Clin. exp. Immunol. (1990) **80**, 69–76

Early antibody responses in human schistosomiasis

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(Accepted for publication 31 October 1989)

SUMMARY

Early diagnosis is important when handling patients with acute schistosomiasis. This state is usually more severe in travellers and tourists than in the immune, resident patients. With increased travelling to areas endemic for schistosomiasis, a tool is needed to solve the problem of differential diagnosis due to the non-specific symptoms of the early stages of the disease. Early appearance of antibodies against excretory/secretory antigens of the intestinal tract in the adult worm was seen in six individuals recently infected with *Schistosoma mansoni*, using an indirect immunofluorescence technique. The antibodies were of IgM, IgG and IgA classes, and of the IgG1, IgG3 and IgA1 subclasses as detected by ELISA using an antigen preparation of adult worm. On immunoblots, using a freeze-dried adult worm antigen, IgG1 and IgG3 antibodies recognized antigens of 32–35 kD. Antibodies against these antigens could thus be a marker of early infection in previously non exposed visitors to endemic areas.

Keywords early schistosomiasis subclasses immunoblotting enzyme immunoassay

INTRODUCTION

An early diagnosis is essential for adequate treatment of acute schistosomiasis. The symptoms may easily be mistaken for other diseases such as malaria, typhoid fever or influenza (Zuidema, 1981; Istre *et al.*, 1984; Stuiver, 1984; Harries & Cook, 1987; Chapman, Wilkinson & Davidson, 1988). The initial symptoms may also be neglected and lead to chronic disease (Harries *et al.*, 1986). The importance of early diagnosis and treatment is also illustrated by case reports describing neurological complications caused by ectopic worms or eggs (Neves *et al.*, 1973; Scully, Mark & McNeely, 1985; Suchet *et al.*, 1987). A visitor to an endemic area who has never been exposed to *Schistosoma*-infected water, in this respect an immunologically naive individual, risks having more severe symptoms than a person from an endemic area when contracting acute schistosomiasis. Current diagnosis of an active infection relies on finding of non-calcified eggs in stool. This is a rather insensitive method (Watt *et al.*, 1986); furthermore, since onset of egg-excretion may be delayed, the diagnosis of early infections will have to depend on serological assays or detection of circulating parasite antigens. It is known that antibodies against intestinal antigens of the adult worm detectable by immunofluorescence appear early during *S. mansoni* infection in humans (van Helden *et al.*, 1975; Nash, 1978; Kanamura *et al.*, 1979; Deelder *et al.*, 1980; Ruppel, Diesfeld & Rother, 1985a; Ruppel, Breterniz & Burger, 1987a) and the appearance of antibodies to a 31/32 kD antigen may be an early event in previously not exposed individuals. Antibodies against this antigen are also found in long-standing infections (Ruppel *et al.*, 1987b; Idris & Ruppel, 1988). The 31/32 kD *S. mansoni* protein antigens cross-react with sera from patients with *S. japonicum* (Ruppel *et al.*, 1987b) and *S. haematobium* infections (Idris & Ruppel, 1988).

The biological basis for the development of particular subclasses of antibodies against specific parasite antigens during the early stage of infection is not fully understood. Knowledge of the kinetics of the antibody subclass responses to different antigens is essential for vaccine development as the expression of immunity seems to be governed by the class and subclass of the antibodies elicited. In addition, it might have importance in the design of serodiagnostic assays, as IgG4 anti-egg antibodies were recently found to display the best correlation to intensity of infection (Butterworth *et al.*, 1988; Dunne *et al.*, 1988). The diagnostic use of subclass distribution of specific antibodies has also been described in other parasitic infections such as filariasis, onchocerciasis and trichinosis (Lal & Ottesen, 1988; Ljungström *et al.*, 1988; Cabrera, Buttner & Parkhouse, 1988).

The problem of early diagnosis is illustrated in a group of six Finnish men who during a 'hunting safari' to the Central African Republic contracted *S. mansoni* infection. Three of the men showed symptoms of an acute schistosomiasis after exposure to contaminated water. This homogenous patient material, although small, gave us the rare opportunity to study

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relatively early immunological responses as opposed to reactions in patients with a chronic *Schistosoma* infection (Iskander, Das & Aalberse, 1981; Evengård *et al.*, 1988). The patients were all immunologically naive with respect to *Schistosoma* antigens and as the time of infection was limited to a few days it is of interest to follow the kinetics of the antibody responses using different assays. In this study we have analysed the kinetics of the antibody responses against *Schistosoma* antigens during the early stages of infection as well as the specificity of the IgG and IgA subclasses involved.

MATERIALS AND METHODS

Patients

Clinical data are presented in detail by Pitkänen *et al.* (1990). In brief, six Finnish men (aged 41–67) on a 2-week safari in the Central African Republic were exposed to schistosome-infected water on two occasions. One week after leaving endemic area, one man (patient 1) developed acute schistosomiasis and after a few weeks two others (patients 2 and 3) had similar symptoms. Serum samples were collected 6–60 weeks after infection. All were given Praziquantel 50 mg/kg 4–10 weeks post-exposure. Faecal examination, performed after finding a positive serology, revealed that five (patients 1–5) of the six men excreted schistosoma eggs. For the sixth patient the diagnosis was made based on anamnesis and serology. No rectal snips were taken.

Sera from five patients with a chronic *Schistosoma* infection were used in immunoblot assay for comparative analysis.

Sera from six individuals, negative in routine schistosomiasis serology, were used as controls.

Antigens

Adult *S. mansoni* worms were recovered from 8-week infected NMRI mice by perfusion using BSS (balanced salt solution; NBL, Stockholm, Sweden) with 2 U heparin/ml.

Worms to be used in the immunofluorescence assay were embedded in tissue-tek (Histolab Products, Gothenburg, Sweden) before freezing. The worms were then cut in a frozen condition and placed on slides and stored at −70°C.

Schistosomes to be used in ELISA (adult worm antigen, AWA) were rinsed in phosphate buffer and homogenized in a Sorval omnimixer 7 × 1 min in an ice-bath. After adding phosphate buffer with 0·3 M sucrose, pH 7·4, the preparation was ultracentrifuged at 100 000 *g* for 1 h. The solubilized antigens contained in the supernatant fluid were dialysed against PBS overnight. The protein content was 8·16 mg/ml.

Worms to be used in immunoblotting studies were homogenized, sonicated five times for 1 min and freeze-dried. The final protein content was 0·8 mg/ml.

Soluble egg antigen (SEA) was prepared by the method of Carter & Colley (1978). *S. mansoni* eggs were recovered from the guts and livers of 8-week-infected mice and homogenized. The homogenate was then centrifuged at 100 000 *g* for 90 min and the supernatant fluid was used as antigen.

Assays

Immunofluorescence assay. Cryostat sections (8 μm) of schistosomes were fixed in acetone for 10 min at room temperature. Incubations with serum diluted in PBS were for 30 min at room temperature. Following three washes in PBS, the slides were incubated for 30 min at room temperature with a 1/30 dilution of FITC-conjugated sheep anti-human immunoglobulin (National Bacteriological Lab., Stockholm, Sweden), a 1/5 anti-IgM or a 1/10 anti-IgA FITC dilution (Behringwerke, Marburg, FRG).

Two types of fluorescence in the adult worm were noted; the fluorescence of the intestinal lumen and the lining of the intestinal tract (gut-associated antigens, GAA) and the fluorescence of the whole structure, the soma, called the somatic antigens (SA).

ELISA. This was performed as described by Voller, Bartlett & Bidwell (1976). Microtitre ELISA plates were coated in coating-buffer with 1 μg/ml of SEA overnight in room temperature, washed three times in physiological NaCl with 0·05% Tween 20 and the sera were incubated at 37°C for 2 h, washed again and incubated with a horseradish peroxidase anti-human immunoglobulin conjugate overnight at room temperature. After three washes substrate was added and the change in colour measured in a Titertek multiscan photometer (Eflab Oy, Helsinki, Finland)

Immunoglobulin subclass measurements. Microtitre ELISA plates were coated with 5 μg/ml of AWA or 1 μg/ml of SEA and subclass distribution of specific antibodies was measured as previously described in detail (Persson, Hammarström & Smith, 1985). All anti-human immunoglobulin subclass antibodies were mouse monoclonal antibodies of IgG1 subclass.

In brief, the serum samples (diluted 1/100 in PBS with 0·05% Tween 20) were incubated overnight on the antigen-coated plates. Commercially available monoclonal antibodies (Unipath, London, UK) against the various human IgG subclasses, purified on an ion-exchange column (FPLC) (Pharmacia Fine Chemicals, Uppsala, Sweden) were added in optimal concentrations (anti-IgG1, Bam 15 diluted 1/2000; anti-IgG2, Bam 10 diluted 1/2000; anti-IgG3, Bam 08 diluted 1/8000; anti-IgG4, Bam 16 diluted 1/12 000). Monoclonal antibodies against the IgA subclasses (Nordic, Tilburg, The Netherlands) were added in optimal concentrations (anti-IgA1, 69-7·1, diluted 1/5000 and anti-IgA2, 512-H5, 1/2000). The incubation with the monoclonals was preceded by washing. After 4 h of incubation at room temperature the plates were washed, and a 1/1000 dilution of rabbit anti-mouse immunoglobulin (Dakopatts, Hägersten, Sweden) was added. After a further 4-h incubation the plates were washed and a 1/1000 dilution of alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin (Sigma Chemical Co., St Louis, MO) was added, after which the plates were incubated overnight. After additional washes, disodium *p*-nitrophenyl phosphate, 1 mg/ml (Sigma) in 10% diethanolamine buffer, was added, and the plates were incubated for 20–30 min. Absorbance was measured at 405 nm with a Titertek multiscan photometer. Sera were considered positive with specific IgG antibodies of the respective subclass when showing an optical density (OD) of $> 0{\cdot}3$ in ELISA.

Immunoblotting

This was performed essentially as described by the manufacturer for use of alkaline phosphatase-conjugated secondary antibodies (Dakopatts). Freeze-dried adult worm antigen was separated on a 7·5% SDS polyacrylamide gel and transferred to nitrocellulose paper (NCP). The NCP strips were blocked for 30 min with 3% BSA in PBS and then washed three times for 5 min with PBS Tween (0·1%) containing 0·1% BSA before incubation with serum diluted 1/100 in washing solution for 2 h

at room temperature in 8-well reservoirs (Flow Lab., Solna, Sweden). The washing was subsequently repeated and the strips were futher incubated in sealed reservoirs with monoclonal antibodies from Unipath (anti-IgG1, Bam 15 diluted 1/2000; anti-IgG2, Bam 10 diluted 1/2000; anti-IgG3, Bam 08 diluted 1/8000; anti-IgG4, Bam 16 diluted 1/10 000; anti IgG, Bam 06 diluted 1/4000) overnight at room temperature on a rocker. The strips were finally incubated with alkaline phosphatase-conjugated rabbit anti-mouse IgG D 314 (Dakopatts) diluted 1/1000 at room temperature for 3 h. After additional washes, 5-bromo-4-chloro-3 indolylphosphate 4 mg/ml and nitro blue tetrazolium (NBT) 1 mg/ml, dissolved in methanol-acetone and diluted in ethanolamine buffer, was used as substrate and distilled water was added to stop the reaction.

Specific IgE antibodies

IgE antibodies to AWA were determined by the radioallergosorbent test (RAST) essentially as described (Johansson, Bennich & Berg, 1971). Five microlitres of AWA, 0·22 mg/ml, were coupled per CNBr-activated filter paper disc. Phadebas anti-IgE (Pharmacia) ^{125}I-labelled tracer was used and the results were expressed in PRU/ml using Phadebas Reference System (Pharmacia Diagnostics).

RESULTS

Antibodies against AWA

The reaction against the somatic structures of the adult worm, using the immunofluorescence technique, was weak in all sera, with titres of < 1/20. However, all patients had significant titres against the intestinal tract (GAA) (Table 1). All patients had increased levels of the three isotypes analysed (IgG, IgM and IgA). During the observation time there was only a slow decline in some of the titres. The titres do not indicate an on-going infection as the patients had been adequately treated.

When monoclonal antibodies against the IgG subclasses were used in immunoblotting a distinct pattern was found (Table 2). The major response was against the antigens in the 32–35 kD region (Fig 1b, patients 1–6). This band was broad and strong and might well consist of several bands indistinguishable with this technique. We observed anti-32–35 kD reactivity with IgG1 antibodies in all patients, IgG3 antibodies in three of the six patients, IgG2 antibodies in two of the patients, and IgG4 antibody in one patient. All sera recognized, although weakly, other antigens with individual patterns of subclass specificities (Table 2).

All patients had increased response of IgG1 against adult worm and two patients also of IgG3 in ELISA using the adult worm antigen (Fig. 1a, patients 1–6). No IgG2 or IgG4 was detected (Fig. 1a, patients 1–6 and data not shown). The discrepancy between the results in immunoblot and in ELISA might reflect differences in the two systems, e.g. polysaccharides which normally induce IgG2 antibodies display a low coating efficiency. For patients 1, 3 and 5 the peak response was early (< 6 weeks) after treatment while for two patients (2 and 4) the maximal response was delayed (Fig. 1a, patients 1–6).

Sera from the five patients with a chronic disease were also analysed using immunoblotting (Fig. 2). An IgG1 and IgG4 pattern was found which corresponded to results obtained in our ELISA system as reported earlier (Evengård *et al.*, 1988).

Table 1. Titres against gut-associated antigen (GAA)

Patient	Weeks post-treatment	Weeks post-infection	IgG	IgM	IgA
1	8	13	640	640	80
	14	18	640	80	80
	27	31	640	80	80
	52	57	320	20	80
2	1	7	320	320	80
	3	13	320	320	80
	13	18	160	320	80
	26	31	320	160	80
	52	60	160	160	80
3	8	13	320	160	10
	14	18	320	80	20
	27	31	320	80	40
	54	60	40	40	10
4	0	6	640	160	80
	7	13	640	160	80
	13	18	640	160	80
	27	31	320	80	80
	53	60	320	160	80
5	1	9	1280	640	160
	7	16	640	320	80
	17	27	640	640	80
	50	60	10	5	0
6	1	11	640	640	40
	7	18	40	320	10
	18	31	40	40	5

Indirect immunofluorescence using frozen sections of *S. mansoni* adult worm as antigen and sera from six individuals with an early infection of schistosomiasis drawn at different intervals post-treatment. Titres are given with reciprocal values.

Monoclonal antibodies against the IgA subclasses were also used in ELISA when assaying antibodies against AWA. Five out of six patients displayed increased levels of IgA1 (Fig. 1a, patients 1-6), whereas no antibodies of the IgA2 subclass were detected (data not shown).

Using RAST, we observed no specific IgE antibodies in any of the sera (data not shown).

Sera from the controls were negative in the subclass ELISA. However, in immunoblot assay a weak reactivity against a 49-kD antigen was found in one subject for all the IgG subclasses and in serum from another control there was a faint reactivity against a 58 and a 62 kD for IgG1 but never with the 31/32 kD band.

Antibodies against SEA

All six patients had specific IgG1 antibodies against egg antigen in ELISA and three patients had raised levels of IgG2 suggesting the existence of polysaccharides in this antigen preparation (Hammarström *et al.*, 1986). No IgG3 or IgG4 was noted. In Fig. 3 a representative pattern for one of the six patients is shown.

As also found in the assay of AWA, all patients had increased levels of IgA1 against egg antigen (Fig. 3) and no IgA2 antibody was detected (data not shown).

Table 2. Kinetics of IgG subclass response in immunoblotting analysis

Mol. wt (kD)	Sub-class	Patient 1 Weeks post-infection 13	18	31	57	Patient 2 Weeks post-infection 7	13	18	31	60	Patient 3 Weeks post-infection 13	18	31	60	Patient 4 Weeks post-infection 6	13	18	31	60	Patient 5 Weeks post-infection 9	16	27	60	Patient 6 Weeks post-infection 11	18	31
32–35	IgG1	+++	+++	+++++	++	+	+	+	+++	+++++	++	+++	+++	+++++	+++	++++	+++++	+++++	+++++	+++++	++	++	−	−	++	++
	IgG2	−	−	−	−	−	−	−	−	−	−	−	+	++	−	++	++	+	+	−	−	−	−	−	−	−	−
	IgG3	++	+	+	+	+	+	+	+++	++	−	−	−	−	+	++	++++	−	−	−	−	−	−	−	−	−	−
	IgG4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−
49	IgG1	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	+	++	−	−	−	+	+	+	+−
	IgG2	+	+	+	+	−	−	−	−	−	−	−	+	+	−	+	+	+	+	−	−	−	−	−	+	−	−
	IgG3	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	++	++	++	++
	IgG4	−	−	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−	−	−	−	−
62	IgG1	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	+	−	+	−	−	−	+	+	+	+
	IgG2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−
	IgG3	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	+	+	−	−	−	−	−	−	−	−
	IgG4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
75	IgG1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	−	+	+	+	+
	IgG2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−
	IgG3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−
	IgG4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
92	IgG1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
	IgG2	−	−	−	−	−	−	++	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−−
	IgG3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−−
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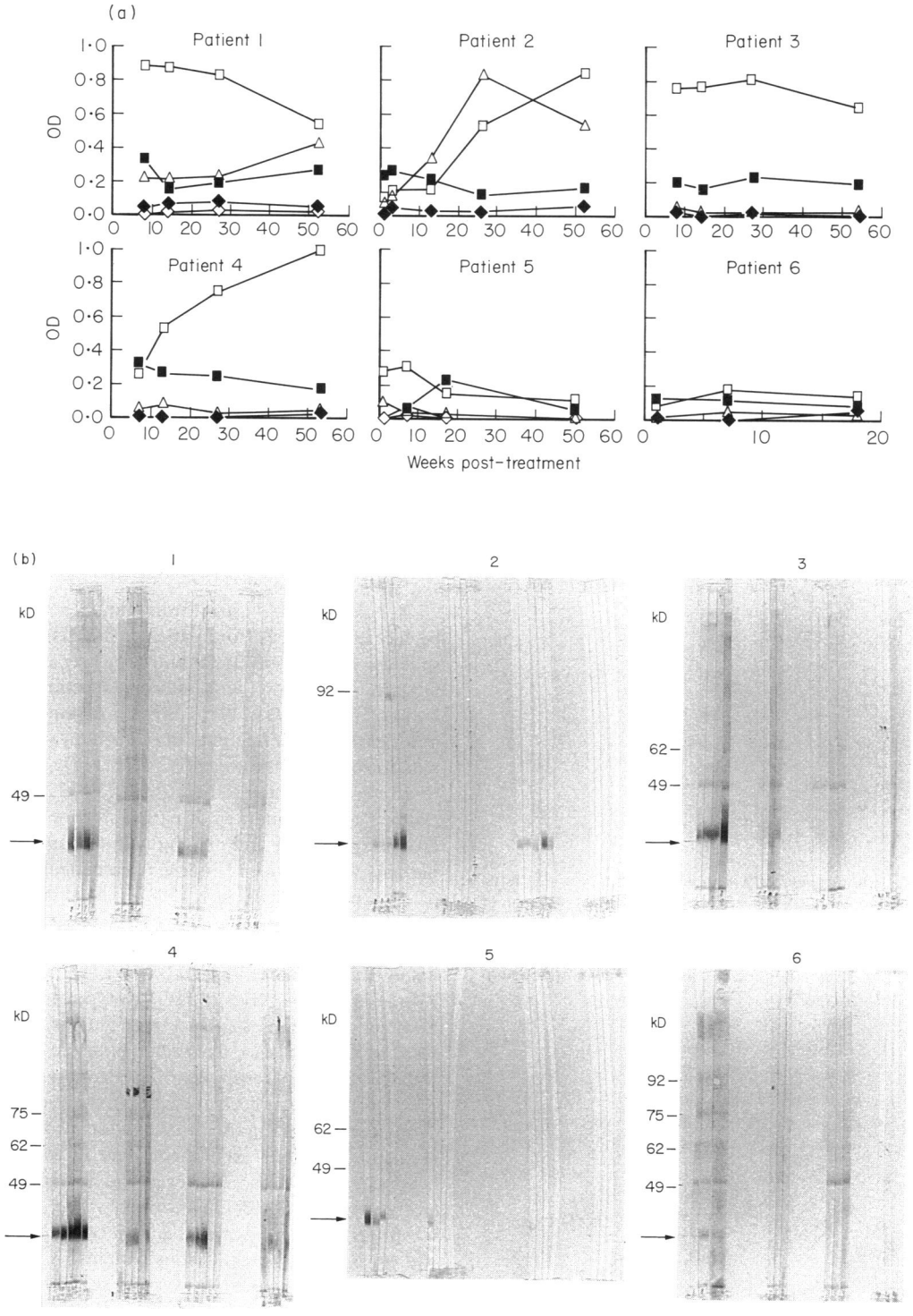

Fig. 1. (a) Kinetic study of the IgG and IgA subclass distribution as determined by ELISA, using adult worm antigen, in consecutive sera from six men (patients 1–6) with an early infection of schistosomiasis. Background values are subtracted. OD, optical density; □, IgG1; ◆, IgG2; △, IgG3; ◇, IgG4; ■, IgA1. (b) The same sera as mentioned above used in western blotting. Adult worm antigen was run on SDS–PAGE and transferred to nitro-cellulose paper before incubation with sera. Each strip in each subclass is incubated with the serum analysed in the corresponding part (a) of the figure. In patient 4 the first strip represents serum from the day of the treatment of the patient. This serum was not analysed in ELISA.

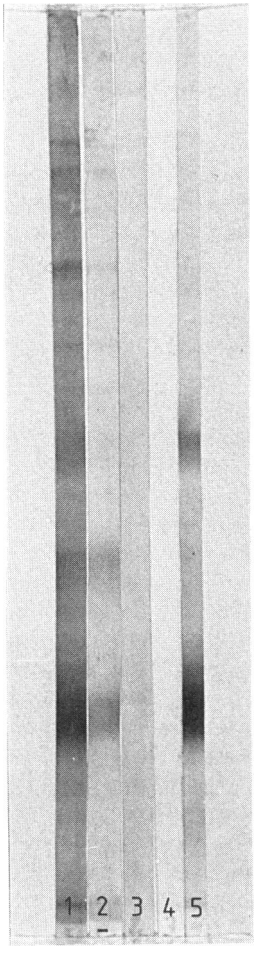

Fig. 2. Analysis of antibody specificity using the western blot technique with adult worm antigen. Sera from a patient with a chronic infection of schistosomiasis as a typical pattern of the five analysed. The revealing probe in strip 1 is a monoclonal antibody (MoAb) against IgG; in 2 a MoAb against IgG1; in 3 against IgG2, in 4 a MoAb against IgG3; and in strip 5 a MoAb against IgG4.

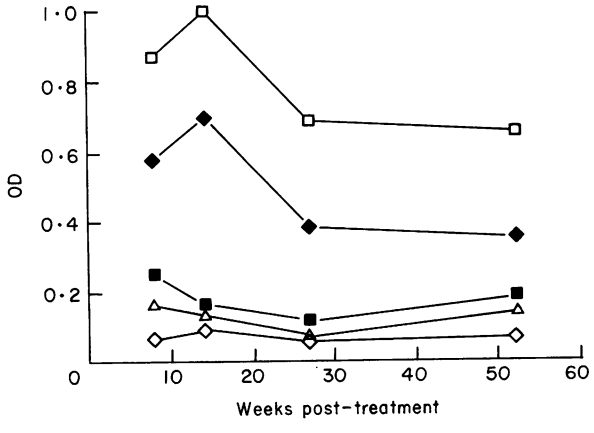

Fig. 3. A representative kinetic study of the IgG and IgA subclass (symbols as in Fig. 1) distribution from one of the six patients with an early infection of schistosomiasis as determined by ELISA using soluble egg antigen.

Correlations with clinical manifestations

Patients 1, 2 and 3 presented with fever, urticaria and/or headache and myalgia as signs of a *S. mansoni* infection. No decline was found in titres against the GAA in sera from these three patients (with the exception of IgM anti-GAA for patient 1). Titres declined in all three isotypes for patients 5 and 6, who had no symptoms.

DISCUSSION

The results presented show that antibody reactions involved in an early immune response against a schistosome inefection are characterized by high titres of IgG, IgM and IgA antibodies against GAA in immunofluorescence; the absence of reaction against somatic structures of the adult worm in the same assay; and the detection of a 32–35 kD antigen by anti-adult worm specific IgG1 antibodies. Three of the six patients investigated had elevated levels of IgG3 antibodies. There were also increased levels of these subclasses as well as IgA1 against AWA in ELISA. Anti-egg specific IgG1 antibodies was found in all six patients, and three also had increased levels of IgG2 against egg antigen in ELISA. All six patients had IgA1 antibodies against egg antigen in ELISA.

Subclass analysis using the immunoblot techniques has been performed with sera from patients with filarial infections using subclass specific polyclonal antibody preparations (Hussain & Ottesen, 1986), and with subclass-specific monoclonal antibodies (Hussain *et al.*, 1986). When analysing sera from patients in an early stage of *S. mansoni* infection, we noted an intense reaction covering the area of 32–35 kD (Fig. 1b, Table 2). These responses are of interest, because the 31/32 kD antigen has been claimed to be a marker of early infection of schistosomiasis especially in non-immune individuals as well as found in patients with a chronic disease from endemic areas (Ruppel *et al.*, 1987b; Idris & Ruppel, 1988). It has been shown to appear before excretion of eggs in mice (Ruppel *et al.*, 1985b) and baboons (Evengård *et al.*, manuscript in preparation). Antibody responses directed against the 32–35 kD antigens were primarily restricted to the IgG1 and IgG3 isotypes as judged by ELISA. Some weak responses against these antigens by IgG2 and IgG4 were noticed in sera from two patients. This might reflect different degree of sensitivity in the two systems. Antigens of 49, 62, 75 and 92 kD molecular weights were also detected (Table 2). Non-specific binding of antibody to schistosome antigens have been noted in our experiments and in those carried out by Ruppel *et al.* (1985a). However, the non-specific reactions did not involve the 32–35 kD antigens.

It has been reported that glycoproteins of 55, 52 and 35 kD are precipitated by sera from patients in the acute phase of infection by *S. mansoni*. It was thus suggested that measuring a ratio of antibodies against 55 and 52 kD glycoproteins to antibodies against high molecular weight glycoproteins may be a way to differentiate an acute from a chronic infection (Norden & Strand, 1985). When sera from patients with a chronic schistosome infection were analysed by immunoblot (Fig. 3) several bands were detected and it seems that as the infection progresses the more antigens are recognized; similar observations have been made by Norden & Strand (1985).

When we used a subclass ELISA to study the response in patients with a chronic infection we found that anti-adult worm IgG1 and IgG4 and specific IgE antibodies increased 3 weeks

post-treatment (Evengård *et al.*, 1988). For those patients with an early infection, increased levels of IgG1 and IgG3 were found against the adult worm whereas no specific IgE was found. All patients also had increased levels of eosinophils (data not shown). The increase of selected immunoglobulin classes, subclasses and eosinophils gives rise to speculations whether T cell lymphokines such as IL-4 and IL-5 are regulating the immunoglobulin switch pattern in a schistosome infection.

We have reported (Evengård *et al.*, 1988) the increased IgA levels, especially IgA1, against the soluble AWA in patients with a chronic infection. These individuals, with an early infection of schistosomiasis presented here, also displayed increased levels of specific IgA1 against the AWA and against the egg antigen. None of our study patients had increased levels of specific IgA2. In a previous study (Jassim, Hassan & Catty, 1987) it was reported that IgA antibodies were selectively produced against egg antigens as opposed to adult worm and larval antigen measured by ELISA. We were not able to confirm these results. The anti-IgA subclass monoclonals used in this assay were previously evaluated (Engström *et al.*, 1988) and were found to be sensitive and specific for their corresponding IgA subclass. Functional differences due to biological properties between the two IgA subclasses is not yet clarified (for a review see Mestecky & Russell, 1986) and the dominance of IgA1 is therefore of unknown relevance.

The different functional properties of the IgG subclasses (Spiegelberg, 1974) such as their different efficiency in complement activation, makes it interesting to study the subclass distribution in the response to a parasite infection. Analysis of antibody subclasses involved in the immune responses against schistosomiasis is of interest for their implications both for disease pathogenesis and blocking antibodies mediating immune protection (Capron *et al.*, 1987; Butterworth *et al.*, 1987, 1988). The subclass distribution of specific antibodies has been described in individuals with a chronic infection of schistosomiasis (Iskander *et al.*, 1981; Jassim *et al.*, 1987; Evengård *et al.*, 1988).

Specific subclass patterns have also been reported in certain clinical manifestations of the parasitic disease. When analysing sera from patients with different stages of established filarial infections, Lal & Ottesen (1988) described enhanced diagnostic specificity in filariasis using an enzyme immunoassay assessing antibody of the IgG4 subclass only. Cabrera *et al.* (1988) report that by using an IgG3-specific ELISA they could distinguish sera from patients with SOWDA (chronic hyper-reactive onchocerciasis) from sera from patients with a generalized onchocerciasis. Hussain, Grogl & Ottesen (1987) showed correlations between distribution of IgG subclass antibodies and clinical manifestations of filariasis. They reported that an increase of specific IgG4 was found in asymptomatic patients with microfilaraemia and IgG3/IgG1 antibodies in patients with elephantiasis and antibodies belonging to different subclasses detected different antigens. Knowledge about the subclasses induced and their functional properties is also of value when designing vaccines as different responses are to be expected not only in different age-groups but also in individuals with different immunological status (Dunne *et al.*, 1988).

El Amin, Wright & Vlug (1986) noted an increase of specific IgG1 and IgG3 in patients with mucosal or visceral leishmaniasis. Scott & Goss-Sampson (1984) showed a similar distribution in sera from patients with an infection with *Trypanosoma cruzi*. A sequential appearance of IgG subclasses is shown in patients with trichinosis (Ljungström *et al.*, 1988) where specific antibodies of the IgG1 subclass appeared before IgG3 (early infection) and was later replaced by IgG4 (chronic infection). It has been suggested that an increased level of specific IgG4 antibodies against parasitic antigens results from a chronic infection as shown in schistosomiasis (Iskander *et al.*, 1981; Evengård *et al.*, 1988), filaria (Ottesen *et al.*, 1985), onchocerciasis (Magnusson *et al.*, 1986) and trichinosis (Ljungström *et al.*, 1988). However, no specific IgG4 antibodies were detectable in an acute or chronic toxoplasmosis, an infection with persisting antigen in humans. IgG1 was the predominant IgG subclass in all phases.

Our findings are in agreement with most of the reported antibody subclass analysis performed so far on parasitic diseases. However, the composition of the pattern of the subclass response depends not only on the length of infection but also on the composition of the antigenic stimuli. Polysaccharides give rise mainly to IgG2 (for a review see Hammarström *et al.*, 1986). In this study, where there is a lack of IgG2 against adult worm in sera from the six patients, the dominant antigen might be a protein epitope.

It is of interest to investigate the subclass reponse to schistosome-antigen on an epitope level, because certain subclasses may bind to different epitopes adjacently located and competitively inhibit further binding by other antibodies. It is also interesting to investigate the role of cytokines in development of the immune response as a distinct pattern of reactions can be distinguished within a population due to the length of time they have been exposed.

ACKNOWLEDGMENTS

We thank Professor Gunnar Johansson for kind help with analysis of specific IgE, Dr Inger Ljungström for reviewing the manuscript and Ms Anette Johnsson for excellent technical assistance.

This research was supported by the Swedish Medical Research Council and the Swedish Agency for Research Co-operation with Developing Countries (SAREC).

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