antigen may also contribute to the development of liver and renal pathology.

We consider the most interesting observation of our study to be the rapid decrease in the concentration of circulating antigens after initiation of specific chemotherapy. With regard to praziquantel this confirms preliminary results in experimental *Schistosomiasis mansoni* (Weltman, 1982; Abdel-Hafez, *et al.*, 1983). In our study, the concentration of antigen in the circulation decreased significantly 4 months after the chemotherapeutic intervention. Furthermore in individual patients, the absolute decrease in the parasite load was closely paralleled by a corresponding decrease in the amount of CCA. Hence, qualification of CCA before and after chemotherapeutic intervention would help to selectively identify patients in whom chemotherapy compl(·:ely or partially failed to eliminate the parasite.

A critical appraisal of the data presented in this study leaves several questions open for discussion. Although the difference in intensity of infection between the Sudanese and the Brazilian patients alone may account for the high false negative rate of antigen detection in the latter group, it cannot be excluded that other factors are also responsible. In view of the paucity of information currently available on the dynamics of circulating schistosome antigens in human infection, the tentative conclusion that the number of adult worms present is the only factor determining the concentration of antigen in circulation is certainly premature. It is conceivable that the concentration and the affinity of antibodies to circulating antigens will also influence the concentration of CCA and/or CAA. Furthermore, the gut-associated antigens might be released in higher amounts by younger worms. Consequently, higher concentrations of circulating antigens would be expected in individuals with frequent reinfection, e.g. from the highly endemic area in the Sudan, than in those living under epidemiological conditions where reinfection is quite unlikely due to interruption of transmission. This is probably the case in our patients from Brazil, as they lived in an area where control measures had been maintained for many years.

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