ISOTYPIC ANTIBODY RESPONSES OF A POPULATION IN AN ENDEMIC AREA OF SCHISTOSOMIASIS JAPONICA AND THEIR EPIDEMIOLOGIC SIGNIFICANCE

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Abstract. The present study was designed to explore if there exsits a correlation between predominant isotype-defined antibody levels and reinfection in low age groups of the population in an endemic area of schistosomiasis japonica in China. One hundred and thirty-eight individuals aged 3-25 years old were selected for serological investigations including the levels of IgG, IgG4, IgM and IgE, detected by ELISA with soluble egg antigen and soluble adult worm antigen. Results show that age is a determinant for SEA-specific IgG, IgG4 and IgE, and SWA-specific IgG and IgG4 antibody levels, which increased with age, and that SEA- and SWA- specific IgG4 antibody levels are risk factors of reinfection, *ie*, the risk of reinfection occurrence of the population with high level of SEA or SWA-specific IgG4 is 2.83 or 2.40 times, respectively, that with low level of SEA or SWA-specific IgG4, suggesting that in the endemic area of schistosomiasis japonica, there exists a possibility that in the population aged 3-25 years, SEA and SWA-specific IgG4 antibodies mediate a blocking immunity response.

INTRODUCTION

Many investigations have demonstrated the slow development with age of resistance to reinfection after chemotherapy of *Schistosoma mansoni* (Sm) and *Schistosoma haematobium* (Sh) infections (Hagi *et al*, 1990; Kigoni *et al*, 1986) and indicated that a specific IgE antibody response appears as a strong correlation of protective immunity in humans (Kigoni *et al*, 1986; Capron *et al*, 1981; Joseph *et al*, 1983; Grzych *et al*, 1982; Bazin *et al*, 1980; Capron *et al*, 1987; Capron *et al*, 1985; Hagan *et al*, 1991) and that the susceptibility to reinfection after chemotherapy is significantly correlated with the presence of high levels of IgM, IgG2 and IgG4 (Butterworth *et al*, 1987; Dunne *et al*, 1988; Khalife *et al*, 1986; Grzych *et al*, 1984), which may block the binding to target antigens on schistosomula surface of protective IgE antibody.

The present study was designed, based on the findings mentioned above, to explore if there exists a similar phenomenon in naturally infected individuals living in the endemic areas of schistosomiasis japonica in China. Therefore, serological investigations on a group of 196 individuals aged 3-25 years old, from Nanshan Island of Poyang Lake in Jiangxi Province, were performed in an attempt to find the age-specific correlation of IgG, IgM, IgE and IgG4 antibody response levels against soluble egg antigen (SEA) and soluble adult worm antigen (SWA), indicated by ELISA, and to show the correlation of reinfection after chemotherapy with exposure to cercariae, with antibody response levels in the low age groups of the investigated population.

MATERIALS AND METHODS

Status of the selected field in endemic area

Nanshan island, a small land mass in Poyang Lake, including three neighboring villages named Qiujia, Chenjia and Shanxi, was selected to act as test field area. The residents on the island have been living on agriculture and fishery; water that they need for living needs (*eg* washing clothes and vegetables) is almost all from the Poyang Lake. There were 1,759 persons on the island, and out of 671 residents aged 3-25 years, 196 persons (115 males, 81 females) infected with *S. japonicum* (Sj) were selected to act as subjects.

Investigation of prevalence and intensity of infection in the endemic area

In February 1993, a survey of the whole population in the field was carried out, which included general items such as name, sex, age, profession, education, history of schistosomiasis, physical examination and detection of parasite eggs in stools by modified Kato Katz method to obtain the data on the prevalence degree and infection intensity.

Collection of sera from the patients infected with *S. japonicum* (Sj)

In February of 1993, all subjects received Praziquantel treatment (60 mg/kg for those persons with eggs in their stools and 50 mg/kg for those without eggs in stools); 45 days later (in April) and before the infectious season began, from 138 individuals who had no Sj eggs in their stools 3 ml blood samples were taken, sera separated and stored at -70°C to be used for detection of specific isotypic antibodies.

Detection of isotypic antibodies by ELISA

Soluble egg antigen (SEA): The eggs were separated from the liver of a rabbit infected with Sj for 45 days, suspended in physiological saline and then homogenized to break egg shells. The homogenized material was centrifuged at 100,000*g* for 60 minutes at 4°C and supernatant was removed to be used for antigen (SEA). The protein amount of SEA was determined to be 4.416 mg/ml by Kalckar's equation (Kalckar, 1947).

Soluble adult worm antigen (SWA): From the portal vein of rabbit infected with Sj for 45 days, Sj adult worms were collected by use of perfusion technique, dried in vacuum and homogenized in physiological saline. After freezing and thawing 5 times, the suspension was centrifuged at 10,000*g* for 30 minutes at 4°C and the supernatant (SWA) was removed and stored in aliquout at -70°C. The protein amount of SWA was measured as mentioned above to be 1.4 mg/ml.

Reference sera

Positive reference sera had two types, *ie* 40 sera from patients with acute Sj infection, provided by Nanjing Anti-schistosomiasis Hospital, were pooled and used as positive reference serum for IgM and IgE detection. Twenty sera from patients with acute Sj infection in the tested area were pooled, to be used as positive reference serum for IgG and IgG4 detection. Forty sera were collected from patients in Nanjing children hospital, who lived in non-endemic area of schistosomiasis, pooled and used as negative controls.

Source of other materials used for ELISA: The ELISA plates were purchased from Huangyan Plastic Factory, Zhejiang Province, China in the same batch. HRP conjugated goat anti-human IgE was purchased from SIGMA company; HRP conjugated mouse anti-human IgM or IgG4 was from ZYMED company, USA; and HRP conjugated rabbit anti-human IgG was from DAKO company, disposed in aliquots by Huamei company, Shanghai, China. The substrate, TMB, was provided by Suzhou Chemical Manufactory, China.

Detection conditions: Based on the method reported by Dunne *et al* (1992), ELISA was performed. The "checkerboard" titration method was used to select the appropriate reaction conditions, such as concentration of antigen used for coating plates, concentration of blocking reagent, dilutions of sera to be tested, incubation condition and dilutions of HRP conjugates.

Detection of SEA-specific IgG, IgM, IgG4 and IgE levels in the sera from individuals: The plate wells were coated with 100 μl each of SEA at 1:400 dilution in carbonate coating buffer, pH 9.6, at 4°C overnight and then washed with PBST (PBS containing 0.05% Tween-20, pH 7.4) three times (5 minutes each), blocked with 200 μl per well of 4% fat-free milk powder at 4°C overnight, washed again with PBST (5 minutes each) three times. The defined reaction conditions of the tested sera and the conjugates were taken as follows: The sera to be tested for IgG, IgM, IgG4 and IgE isotypic antibody detections were used at dilutions of 1:400, 1:400, 1:80 and 1:80, respectively. The conjugates were diluted to 1:5,000, 1:1,500, 1:600 and 1:2,000 and used for IgG, IgM, IgG4 and IgE detection, respectively. One hundred microliters per well of sera were incubated with plates at 37°C for 30 minutes for IgG, IgM and IgG4 detection and at 4°C overnight for IgE detection. After antibody reaction, the plate wells were washed with PBST three times (5 minutes each) and reacted with different conjugates at 37°C for 60 minutes. After conjugate reaction, the plate was washed with PBST four times (5 minutes each), and incubated with TMB/

H_2O_2 solution (100 µl/well). When the substrate reaction was completed, 50 µl of 2M H_2SO_4 solution were added to stop the reaction and the ELISA results were demonstrated by OD value on DG 3022 ELISA READER at 450 nm wavelength.

Detection of SWA-specific IgG, IgM, IgG4 and IgE levels in the sera from individuals: The ELISA plate wells were coated with 100 µl/well of SWA at 1:150 dilution in carbonate coating buffer, pH9.6 at 4°C overnight and then washed, blocked as above. Sera to be tested for IgG, IgM, IgG4 and IgE isotypic antibody detection were used at 1:200, 1:100, 1:20 and 1:80, respectively. Other steps and reaction conditions were the same as those used in SEA-specific isotypic antibody detection.

Standardization of OD value for evaluating the levels of specific antibodies against Sj antigens: Because of large numbers of samples in the present study, if the usual final titration method is used for evaluation of the antibody levels, the work not only needs a lot of materials but also is time-consuming and affects the precision of the results. Therefore, referring to other reports (Hagan *et al*, 1991; Dunne *et al*, 1988; Rihet *et al*, 1991), the OD-value method was adopted for evaluating the levels of isotypic antibodies. A formula was used to correct errors resulting from the different ELISA plates: each specimen was tested in triplicate on different plates and standardized OD values were obtained (Wu *et al*, 1995).

RESULTS

Age-specific distributions of different isotype-restricted antibody levels

The age distribution of specific isotypic antibody levels in the tested population are presented in Table 1.

In Table 1 the numbers are the means of specific isotypic antibody levels of different age groups and the distribution of antibody levels was analysed by using the non-parametric Kraskal-Wallis test. The table shows that age is a determinant for SEA-specific IgG, IgG4 and IgE, and SWA-specific IgG and IgG4 antibody levels. The grade correlation analysis of age and specific isotypic antibody levels indicates that SEA-specific IgG, IgG4 and IgE, and SWA-specific IgG and IgG4 levels are all correlated positively with age, which suggests that in the test individuals with age of 3-25 years, the specific isotypic antibody levels increase with age (Table 2).

The effects of specific antibody levels, exposure intensity and age on the possibility of reinfection

Whether reinfection occurred or not after chemotherapy was used as a causal variable and age, exposure degree, and specific isotype-restricted antibody levels were used as autovariables. Then, logistic regression (SPSS) was utilized to do single-factor or multiple, non-conditional logistic regres-

Table 1

Distribution of specific isotypic antibody levels in different age groups.

Age	SEA-IgG	SEA-IgM	SEA-IgG4	SEA-IgE	SWA-IgG	SWA-IgM	SWA-IgG4	SWA-IgE
3-10	0.000	0.754	0.738	0.546	0.886	0.891	0.693	0.315
11-15	0.774	0.775	0.934	0.688	0.948	0.910	0.874	0.264
16-20	0.968	0.802	0.976	0.736	0.971	0.902	0.935	0.305
21-25	0.964	0.785	0.994	0.707	0.985	0.915	0.981	0.374
x2	6.2185	1.0658	31.3287	13.5480	19.6568	1.003	31.2095	0.7625
p	0.0000	0.7853	0.0000	0.0036	0.0002	0.8005	0.0000	0.8584
	**	ns	**	*	**	ns	**	ns

* = significant, ** = very significant, ns = not significant

Table 2

The grade correlations of age and the specific isotypic antibody levels in the tested population.

Ab	SEA-IgG	SEA-IgM	SEA-IgG4	SEA-IgE	SWA-IgG	SWA-IgM	SWA-IgG4	SWA-IgE
Age	0.43**	0.13ns	0.46**	0.29**	0.34**	0.12ns	0.41**	0.04ns

** $p < 0.001$, ns $p > 0.01$

Table 3

Results of single factor, non-conditional, logistic regression analysis.

Variable	Coefficient of regression	Standard error of regression coefficient	p-value	Odds ratio
Age (3-25y)	0.8005	0.4140	0.0532	2.2266
Exposure intensity	0.5016	0.2094	0.0166	1.6514
SEA-IgG4	1.2391	0.5777	0.0320	3.4524
SEA-IgE	0.9525	0.5000	0.0566	2.5922
SWA-IgG4	1.0498	0.4286	0.0143	2.8571

sion analysis. The results indicated that out of 9 analysed variables (age, exposure degree, SEA-specific IgG, IgG4, IgM and IgE levels, and SWA-specific IgG, IgM and IgG4 levels, age, exposure intensity) SEA-specific IgG4, IgE and SWA-specific IgG4 antibody levels were all the risk factors of reinfection in the population aged 3-25 years (Table 3).

In order to evaluate the factors affecting occurence of reinfection, to control effectiveness and to analyse cross influences among these factors, age, exposure intensity, SEA-specific IgG4 and IgE antibody levels, and SWA-IgG4 antibody level were put into multiple, non-conditional logistic regression formulae, and the largest similar estimation method was used to select the factor that was most significantly relevent to reinfection in the population aged 3-25 years. The results are presented in Table 4, which shows that SEA and SWA-specific IgG4 antibody levels were the risk factors of reinfection, and the risk of reinfection occurrence in the population with high level of SEA or SWA-specific IgG4 is 2.83 or 2.40 times, respectively of that with low level of SEA or SWA-specific IgG4.

The results mentioned above suggest that in an endemic area of schistosomiasis japonica, there is a possibility that in the population aged 3-25 years,

Table 4

The results of multiple and non-conditional logistic regression analysis.

Variable	Coefficient of regression	Standard error of regression coefficient	p-value	Odds ratio
SEA-IgG4	1.0410	0.5905	0.0479	2.8321
SWA-IgG4	1.8748	0.4396	0.0466	2.3983

SEA and SWA-specific IgG4 antibodies mediate a blocking immune response.

DISCUSSION

From the study, we found that SEA-specific IgG, IgG4 and IgE, and SWA-specific IgG and IgG4 antibody levels were significantly correlated with age. This is very similar to the findings reported by other researchers (Hagi *et al*, 1990; Butterworth *et al*, 1987). We also found that SEA-specific IgE antibody level increased with age (3-25 years old). However, in contrast to the results reported by Hagan *et al* (1991) and Dunne *et al* (1992), neither SEA nor SWA-specific IgG and IgG4 antibody levels showed significant decline with increasing over 3-25 years. This may be attributed to the features of reinfection in the population in the endemic area of schistosomiasis japonica which differ to that of the populations in endemic areas of other kinds of schistosomiasis.

Single-factor and non-conditional logistic regression analysis showed that age, exposure level, SEA-specific IgG4 and IgE and SWA-specific IgG4 antibody levels all correlated with reinfection, but when we put these factors simultaneously into multiple and non-conditional logistic regression formula, the autovariables age, exposure level and SEA-specific IgE were excluded from the formula. This suggests that the levels of SEA and SWA-specific IgG4 are the most important risk factors for reinfection occurrence although age and exposure level are still possible risk factors, and that the studied population in an endemic area of schistosomiasis japonica may present blocking immune responses mediated by IgG4 antibody. These results are similar to those obtained from the studies on schistosomiasis mansoni and schistosomiasis haematobium (Hagan *et al*, 1991; Butterworth *et al*, 1987). Hagan *et al* (1991) reported that not only SEA-specific IgG4 was a risk factor for reinfection, but also SWA- or SEA-specific IgE was a protective factor for resisting reinfection, but we did not find that SEA- and SWA-specific IgE antibody levels were the protective factors for resistance to reinfection in low age groups (less than 25 years). Yun *et al* (personal communication) found that age was a significant target of reinfection after population chemotherapy. The average age of the susceptible group was 14.8 years, while in the resistant group the average age was 26.5 years old. Although water-contact levels between the two groups did not show significant differences, the rate and intensity of reinfection after chemotherapy presented remarkable variation. This suggests that in the endemic area of schistosomiasis japonica, the population may develop acquired immunity slowly. Therefore, during reinfection a protective immune response mediated by specific IgE antibody may develop in groups aged over 25 years. The high rate of reinfection and wave-shaped curve of age to infection rate and age to infection intensity of population in the endemic area of schistosomiasis japonica indicate that the immune memory time resulting from *S. japonicum* infection was shorter than with other schistosome infections. Therefore, it cannot be excluded that *S. japonicum* infection may generate acquired immunity similar to that of *S. mansoni* and *S. haematobium* infections.

Referring to the studies of the Butterworth and Hagan groups (1987), which indicated that acquired immunity in older or younger than the 12-15 age group showed significant differences, the present study selected the population aged less than 25 years for analysis. However, according to the results from the present study and that of Yun *et al* (Personal communication), it is evident that selecting the population aged less than 25 years for studying the predominant antibody response indicating resistance to reinfection with *S. japonicum* may not be appropriate. Therefore, the age range of subjects studied should be increased to explore the possible mechanism of acquired immunity of the population in the endemic area of schistosomiasis japonica. The results in the present study indicate that SEA-specific IgG, IgG4, IgE and SWA-specific IgG, IgG4 antibody level distribution in the population were dependent on age, *ie* they increased with age, but we could not draw the conclusion that SEA-or SWA-specific IgE are resistant factors for reinfection. Therefore, it is necessary to amplify the age range for further study. However, from the present study, it is clear that SEA- and SWA-specific IgG4 are significant risk factors for reinfection occurrence.

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