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Clinical, parasitological and immunological features of canal cleaners hyper-exposed to *Schistosoma mansoni* in the Sudan

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SUMMARY

The present work was a longitudinal study on *Schistosoma mansoni* infection in occupationally hyperexposed canal cleaners in the Sudan and the influence of therapy on the parasitological and humoral immune parameters. Chronically infected canal cleaners (n=28) were more resistant to reinfection (Fisher's exact test, P<0·05) than newly recruited canal cleaners (n=17). Chronically infected canal cleaners had a significantly higher degree of Symmers' fibrosis (χ^2=19·1, P<0·0001), significantly larger portal vein diameter (P<0·05) and enlarged spleen (χ^2=4·2, P<0·05) than recently infected, newly recruited canal cleaners. ELISA was used to detect IgG, IgA and IgM in response to whole worm homogenate (WWH) and cercarial homogenate (CH). Chronically infected canal cleaners had significantly higher IgG to WWH antigen than newly recruited canal cleaners and normally exposed individuals (P<0·05), while both chronically infected and newly recruited canal cleaners had higher IgG levels to CH antigen than normally exposed individuals (P<0·05). The newly recruited canal cleaners had a significantly higher IgM level to CH antigen than chronically infected canal cleaners (P<0·05). The IgG level to WWH antigen increased significantly after treatment in newly recruited canal cleaners and normally exposed individuals (P<0·05). The IgA level to CH antigen increased significantly after treatment in the chronically infected group (P<0·05). Comparison of the serological parameters between the different study groups with regards to infection and treatment is discussed.

Keywords canal cleaners *Schistosoma mansoni* whole worm homogenate cercarial homogenate

INTRODUCTION

Schistosomiasis mansoni is still a serious cause of morbidity in the Sudan. Prevalence and infection are very much related to the pattern and degree of exposure. Infection, if not treated early, may lead to severe morbidity and irreversible pathological changes with advanced periportal fibrosis in the liver [1] and the sequelae of the development of oesophageal varices with the serious risk of fatal haematemesis [2]. Chemotherapy (praziquantel) has been shown to reduce the worm burden and, if given on an annual basis, can markedly reduce the disease associated with infection [3].

Man can develop partial immunity to a schistosome infection [4]. Eosinophils and IgG obtained from infected humans can destroy the immature parasite when placed in the presence of complement [5]. Furthermore, aggregated IgE complexes enhance macrophage-mediated cellular killing [6,7]. Recently, two sets of epidemiological studies have brought convergent evidence for a protective role of IgE in human schistosomiasis infection [8, 9]. Several studies demonstrated a role for IgM and some of the IgG subclasses in blocking the expression of protective immunity [10–12]. Such antibodies were shown to modify immunity and allergic reactivity [13].

The present study was carried out to examine the host–parasite relationship in chronically infected irrigation canal cleaners, who are occupationally hyperexposed to *S. mansoni*. Their schistosome-specific antibody responses were qualitatively and/or quantitatively compared with newly recruited canal cleaners and with naturally infected individuals from the endemic region. The immunological profiles and the effect of praziquantel treatment on the clinicopathological and parasitological status of these groups were evaluated.

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MATERIALS AND METHODS

Study area and study population

The study population was selected from the Gezira irrigation scheme which lies in a triangle of land south of the junction of the Blue and White Niles in Central Sudan. In order to select the

study groups, a survey of the area was undertaken to map the operating canals. Vegetation plays a considerable role in obstructing the flow of water along the irrigation canals. Clearance of the canal from the blocking vegetation is absolutely mandatory. In most cases canals are cleaned manually to remove the submerged and emergent rotted and floating vegetation during the irrigation season of the year. Canal cleaners use machetes to cut weeds. They are hyperexposed to cercariae of schistosomes during their weed-clearing job, which is usually carried out at the time of day when cercarial shedding is at its peak [14].

The groups selected for the present study were age-matched (25–55 years) canal cleaners in the Gezira area who had been on the job for variable periods. Group 1, chronically infected canal cleaners ($n = 28$) who were on the job for more than 5 years; group 2, newly recruited canal cleaners ($n = 17$) who were in service for only 8 months (one transmission season) before the initiation of the field phase of the study. They were newcomers to Gezira and were included to study the effect of hyperexposure to schstosome cercariae on people without previous exposure; group 3, a group of local farmers infected with *S. mansoni* ($n = 11$) was included as normally exposed individuals; group 4, a control group ($n = 12$) selected from Khartoum, who were not infected.

Parasitological and clinical examination

Stool and urine samples were collected on 3 consecutive days. The presence and intensity of *S. mansoni* infection was determined according to the modified Kato technique (three Kato smears for each sample) [15]. A deposit of 10 ml of mid-day urine was examined for *S. haematobium*. Study groups were followed parasitologically at 3, 6, 12 and 48 weeks post-treatment. A hatching test of the egg-positive samples was done at 12 weeks post-treatment.

Study groups were thoroughly examined clinically (general physical, ECG, ultrasonography, etc). The size of the liver and spleen along with the extent of Symmers' periportal fibrosis were determined by ultrasound examination as previously described [1].

Treatment

All positive patients were treated using 40 mg/kg body weight of praziquantel (Biltricide, Bayer) in two doses, about 4–6 h apart (2×20 mg/kg) [16].

Blood collection

After informed consent was received, venous blood samples (10 ml) were obtained from each individual before and 3 months after treatment. The blood was defibrinated with glass beads, centrifuged and the serum was frozen in small aliquots in liquid nitrogen (-196°C).

Antigen preparation

Whole worm homogenate antigen. Suspensions of adult worms of *S. mansoni* were collected from infected mice [17], washed with sterile H_2O, suspended in PBS and homogenized in a tissue grinder on ice and then ultracentrifuged at 100 000 ***g*** at 4°C for 1 h. Supernatant was collected and used as whole worm homogenate (WWH) antigen.

Cercarial homogenate antigen. Cercariae were collected from shedding snails (Sudanese strain of *S. mansoni*) and stored overnight at 4°C to reduce their motility. They were then washed three times in PBS. The pooled cercariae were then sonicated on ice for 30 s and ultracentrifuged at 100 000 ***g*** at 4°C for 1 h. Supernatant was then used as cercarial homogenate (CH) antigen. The protein content of WWH and CH antigens was determined according to Bradford method [18].

ELISA

Basically, the procedure described by Voller *et al.* [19] was followed. The appropriate concentrations of antigens, sera and anti-human serum probes were determined by checkerboard titration. ELISA plates (Nunc–Immunol, Roskilde, Denmark) were coated with 1·4 μg/ml of WWH antigen or 3·5 μg/ml of CH antigen in carbonate–bicarbonate buffer pH 9·6 (100 μl/well). Coating was carried out overnight at 4°C. One percent bovine serum albumin (BSA; Sigma Chemical Co., St Louis, MO) was added at 100 μl/well for 1 h to block the free binding sites of wells. Serum samples were diluted at 1 : 50 for WWH antigen and 1 : 100 for CH antigen in PBS with 0·05% Tween 20. Diluted sera (100 μl) were added to each well and the plates were incubated at 37°C for 1 h. A horseradish peroxidase-labelled sheep anti-human antibody was diluted at 1 : 2000 for IgG and 1 : 1000 for IgA or IgM (Sigma) in PBS with 0·05% Tween 20. One hundred microlitres were added to each well and the plates were incubated at 37°C for 1 h. *o*-phenylenediamine dihydrochloride (OPD; Sigma) substrate was used at a concentration of 0·4% (w/v) in citrate buffer pH 5 with 0·01% (v/v) H_2O_2. The reaction was stopped after 30 min by the addition of 20% sulphuric acid (100 μl/well). The absorbance was read at 492 nm on an ELISA reader (Minireader II; Dynatech Laboratories, Inc.). The detected optical density (OD) readings were corrected to the set reference positive according to the method of Voller *et al.* [20] Only interassay variation of $<10\%$ was accepted.

Statistical analysis

Student's *t*-test, the Fisher exact test, χ^2 test, one-way analysis of variance (Scheffe) and correlation analyses were applied where appropriate.

RESULTS

Clinical and ultrasonic examination

Of the chronically infected canal cleaners, 67% had Symmers' fibrosis, while no Symmers' fibrosis was observed among the newly recruited canal cleaners. Of the normally exposed individuals, 43% had Symmers' fibrosis, but this was not significantly different from the chronically infected canal cleaners. Of the chronically infected canal cleaners, 83% had moderate (grade 2) to advanced grade (grade 3–4), while all normally exposed individuals had a mild grade of fibrosis (Fisher's exact test, $P < 0{\cdot}05$). The liver was shrunken in 26% of chronically infected cleaners, 6% of newly recruited cleaners ($n = 1$) and 44% of normally exposed individuals. However, the difference was significant between newly recruited cleaners and normally exposed individuals only ($\chi^2 = 5{\cdot}6$, $P < 0{\cdot}05$). The spleen in chronically infected canal cleaners was significantly larger than in newly recruited cleaners ($\chi^2 = 4{\cdot}2$, $P < 0{\cdot}05$) and normally exposed individuals ($\chi^2 = 6{\cdot}8$, $P < 0{\cdot}01$).

Parasitological examination

Table 1 shows the faecal egg counts of the infected groups before and after treatment. Chronically infected canal cleaners showed the highest geometric mean of egg/g before treatment. Newly recruited

Table 1. Faecal egg counts of *Schistosoma mansoni*-infected study groups

	Before treatment	Post-treatment follow up			
		3 weeks	6 weeks	12 weeks	48 weeks
a. Group 1 ($n = 28$)					
Geometric mean	247·3	1·2	2·0	3·8	11·3
Range	0–2847	0–13	0–165	0–533	0–773
Percent positives	96	7	30	36	55
b. Group 2 ($n = 17$)					
Geometric mean	148·4	1·5	3·9	7·2	49·6
Range	0–1340	0–191	0–351	0–253	0–573
Percent positives	94	7	31	50	78
c. Group 3 ($n = 11$)					
Geometric mean	81·51	1·2	0·0	1·3	3·3
Range	13–1247	0–6	0–0	0–9	0–120
Percent positives	100	10	0	11	27

Group 1, Chronically infected canal cleaners; group 2, newly recruited canal cleaners; group 3, normally exposed individuals.

cleaners showed a lower geometric mean. Normally exposed individuals showed the smallest geometric mean. The highest intensities of infection were found among the chronically infected cleaners, with a maximum of 2847 eggs/g. At 3 weeks following treatment, all patients stopped passing ova except four (two chronically infected cleaners, one newly recruited cleaner and one normally exposed individual). Six weeks after treatment, 30% of the chronically infected canal cleaners and 31% of the newly recruited cleaners passed ova, whereas none of the normally exposed individuals did. A hatching test was done 12 weeks after treatment to examine the viability of the excreted eggs.

At 12 weeks post-treatment, 36% of the chronically infected cleaners, 50% of the newly recruited cleaners and 11% of the normally exposed individuals were positive for *S. mansoni* ova test. Of the chronically infected canal cleaners that were passing ova 12 weeks after treatment, 20% excreted viable eggs (hatched), while 100% of the newly recruited canal cleaners and normally exposed individuals were excreting viable eggs. One year after treatment, 55% of the chronically infected cleaners compared with 78% of the newly recruited cleaners and 27% of normally exposed individuals were positive for *S. mansoni* ova. The highest geometric mean of egg/g 1 year after treatment was found in newly recruited canal cleaners (49·6 eggs/g) compared with 11·3 in chronically infected canal cleaners and 3·3 in normally exposed individuals. Egg counts for 1 year following therapy were significantly higher in newly recruited canal cleaners compared with egg counts in chronically infected canal cleaners (Fisher's exact test, $P < 0{\cdot}05$). *Schistosoma haematobium* infection was not found in any of the study individuals.

Comparison of mean IgG, IgA and IgM levels to WWH and CH antigens between the study groups

Chronically infected canal cleaners had significantly higher IgG levels to WWH antigen than newly recruited canal cleaners and normally exposed individuals ($P < 0{\cdot}05$) (Fig. 1) before treatment. No significant difference between the infected groups was seen in IgA and IgM response to WWH antigen before treatment. Chronically infected canal cleaners and newly recruited canal cleaners had a significantly higher IgG response to CH antigen than normally exposed individuals ($P < 0{\cdot}05$) (Fig. 2).

With regard to IgM levels before treatment, we noted that newly recruited canal cleaners had the highest level, and this was significant compared with chronically infected canal cleaners ($P < 0{\cdot}05$) (Fig. 2).

Among all antibody isotypes studied, the IgG response to WWH antigen showed a significant correlation with infection

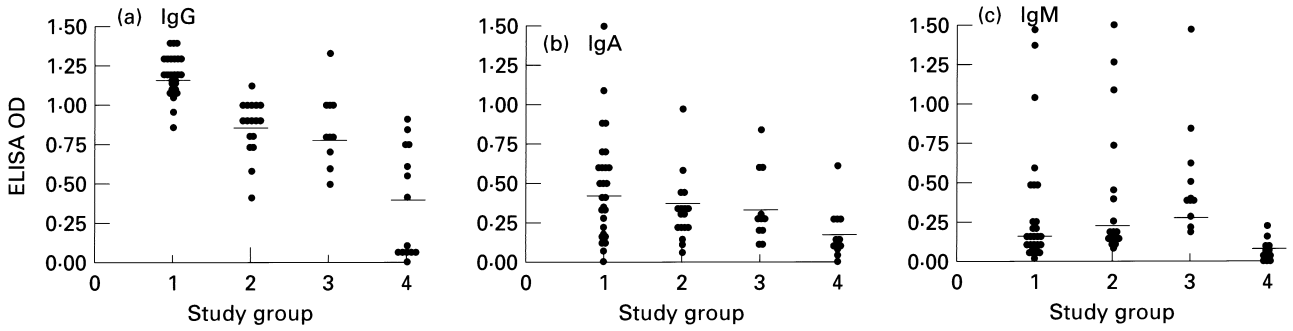

Fig. 1. Immunoglobulin levels in response to *Schistosoma mansoni* whole worm homogenate in chronically infected canal cleaners (1), newly recruited canal cleaners (2), normally exposed individuals (3) and uninfected controls (4). The solid lines represents the mean. (a) Chronically infected canal cleaners had significantly higher IgG than other groups ($P < 0{\cdot}05$).

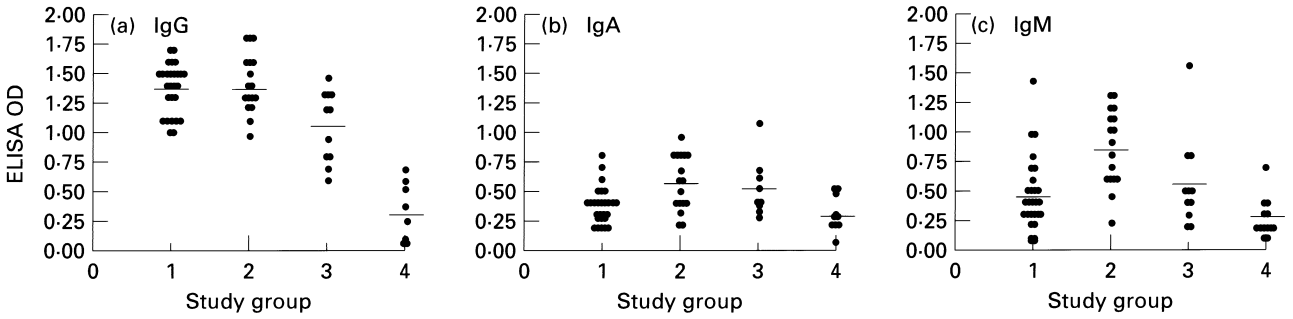

Fig. 2. Immunoglobulin levels in response to *Schistosoma mansoni* cercarial homogenate in chronically infected canal cleaners (1), newly recruited canal cleaners (2), endemic infected individuals (3) and uninfected controls (4). The solid line represents the mean. (a) Chronically infected and newly recruited canal cleaners had significantly higher IgG than normally exposed individuals ($P < 0{\cdot}05$). (c) Newly recruited canal cleaners had the highest IgM level, and the difference was significant compared with chronically infected canal cleaners ($P < 0{\cdot}05$).

intensity (Spearman's correlation coefficient $= 0{\cdot}43$; $P < 0{\cdot}001$; Fig. 3).

Antibody responses in the different study groups before and after treatment (paired samples)

The effect of treatment on immunoglobulin classes IgG, IgA and IgM is shown in Tables 2 and 3. IgG levels increased significantly 3 months after treatment in newly recruited canal cleaners and normally exposed individuals ($P < 0{\cdot}05$) (Table 2). There was no significant change after treatment in IgA and IgM response to WWH antigen of all groups.

With regard to the CH antigen, newly recruited canal cleaners showed a significant decline in IgG level after treatment ($P < 0{\cdot}05$) (Table 3), while chronically infected canal cleaners and normally exposed individuals showed no significant difference. Chronically infected canal cleaners showed a significant increase in IgA level to CH antigen, following treatment ($P < 0{\cdot}05$), but this was not observed in other groups (Table 3). Chronically infected canal cleaners also showed a significant decrease in IgM level to CH antigen after treatment ($P < 0{\cdot}05$), while IgA and IgM levels to CH antigen showed no significant change after treatment in newly recruited canal cleaners and normally exposed individuals (Table 3).

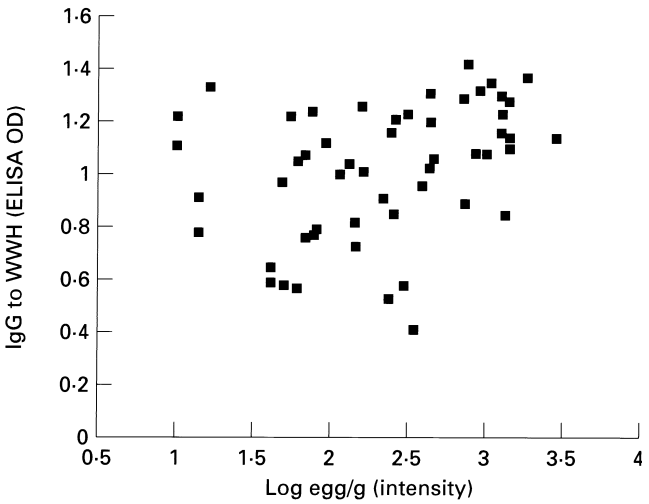

Fig. 3. Correlation between intensity of infection and IgG antibodies in response to whole worm homogenate (Spearman's correlation coefficient $= 0{\cdot}43$, $P < 0{\cdot}001$). WWH, Whole worm homogenate.

DISCUSSION

The clinical and pathological manifestations of *S. mansoni* infection are governed by many factors. Intensity of infection, including the frequency and severity of exposure, and the immunological status of the host are the most important factors. Mortality in *S. mansoni* infection is always secondary to the development of Symmers' hepatic preportal fibrosis and the subsequent portal hypertension, oesophageal varices and haematemesis [21,22].

Canal cleaners undertaking manual cleaning of weeds from the irrigation canal system experience an unusual exposure pattern and risk of infection with schistosomiasis representing an unusual host–parasite relationship. The disease pattern, clinicopathological and immunological parameters of such individuals experiencing this exposure pattern have not been well studied before.

Long-term occupational hyperexposure without adequate treatment produces, as expected, high levels of infection (geometric mean 247 eggs/g) and advanced irreversible pathological changes in chronically infected canal cleaners. Of chronically infected canal cleaners, 67% had Symmers' fibrosis, whereas no newly recruited canal cleaner showed any signs of Symmers' fibrosis. In comparison, 43% of normally exposed infected individuals had Symmers' fibrosis. Previously, Homeida *et al*. [1] found 12–18% Symmers' fibrosis prevalence in two Sudanese villages. The difference in prevalence between our normally exposed infected group and Homeida's group may be due to the fact that in our study only adults were selected, while he studied the whole village cross-sectionally.

Treatment with praziquantel was effective in 92% of all

Table 2. IgG levels in response to WWH before and after treatment in *Schistosoma mansoni*-infected groups (paired samples)

	IgG mean ELISA OD ± s.d.		
Study group	Before treatment	After treatment	*P*
Group 1 ($n = 25$)	$1{\cdot}20 \pm 0{\cdot}11$	$1{\cdot}23 \pm 0{\cdot}13$	NS
Group 2 ($n = 14$)	$0{\cdot}87 \pm 0{\cdot}20$	$1{\cdot}02 \pm 0{\cdot}19$	**
Group 3 ($n = 8$)	$0{\cdot}73 \pm 0{\cdot}26$	$0{\cdot}91 \pm 0{\cdot}29$	*

*$P < 0{\cdot}05$; **$P < 0{\cdot}01$. NS, Not significant.

Group 1, Chronically infected canal cleaners; group 2, newly recruited canal cleaners; group 3, normally exposed individuals.

Table 3. Immunoglobulin levels in response to cercarial homogenate before and after treatment in *Schistosoma mansoni*-infected groups (paired samples)

	Immunoglobulin type (mean ELISA OD ± s.d.)								
	IgG			IgA			IgM		
Study group	Before*	After*	*P*	Before	After	*P*	Before	After	*P*
Group 1 ($n = 25$)	1·40 ± 0·21	1·43 ± 0·19	NS	0·40 ± 0·21	0·55 ± 0·26	†	0·49 ± 0·31	0·33 ± 0·25	†
Group 2 ($n = 14$)	1·50 ± 0·24	1·30 ± 0·21	†	0·60 ± 0·23	0·59 ± 0·24	NS	0·89 ± 0·33	0·82 ± 0·33	NS
Group 3 ($n = 8$)	1·09 ± 0·37	1·16 ± 0·33	NS	0·47 ± 0·27	0·43 ± 0·35	NS	0·45 ± 0·24	0·38 ± 0·26	NS

*Before and after treatment.
†$P < 0·05$.
Group 1, Chronically infected canal cleaners; group 2, newly recruited canal cleaners; group 3, normally exposed individuals.

individuals studied at 3 weeks post-treatment. Only 20% of chronically infected canal cleaners that passed ova were passing viable eggs, while all egg-positive individuals in newly recruited canal cleaners passed viable eggs. When examined 1 year after treatment, 78% of newly recruited canal cleaners had acquired infection, compared with 55% of chronically exposed canal cleaners. Newly recruited canal cleaners seemed to be more susceptible to reinfection, especially when viability of their eggs was taken into consideration (Fisher's exact test, $P < 0·05$).

Regulation of the schistosome-specific antibody class response was studied in relation to the effect of chemotherapy. Chronically infected canal cleaners were found to have the highest IgG levels to WWH antigen. This is in line with the findings of Sher *et al*. [23] and Feldmeier *et al*. [24], who stated that anti-schistosome IgG and IgE levels correlate positively with the intensity of infection. Such correlation was found also in the present study between intensity of infection and IgG to WWH antigen (Spearman's correlation coeffecient = 0·43; $P < 0·001$). IgG levels in chronically infected canal cleaners showed a slight, but not significant, decrease after treatment. The level of the IgG to WWH antigen in newly recruited canal cleaners and parasite-positive normally exposed controls increased significantly after treatment ($P < 0·01$, $P < 0·05$, respectively). Jassim *et al*. [25] did not find changes in IgG levels following chemotherapy of chronically infected canal cleaners, early infected cases and acute hospital Sudanese cases. Barsoum *et al*. [26] found that after chemotherapy, most patients expressed elevated antibody responses to WWH and CH antigens, which persisted for at least 9 months. Although our study groups, especially canal cleaners, showed a different pattern in their specific anti-schistosome IgG response, there was no difference in total serum IgG between the chronically infected group and the newly recruited group (data not shown). In fact, newly recruited canal cleaners had slightly higher total serum IgG. This finding suggests that there may be differences in the percentage of IgG antibodies which are parasite-specific between the different groups. It also indicates the need to examine the possible variations in IgG subclasses between the different study groups.

The IgA and IgM levels before and after treatment were low in all study groups except newly recruited canal cleaners, who showed significantly higher IgM to CH antigen in comparison with the chronically infected group ($P < 0·05$). This may be related to the recent infection of the previously naive individuals of the newly recruited canal cleaners. This IgM may have a blocking effect on the other protective antibodies [10,12]. In fact, as already shown, newly recruited canal cleaners were more susceptible to reinfection than chronically infected canal cleaners. It has been found that susceptible children had significantly higher levels of IgM antibodies against 38-kD schistosomular protein [10]. IgG antibodies against this antigen moiety mediate antibody-dependent cellular cytotoxicity, which can effectively be blocked by IgM [12]. Persistence of high IgM complex levels was also demonstrated by Jassim *et al*. [25] in chronic schistosomiasis infection. In Jassim's study all groups studied (chronically infected, acutely infected and school children) showed similar IgM responses to worm and egg antigens, but, except for the acute cases, a minority had IgM antibodies to larval antigens. The antigen of significance as a stimulus for such blocking IgM antibodies was identified as carbohydrate epitopes expressed both on egg polysaccharides and on schistosomulum surface antigen [27].

High levels of schistosome-specific serum IgA were reported in schistosomiasis patients, and these levels tended to increase with severity of the disease, being highest in cases with ascites [28]. In our study, low levels of IgA were found in all groups. In chronically infected canal cleaners IgA antibodies in response to CH antigen increased significantly after treatment. A similar observation was made by Auriault *et al*. [29], who observed a significant increase in IgA response to the recombinant p28 glutathione-S-transferase and its peptides in Kenyan children, both in resistant and susceptible groups. This antigen has been identified among adult worm proteins and on the schistosomulum surface, and can be considered an important molecule for a potential vaccine [30]. Auriault *et al*. [29] found the specific IgA that bound to the 140–153 peptide of the p28 to be two-fold higher in resistant Kenyan children than in the susceptible group. However, this difference in IgA response was less marked after oxamniquine treatment. Although anti-schistosome-specific IgA could play an important role in immunity to schistosome infection, neither our nor Auriault's results prove this.

Studies in the mechanisms underlying the regulation of antigen-specific antibody classes and subclasses are essential for better understanding of immunity to schistosomiasis and the effect of chemotherapy. Detailed studies on the IgE and IgG subclasses and immune complex formation and clearance are needed in order to explain how the serological responses are regulated. Such studies are now in progress.

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