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Subclass distribution and IgE responses after treatment in human schistosomiasis

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SUMMARY

The IgG and IgA subclass distribution of specific antibodies as well as the distribution of total and specific IgE in 15 patients with schistosomiasis was determined in consecutive samples before and after initiation of treatment. An adult worm antigen preparation and a soluble egg antigen preparation were used as antigens in the ELISA assays. After initiation of treatment a rise was noted in certain subclasses and a correlation was found for specific IgG1 and IgG4 serum levels in the egg-excreting patients against adult worm antigen and for specific IgG4 and IgE levels in sera from the eight patients with a chronic disease. They also had a rise of the specific IgA1 titre and six of them also of specific IgA2. Members of eosinophilic granulocytes reached a peak after 2 weeks in seven of the eight patients. The increase of eosinophils was an early event as opposed to the incidence of peak of the determined specific isotypes. The associated rise in IgG1, IgG4 and IgE antibody concentrations and eosinophils may suggest a causal relation possibly induced by common interleukins.

Keywords schistosomiasis subclasses IgE eosinophils

INTRODUCTION

In recent years the IgG subclass distribution of the antibody response in parasitic diseases has been a topic of rising interest as differences could have an important pathogenetic significance. The antibody response is essential for the development of immunity and antibodies seem to mediate immunity not only to reinfection but also to primary infection as shown by passive transfer and vaccination studies (Capron & Dessaint, 1985). IgE antibodies seem to be essential in elimination of schistosomes by mediating binding of the parasite to effector cells. Antibodies of different classes and subclasses have different biological functions. The current hypothesis suggests that IgG4 production results from chronic or repetitive antigenic stimulation both by bacterial (Aalberse, van der Gaag & van Leeuwen, 1983), viral (Sundqvist, Linde & Wahren, 1984) and parasitic (Iskander, Das & Aalberse, 1981; Catty *et al.*, 1986; Jassim, Hassan & Catty, 1987) antigens. IgG antibodies to protein antigens are mostly of IgG1, IgG3 and IgG4 subclass whereas carbohydrates mainly induce IgG2 (for review, see Hammarström *et al.*, 1984a).

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A causal relation between the IgE and IgG4 isotypes is suggested by Magnusson *et al.*, 1986 as IgG4 and IgE serum levels correlated in patients with atopy, lymphatic filariasis and onchocerciasis. Such a correlation was also found by Hammarström *et al.*, 1986 in the case of 137 IgA-deficient donors. In the present study we measured the antibody levels of the different IgG and IgA subclasses as well as total and specific IgE at regular intervals after initiation of treatment of schistosomiasis.

MATERIALS AND METHODS

Patient population

Sera were collected from 12 immigrants from regions endemic for schistosomiasis and from three patients born in Sweden who were given treatment for schistosomiasis at Roslagstull Hospital, Stockholm, Sweden. They were all hospitalized during treatment and afterwards closely supervised at the out-patient clinic. The characteristics of the group is shown in Table 1. Sera were taken before treatment and mostly on days 7, 14, 21, 28, 35 and 180 after initiation of drug therapy. The number of serum samples varied from 4 to 9. In 10 patients eggs were found in rectal snips, stool or urine. Treatment was given to five patients in whom egg excretion could not be demonstrated. They had a history of contact with schistosome-contaminated fresh water

Table 1. Study population

Nr	Sex	Age	Species	Type of infection	Diagnosis	Concurrent parasites	Treatment	No. of samples
1	m	25	*mansoni*	A	eggs		O	6
2	m	32	*mansoni*	A	eggs		O	7
3	m	24	*mansoni*	A	eggs		O	5
4	m	24	*mansoni*	A	eggs		O	8
5	m	20	*mansoni*	A	eggs		O	7
6	m	37	*mansoni*	A	eggs	*Hym. nana*	O	7
7	m	36	*mansoni*	A	eggs		O	8
8	m	27	*mansoni*	A	eggs		O	6
9	m	40	*mansoni*	B	eggs		O	9
10	f	25	*haemat*	B	eggs		P	6
11	m	33	—	A	serology		M+P	6
12	m	22	—	A	serology		O	6
13	m	15	—	A	serology	*Strong., Giard.*, Ankylostoma,	O	7
14	m	24	—	A	serology	*Hym. nana*	O+P	4
15	m	39	—	A	serology		O+P	7

A = chronic disease. B = acute disease. *Hym. nana* = *Hymenolepis nana*. *Strong.* = *Strongyloides stercoralis*. *Giard.* = *Giardia lamblia*. O = Oxamniquine. P = Praziquantel. M = Metrifonate.

and significant serological titres using an indirect immunofluorescence assay for antibodies against adult worms (F/P molar ratio 3·0, NBL, Stockolm, Sweden) and unfixed frozen section of schistosomes as antigens employing a FITC anti-human IgG conjugate and an enzyme immunoassay ELISA for *S. mansoni* egg antigen using a horseradish peroxidase anti-human Ig conjugate.

Eight of the 10 patients excreting eggs had lived for a long time in endemic areas with frequent exposure to parasites and were considered to suffer from chronic schistosomiasis (Table 1). Two had been exposed to contaminated fresh water less than 6 months before treatment and were considered to have an early disease. The five with a diagnosis based on serology had been exposed several times for more than 5 years before treatment.

Oxamniquine 30 mg/kg was given to 11 patients, Praziquantel 40–50 mg/kg to one patient, Metrifonate 7–10 mg/kg repeated after 14 days and Praziquantel later to one and Oxamniquine and Praziquantel to two patients (Table 1).

Antigens

Adult worm antigen preparation (AWA). Adult *S. mansoni* worms were recovered from 8-week infected NMRI mice, rinsed in phosphate buffer and homogenized in a Sorwall 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 supernatant was dialysed against PBS overnight.

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

Immunoglobulin subclass measurements. Microtitre ELISA plates were coated with 5 μg/ml of AWA and 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 Ig subclass antibodies were mouse monoclonal antibodies of IgG1 subclass. The monoclonal antibodies have different avidity and the assay was standardized using myeloma proteins as subclass antigens (Persson, Hammarström & Smith, 1985).

IgE determinations. IgE was measured using an enzyme immunoassay technique (Phadezym IgE PRIST, Pharmacia Diagnostics Uppsala, Sweden) following the recommendations of the manufacturer.

Specific IgE ab. IgE antibodies to the schistosoma antigens from adult worms and eggs (see above) were determined by the radioallergosorbent test, RAST, essentially as described (Johansson, Bennich & Berg, 1971). Five microlitres of adult antigen, 0·22 mg/ml, and five microlitres of egg antigen, 0·82 mg/ml, respectively, were coupled per CNBr-activated filter paper disc. Phadebas anti-IgE (Pharmacia Diagnostics AB, Uppsala) 125-I labelled tracer was used and the results were expressed in PRU/ml using Phadebas Reference System (Pharmacia Diagnostics AB, Uppsala). No significant non-specific binding (< 0·35 PRU/ml) of non-antibody active IgE was noted for IgE levels below 3,000 kU/l.

Differential counts. Thin blood films on slides were fixed in methanol for 4 min followed by staining in May–Grünewald for 3 min and Giemsa for 13 min.

Statistics. Student's *t*-test was used for parametric and Wilcoxon's ranking test for non-parametric analysis. Correlation coefficients were calculated with a *P*-value to evaluate the results.

RESULTS

Before treatment, we found mostly specific IgG1 and IgG4. The absorbance values were well above background levels and had, with few exceptions, the same relation as later in the kinetic study.

The kinetics of the immunoglobulin subclass pattern of anti-schistosome antibodies in sera from two patients with chronic

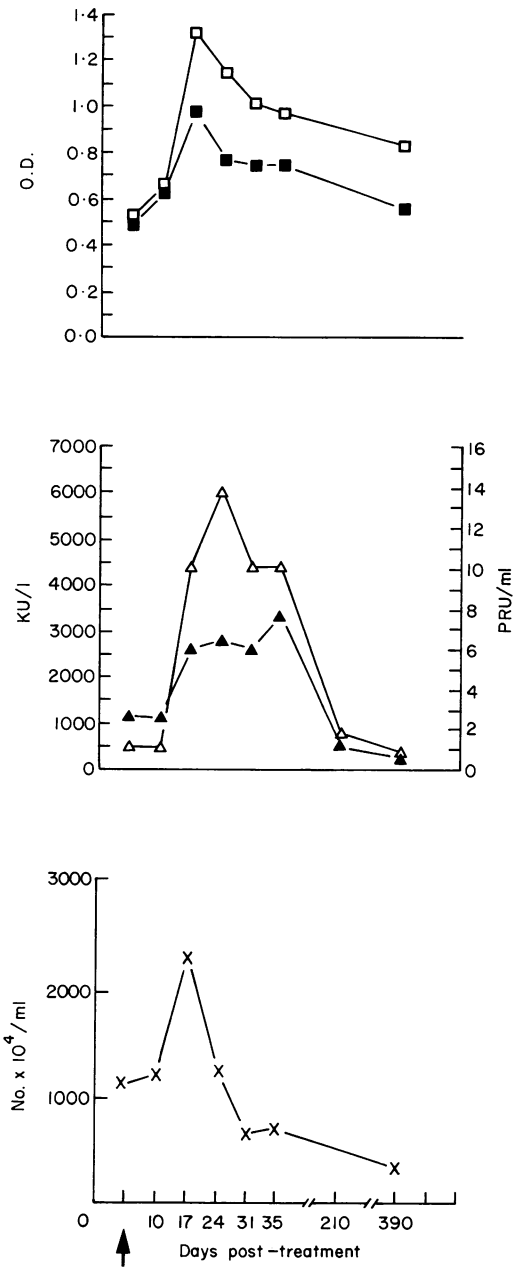

Fig. 1. Antibody activity as measured by ELISA using an adult worm antigen preparation in consecutive sera from a patient with a chronic schistosomiasis. Treatment is indicated by the arrow. Total level of IgE (kU/l), specific IgE antibodies (PRU/ml) and amount (× 10/ml) of eosinophils are also demonstrated. (□) IgG4; (■) IgG1; (△) IgE tot; (▲) IgE spec; (×) eosinophils.

schistosomiasis is shown in Fig. 1. This pattern is typical for the eight patients with a chronic disease and with non-calcified eggs in faeces or rectal snip. Figure 2 demonstrates the peak values of immunoglobulin subclasses, IgE and eosinophils after treatment in these eight patients.

The five patients with a diagnosis based on serology, showed some variation in patterns with regard to peak antibody response after drug administration. One gave no reaction at all. The two with an acute infection also showed individual patterns.

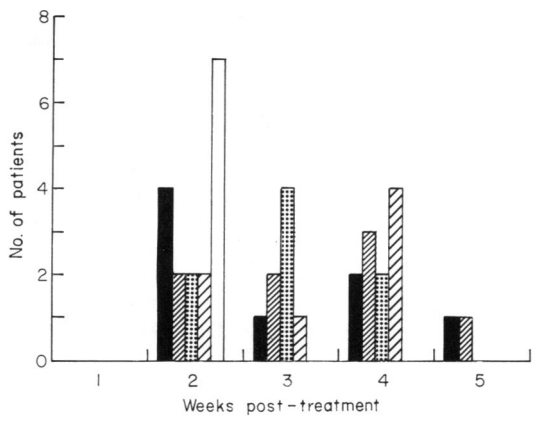

Fig. 2. Peak values of immunoglobulin subclasses, IgE and eosinophils as measured at various time intervals after treatment in the eight patients with a chronic disease. (■) IgG1; (▨) IgG4; (▦) IgA1; (▨) IgE; (□) eosinophils.

Table 2. Increase of IgG1 and IgG4 and its significance expressed as absorbance value at peak/absorbance value pretreatment in ELISA using (a) adult worm antigen and (b) soluble egg antigen.

(a)		
IgG1	8/14	>150%*
	6/14	105–146%
IgG4	1/14	<100%
	10/14	>150%**
	3/14	121–148%
(b)		
IgG1	2/14	<100%
	2/14	>150%
	10/14	105–149%
IgG4	3/14	<100%
	7/14	>150%**
	4/14	114–149

U chosen to 1·50 (=increase of 50%)
t-test **0·1% $< P <$ 1%, * 1% $< P <$ 5%.

IgG subclasses

A significant rise of IgG4 against both tested antigens was noted (Table 2) in the total population as was the rise of IgG1 against adult worm antigen. Table 3 shows the correlation coefficients both for IgG subclasses and total IgE levels. There is a strong correlation ($r = 0{\cdot}93$, $P < 0{\cdot}001$) between the increase of IgG4 and IgG1 against adult worm antigen expressed as absorbance value of peak/absorbance value pretreatment for the 10 patients excreting eggs. The correlation coefficient is 0·83, $P < 0{\cdot}05$ for IgG4 and specific IgE calculated in the same manner against adult worm antigen for the eight egg-excretors with a chronic disease.

There is a correlation (Table 4) between incidence in time of the peak for IgG4 and IgG1 against adult worm antigen ($r = 0{\cdot}87$, $P < 0{\cdot}001$) and soluble egg antigen ($r = 0{\cdot}89$,

Table 3. Correlation coefficients of the increase of IgG4, IgG1 and total IgE expressed as absorbance value of peak/absorbance value pretreatment. No. of patients giving a value > 1·00 are indicated.

	Patients excreting eggs ($n=10$)							
	AWA			SEA				
Ig	G4	A1	A2	G1	G4	A1	A2	E
	***9	10	10	**9	8	10	10	7
G1	0·93	−0·02	0·23	0·81	−0·26	0·19	−0·01	0·49
		9	9	8	8	9	9	6
A G4		0·20	0·42	0·44	0·14	0·38	−0·02	0·77
W			10	9	8	10	10	7
A A1			0·73	0·14	−0·53	0·49	0·28	0·60
				9	8	10	10	7
A2				0·60	−0·62	0·18	0·38	0·35
					8	9	10	7
G1					−0·37	−0·25	0·36	0·07
						8	8	7
S G4						−0·12	−0·42	0·48
E							10	7
A A1							0·17	0·30
								7
A2								−0·14

The significance is indicated by stars, *** = $P<0·01$. ** = $P>0·01$.

Table 4. Correlation coefficients of incidence of peak in time (weeks) for antibodies of IgG4 and IgG1 subclass and total and specific IgE using (a) adult worm antigen and (b) egg antigen.

(a)	Ig	G4 ***	E	specific IgE
	G1	0·87	0·46	−0·58
	G4		0·33	−0·64
		***		
(b)	Ig	G4	E	−0·27
	G1	0·89	0·11	−0·06
	G4		0·06	

$n=14$. *** = $P<0·001$.

$P<0·001$). All showed a decrease in antibody activity after 6 weeks. Serum IgG2 anti-SEA levels were increased in 3–15 patients and serum IgG3 anti-SEA levels were increased in 5–15 (calculated as > 2 values > 2-fold background OD values). One patient had an increase of both subclasses. No one showed an increase of these two subclasses against adult worm antigen.

IgA subclasses

All eight egg-excretors with a chronic disease had an increase (as defined above) of IgA1 against AWA from 1·3 to 3·5 times the pretreatment value within 2–4 weeks and 6–8 showed an increase of IgA2 against the same antigen. Two of the other seven had an increase of 1·8 times pretreatment value of IgA1 against AWA with no increase for the others. None had an increase of IgA2. The IgA response against SEA was low .

IgE

IgE was measurable in seven of the eight patients with a chronic disease. In these seven patients specific IGE anti-AWA was detectable. The correlation coefficient for absorbance value at peak/absorbance value pretreatment was 0·81, $P<0·05$ for total IgE and specific anti-AWA IgE. The two egg excretors with an acute disease had detectable but low levels of IgE but no specific IgE.

Effector cells

Seven of the eight egg excretors with a chronic disease had an increase in eosinophils from 1·8 to 4·9 times the pretreatment value ($P=0·01$, Wilcoxon signed rank test for paired observations). Seven of these eight patients had a peak after 2 weeks and no reaction was seen in one patient. The latter individual had undetectable levels of total IgE. For these eight patients the neutrophil counts varied from 0·54 to 1·0 of the pretreatment value.

DISCUSSION

Before treatment measurable levels of specific antibodies of IgG1 and IgG4 subclass were noted. Three weeks after treatment we observed an increase of either IgG1, IgG4 or both against adult worm antigen in 14 of the 15 patients. This increase suggests that released worm antigens give rise to a subclass-restricted response. The likely explanation is a booster effect caused by released parasite antigens. As it is sometimes necessary to treat a patient due to high serological titres and a positive anamnesis the increase of specific immunoglobulin 2–5 weeks after treatment and a subsequent decrease in titres after 3 months might confirm the diagnosis.

IgG4 and IgE have been shown to have a similar specificity in sera from patients with filariasis although not shown at the

epitope-level (Hussain & Ottesen, 1986). IgE antibodies play an important role in the ADCC which appears as a primary mechanism of defence against schistosomes in *in vitro* experiments (Capron & Dessaint, 1985). IgG4 antibodies may therefore interfere with the binding of protective IgE class antibodies and act as a blocking agent (Khalife *et al.*, 1986).

There are two IgA subclasses in man and an immune response normally results in antigen specific antibodies of both subclasses (for a review, see Mestecky & Russell, 1986). IgA1 is normally the dominating IgA subclass against protein antigen. The highest increase of specific IgA antibodies is in the IgA1 subclass.

The mechanisms that regulate the isotype composition of antibodies during the immune response are largely unknown. A B cell clone that has switched from IgM to the production of a new isotype may theoretically also undergo additional isotype switches (Teale & Abraham, 1987). It has been suggested (Flanagan & Rabbitts, 1982) that the order of the immunoglobulin heavy-chain genes on chromosome 14 is 5′-γ_3-γ_1-pseudo ε-α_1-γ_2-γ_4-ε-α_2- 3′. Using a protein antigen a sequential development of IgG1, IgA1 and IgG4 was suggested (Hammarström, Persson & Smith, 1984b) supporting a distinct pattern of class and subclass switches. In the chronically ill patients the observed parallelism between IgG1 and IgG4 may suggest that a switch from IgG1 to IgG4 does not occur to any major extent or that a consecutive switch is a rapid event. However, in our patients with a chronic infection the switch phenomenon may differ from that in a primary response.

We found a close correlation between specific IgG4 and IgE and after 2 weeks there was also a significant rise in eosinophils. It is interesting to note in this context the existence of interleukin 4. This interleukin, which recently has been isolated in man (Yokota *et al.*, 1986) has many biological properties and in the mouse causes an increase in IgG1 and IgE production (Lee *et al.*, 1986; Noma *et al.*, 1986). IL-4 is also a mast cell growth factor so its actions may also be a stimulus on IgE-mediated eosinophil cytotoxicity (Lee *et al.*, 1986; Smith & Rennick, 1986). Furthermore, the eosinophil differentiation factor (Sanderson, Warren & Strath, 1985; Sanderson *et al.*, 1986), also named IL-5 (Azuma *et al.*, 1986), was described as having the capacity to enhance eosinophil mediated killing of schistosomula. It has recently been shown that *in vivo* IgE formation in *Nippostrongylus brasiliensis*-infected mice (Lebrun & Spiegelberg, 1987) is linked to IgG1 formation suggesting that both IgE and IgG1 formation is dependent on T-helper cells forming IL-4 as also reported by Coffman *et al.*, 1986. The similar functional properties of mouse IgG1 and human IgG4 as lacking the ability to fix complement and having an anodal electrophoretic mobility might suggest that IgG4 is the subclass linked to the production of IgE (Lebrun & Spiegelberg, 1987). It is tempting to speculate that one or two interleukins acting alone or in concert are involved in the processes behind the present observations of IgG subclasses, IgE and eosinophils in man.

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