HUMAN ANTIBODY ISOTYPE RESPONSES TO *SCHISTOSOMA JAPONICUM* EGG ANTIGEN

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Abstract. *Schistosoma japonicum* - infected subjects from Hubei province of China were investigated to determine the class and subclass of the antibody response to soluble egg antigen (SEA), using an enzyme-linked immunosorbent assay. The subjects were 50 acute and 55 chronic cases. In acute cases, the mean OD values for IgA, IgE and IgG3 were very high, while the positive ratios of IgA and IgE were only 78% and 74%, respectively. The positive ratios of IgG, IgM, IgG1, IgG3 and IgG4 were all above 90%. In chronic cases, the mean OD values for IgG, IgG3 and IgG4 were very high, and the positivity rates of IgG, IgG1, IgG3 and IgG4 were all above 90%. Comparing the two study groups, the mean OD values of IgM, IgA, IgE were higher in acute cases than those of chronic cases ($p < 0.0001$), while the mean OD values of IgG, IgG4 were higher in chronic cases than in acute cases ($p < 0.05$). The mean OD values of IgG3 in both groups were high and those of IgG2 in both groups were low.

INTRODUCTION

Schistosomiasis is a parasitic helminth infection that affects an estimated 200 million people in tropical regions. Infections are chronic in nature, with adult worms living for several years in the host's blood stream. Of the many factors that determine the outcome of schistosomiasis in man, the quality of the antibody response may well be critical. The role of antibody in protective immunity against schistosomiasis has been investigated by many workers (reviewed by Smithers and Doenhoff, 1982; Capron *et al,* 1982, 1985). Human antibody class and subclass responses to *S. mansoni* and *S. haematobium* antigens have been reported (Jassim *et al,* 1987; Dunne *et al,* 1988; Evengard *et al,* 1990; Langley *et al,* 1994; Rabello *et al,* 1995), but there has been no report on human antibody class and subclass responses to *S. japonicum* antigen. Knowledge of the antibody subclass responses to different antigens is essential for vaccine development and diagnosis of *S. japonicum* infection. In this study, we have analysed human antibody class and subclass responses against *S. japonicum* egg antigens in acutely and chronically infected cases.

MATERIALS AND METHODS

Human sera

Acute case sera were obtained from 50 Chinese patients with *S. japonicum* eggs in their stool attending the clinic of Hubei Institute of Schistosomiasis Control (People's Republic of China), about one month after their contact with water contaminated with cercariae of *S.japonicum.* The subjects presented fever, diarrhea and liver enlargement.

Chronic case sera were obtained from 55 Chinese patients aged 10-57 years with *S.japonicum* eggs in their stool in the highly endemic area of Gongan country, Hubei province, People's Republic of China. Most of the patients had minimal or no hepatosplenomegaly; all had a high level of IgG anti-soluble egg antigen antibody.

Normal human sera were collected from students of Dokkyo University School of Medicine, Tochigi Prefecture, Japan, with no history of schistosomiasis.

Parasite antigen

S. japonicum Yamanashi strain was maintained in *Oncomelania nosophora* snails and ICR strain mice. *S. japonicum* eggs were isolated from the intestines of infected mice by digestion with pronase and collagenase, lyophillized, and stored -70°C until use (Matsuda *et al,* 1984). Soluble egg antigen (SEA) refers to the supernatant fluid obtained from eggs that had been homogenized in carbonate buffer, pH9.6.

IgG, IgM, IgE, IgA and IgG subclass responses to *S. japonicum* SEA

The ELISA methods used were essentially those described elsewhere (Matsuda *et al,* 1984).

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The optimal condition for ELISA were determined by a checker-board titration. Microtiter plates were coated at 37°C for 2 hours with 10 μg SEA/well and then kept at 4°C overnight. Plates were then washed in 0.5% Tween 20 in phosphate buffered saline. For total specific IgG and IgM antibody assays, sera were tested at a dilution of 1:200; for IgA and IgE assays, sera were diluted at 1:10. Plates were washed after 45 minutes incubation at 37°C. Goat anti-human IgG (r chain) (TAGO Inc, Japan: no 2390), anti-human IgM, anti-human IgA, and anti-human IgE (Miles Scientific, Israel Nos. 61-132, 61-131, 61-133.) were used to determine total IgG, IgM, IgA and IgE responses. Plates were incubated at 37°C for 60 minutes and then developed using ABTS (2-2′ - azino-di-3-ethylbenzthiazoline sulfonic acid). The optical density of each well was determined at a wavelength of 415 nm.

For IgG subclasses assays sera were tested at a dilution of 1:50; plates were washed after 1 hour incubation at 37°C and then mouse anti-human IgG1, IgG2, IgG3 and IgG4 monoclonal anitbodies (Cosmo Bio, Japan: Nos. 0280, 0281, 0282, 0283) were added and the plates were incubated at 37°C for 2 hours. After washing, goat anti-mouse IgG1 conjugated with horse-radish peroxidase (Zymea Japan: No.61-0120) was used to determine IgG subclass respones; and plates were incubated at 37°C for 60 minutes. After washing, plates were developed using ABTS substrate and the optical density of each well was determined at a wavelength of 415 nm.

Statistical analysis

Optical densities greater than the mean value plus 3 standard deviations given by the normal sera were considered as positive. The statistical significance of differences between means was analysed by using Student's *t*-test.

RESULTS

The relative amounts of antibodies to SEA in all immunoglobulin classes and IgG subclasses in serum were determined in all individuals of the two study groups. The data are summarized in Table 1 which gives the mean optical density (OD) readings by subject group, and isotype. Figs 1-3 show the range of class and subclass of antibody responses in acute and chronic Chinese schistosomiasis cases and normal control subjects. Figs 4 and 5 show the comparison between mean OD value and positive rates for acute and chronic cases and the normal control subjects.

It can be seen from Table 1 that total IgG antibody responses to SEA in both of schistosomiasis patients were high, with few individuals failing to respond. It appears that the reaction in chronic cases was stronger than that in acute cases ($p < 0.05$, Fig 4). However, the IgG subclass antibody responses to SEA were different in acute and chronic patients. In acute cases, the IgG3 antibody response was very strong, the responses of the other IgG subclasses, IgG1, IgG2, IgG4 were weaker. In the chronic cases, IgG3 and IgG4 responses were very strong and IgG1 and IgG2 responses are weaker. Comparing the IgG subclass responses between the two infected study groups, the IgG1 and IgG3 responses were similar ($p > 0.1$, Table 1), but the IgG2 and IgG4 responses differed ($p < 0.05$). The IgG2 response in acute cases was stronger than in chronic cases, while the IgG4 response was stronger in chronic than in acute cases. Although the IgG1 response was not strong in either group, the positivity rates were still higher than 90%. the positivity rates of total IgG, IgG1, IgG3 and IgG4, but not IgG2 were also higher than 90% (Fig 5).

With the IgM, IgA and IgE responses to SEA, there were some important differences between the acute and chronic groups. In acute cases, IgA and IgE responses were very strong and the IgM response was less so. In chronic cases, all of these responses were very weak. All three responses in the acute group were stronger than those in the chronic group ($p < 0.0001$, Fig 4). However, the positivity rate of IgM in acute group reached a maximum of 98%, while the positivity rates of IgA and IgE in the same group were only 78% and 74%, respectively; the positive rates of IgM, IgA and IgE in the chronic group were much lower than those in the acute group, only 29.1%, 21.8% and 12.7%, respectively (Fig 5).

DISCUSSION

In recent years, the IgG subclass distribution of the antibody response in parasitic diseases has been a topic of increasing interest as differences could have an important pathogenetic significance. The antibody response is essential for the development of immunity and antibodies appear to mediate immunity not only to reinfection but also to primary infection. Antibodies of different classes and subclasses have different biological functions. Previous studies suggested that IgG4 production results from chronic or repetitive antigenic stimulation by bacterial (Aalberse *et al,* 1983), viral

Table 1
Prevalence and relative concentration of *S.japonicum* antibodies in infected subject from China detected by ELISA using SEA (soluble egg antigen).

Subject groups (no.)	antibody class and IgG subclass							
	Total IgG	IgM	IgA	IgE	IgG1	IgG2	IgG3	IgG4
Normal subjects	(29)							
Mean OD415#	0.079	0.042	0.078	0.075	0.013	0.017	0.013	0.047
Mean+3SD	0.205	0.084	0.381	0.474	0.031	0.038	0.043	0.065
Acute subjects	(50)							
Positive (%)*	100	98	78	74	92	60	100	90
Mean	0.993	0.711	1.449	1.485	0.338	0.17	0.909	0.515
SD	0.415	0.633	0.944	1.053	0.306	0.225	0.74	0.495
Chronic subjects	(55)							
Positive (%)	98.2	29.1	21.8	12.7	100	50.9	100	94.5
Mean	1.245	0.078	0.27	0.262	0.315	0.071	0.932	0.941
SD	0.407	0.059	0.307	0.378	0.224	0.102	0.676	0.799
Significance (p value), acute *vs* chronic cases	<0.05	<0.0001	<0.0001	<0.0001	>0.1	<0.05	>0.1	<0.05

* The percentage of subjects in each infection group with sera giving OD415 values>3SD above the mean of normal sera for the respective isotypes.

The mean OD415 ELISA values with standard deviation SD for all subjects'sera in the group irrespective of whether they were positive or negative (see above) in the assay.
p values determined by Student's *t*-test.

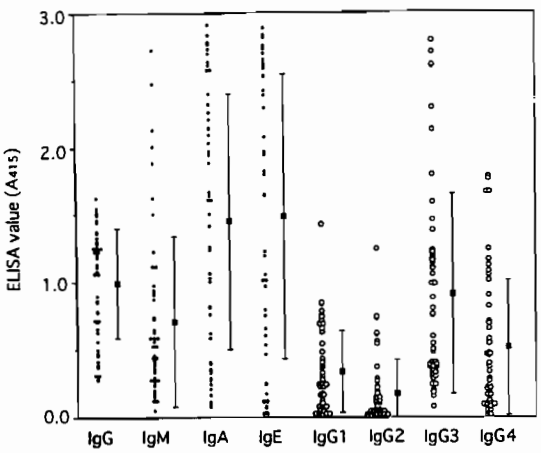

Fig 1–Scatter diagram of Ig class and IgG subclass antibodies against SEA in Chinese acute cases.

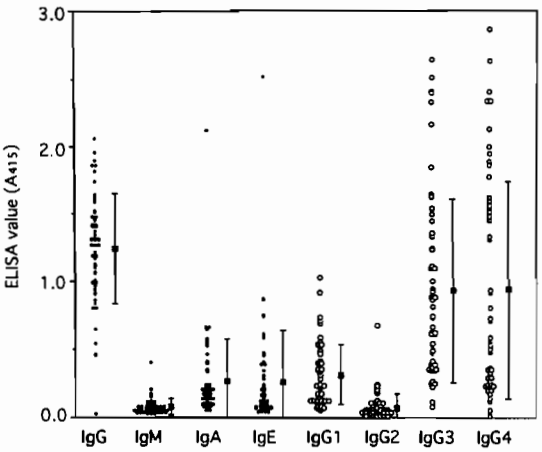

Fig 2–Scatter diagram of Ig class and IgG subclass antibodies against SEA in Chinese chronic cases.

(Sundqvist *et al,* 1984) and parasitic antigens. IgG antibodies to protein antigens are mostly of the IgG1, IgG3 and IgG4 subclasses whereas carbohydrates mainly induce IgG2 (for review, see Hammastrom *et al,* 1984a). Specific subclass patterns in schistosomiasis mansoni have also been reproted. Both Evengard *et al* (1990) and Boctor (1990) reported that in acute and chronic cases, the main antibody responses to SEA were IgG1 and IgG2. But Jassim *et al* (1987) and Evengard *et al* (1988) found that in chronic cases, IgG1 and IgG4 responses to SEA were very strong. Their results differed somewhat from ours. concerning the IgG1, IgG2 and IgG4 responses, our findings were almost identical with

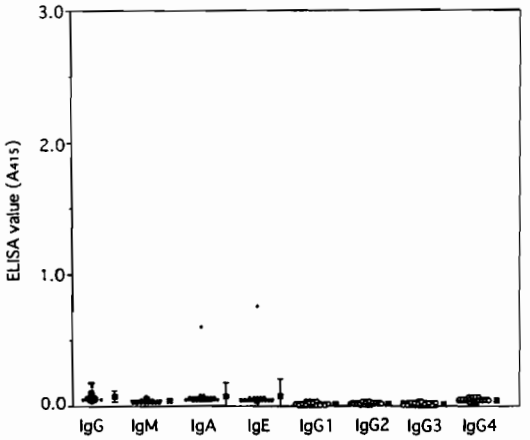

Fig 3–Scatter diagram of Ig class and IgG subclass antibodies against SEA in Japanese normal cases.

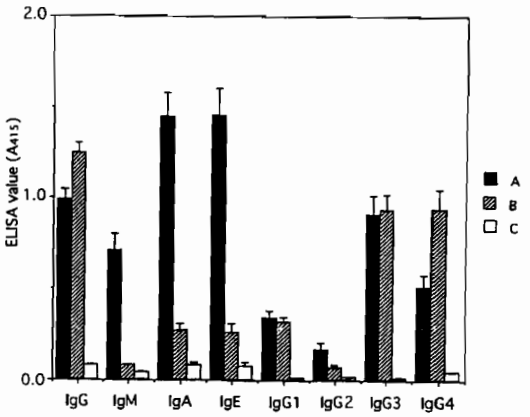

Fig 4–Mean absorbance value at 415 nm (OD) in ELISA using sera from patients with (A) n = 50 acute, (B) n = 55 chronic schistosomiasis japonica and (C) n = 29 normal control.

those of the above authors. But IgG3 response to SEA in schistosomiasis mansoni was not strong. Our results showed that, in both acute and chronic cases, the IgG3 response to SEA was very strong. This may reflect a difference between *S.mansoni* and *S.japonicum*. Specific subclass responses have also been reported in certain clinical manifestations of the parasitic diseases. Cabrera *et al* (1988) reported that, by using an IgG3 specific ELISA, they could distinguish sera from patients with sowda (chronic hyper-reactive onchocerciasis) from sera from patients with generalized onchocerciasis. Hussain *et al* (1987) showed correlations between the distribution of IgG subclass antibodies and clinical manifestations of filariasis. They reported that an increase of specific IgG4 was found in asymptomatic patients with microfilaremia and IgG3/IgG1 antibodies in patients with elephantiasis; antibodies belonging

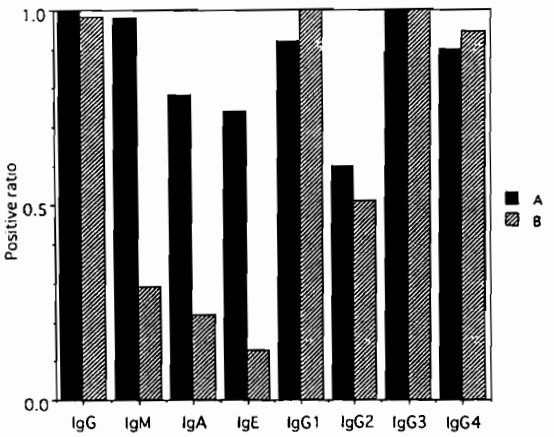

Fig 5–Positive rations of tested samples each group. (A) acute cases, n = 50 (B) chronic cases, n = 55.

to different subclasses detected different antigens. Evengard *et al* (1990) thought the pattern of the subclass response depended not only on the length of infection but also on the composition of the antigenic stimuli. The difference in IgG3 responses to SEA between *S.mansoni* and *S.japonicum* may be caused by differences in the antigen epitopes or the existence of species-specific antigens.

In a previous study; using ELISA (Jassim *et al*, 1987), it was reproted that IgA antibodies were selectively porduced against egg antigens as opposed to adult worm and larval antigens. Our study showed that the IgA antibody level to SEA was significantly higher in acutely infected patients with *S.japonicum* than in the chronically infected group. The results obtained were indentical with the foundings of Rabello *et al* (1995) and Evengard *et al* (1988, 1990). As an IgM response always reflects a recent infection, it was not surprising that the IgM antibody level to SEA was higher in the acutely infected than in the chronically infected group. However, the IgE response in chronic schistosomiasis mansoni cases is stronger than that in acute cases, (Evengard *et al*, 1988, 1990; Ottesen *et al*, 1981). These results differed from ours, possibly due to the difference in the antigen used. Ottesen *et al* (1981) suggested that, while IgE antibodies are induced against antigens common to all three stages of the parasite (cercariae, adult worm and egg) in chronically infected patients, it is the egg which serves as the most important source of allergens in acutely infected individuals. IgE directed against various stages of the parasite life cycle are significantly correlated with low intensities of reinfection and have the ability to protect against reinfection (Hagan *et al*, 1991;

Dunne *et al,* 1992; Rihet *et al,* 1991).

Examination of specific Ig isotypes has permitted a more detailed analysis of the immune response during schistosomiasis, in regard to both the particular antigens recognized by each isotype and the appearance and disappearance of specific isotypes during the course of infection. Research on the relationship between patient's age, intensity of infection, course of infection and antibody isotype resopnses is necessary to define in more detail the function and turnover of the different antibody isotypes in schistosomiasis japonica.

ACKNOWLEDGEMENTS

This study was supported by the Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (No. 07670289) for HM.

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