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IgE response in schistosomiasis japonica: characterization of a cercarial allergen in comparison with purified egg allergens

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Abstract

The relationship between the cercarial allergen and two previously isolated egg allergens (J1, J2) of *Schistosoma japonicum* was examined especially in terms of the cross-reactivity between them. Semi-purified cercarial allergen (JAC) was obtained from the crude extract of *S. japonicum* cercariae by gel chromatography on Sephadex G-200. The apparent molecular weight of JAC was estimated approximately as 60–100 kDa. JAC could bind to Con A-Sepharose, indicating its glycoprotein nature. Three groups of BALB/c mice were immunized with JAC, J1 or J2 using Al(OH)₃ as adjuvant, and the cross-reactivity of each anti-serum was examined by PCA. Anti-JAC, anti-J1 or anti-J2 serum was highly specific to the corresponding antigen. When IgE-ELISA of *S. japonicum* patient sera was performed using JAC, J1 or J2 as an antigen, the correlation between anti-J1 and anti-J2 ($r = 0.78$) was high, whereas the correlation between anti-JAC and anti-J1 ($r = 0.27$) or between anti-JAC and anti-J2 ($r = 0.12$) was low. These results suggest that most IgE epitopes on cercarial allergen are independent from those on egg allergens in *S. japonicum*.

Introduction

Schistosomiasis japonica exhibits the most severe clinical symptoms among three types of schistosome infections in human species mainly because of the large number of eggs laid in the liver or intestine (Warren *et al.*, 1975). We and others have shown the importance of immediate type hypersensitivity to allergens of the eggs for granuloma formation around the deposited eggs in local sites in *Schistosoma japonicum* infection (Ishii *et al.*, 1982; Tracy & Mahmoud, 1982; Owhashi *et al.*, 1989, 1990; Horii *et al.*, 1990). To further clarify this, we successfully isolated two major allergenic components (J1 and J2) from the crude extract of *S. japonicum* eggs with apparent molecular weights of 260K and 46K, respectively (Owhashi *et al.*, 1987a). On the other hand, immediate type hypersensitivity to invading schistosome also possesses another medical importance because it is concerned with protective immunity against schistosome infections.

IgE-dependent cytotoxicity of schistosome has been shown by many investigators (Anwar *et al.*, 1980; Capron *et al.*, 1981; Kojima *et al.*, 1985; Verwaerde *et al.*, 1987). However, the relationship of the IgE epitopes of between the egg and cercariae allergens still remains unclear in schistosomiasis japonica. In the present study, therefore, we tried to characterize the cercarial allergen of *S. japonicum* especially in terms of cross-reactivity to the egg allergen.

Materials and methods

Mice

Female BALB/c mice were obtained from Japan SLC (Hamamatsu, Japan), and used at 10 weeks of age.

Preparation of cercarial extract

Preparation of cercarial extract was performed according

to the method described previously (Horii *et al.*, 1985). In short, *S. japonicum*-infected snails (*Oncomelania nosophora*, Kofu strain) were crushed and put in fresh water, then placed under light for 30 min. Cercariae were filtrated with a No. 2 filter paper (Whatman, Springfield, UK), and were washed with distilled water. The filter paper was then rinsed with distilled water in an ice-chilled glass dish to collect the cercariae. Concentrated cercariae were frozen at -20°C and lyophilized. Lyophilized cercariae were resuspended in 5.8 mM PBS (pH 7.4) and homogenized with a Teflon homogenizer in an ice-chilled water bath. The homogenate was incubated with mixing overnight at 4°C , and then centrifuged at $100,000 \times g$ for 1 h. The supernatant was used as crude soluble cercarial extract (Ce-ext). Protein concentration was measured by the method of Lowry *et al.* (1951) using bovine serum albumin (BSA, A-4738, Sigma Chemical Co., St. Louis, Missouri, USA) as a standard.

Preparation of purified egg allergens

BALB/c mice were infected with *S. japonicum* (Japanese strain) by intraperitoneal injection of 30 cercariae. Eight weeks later, eggs were harvested from the intestine according to the method described previously (Ishii *et al.*, 1982). In short, the intestine was squeezed and disrupted in PBS in Waring blender, and digested with 0.01% pronase (Kaken, Tokyo, Japan) in PBS at 37°C for 2 h. The solution was centrifuged at $2000 \times g$ for 5 min, and the sediment containing eggs was digested again with 0.01% pronase and 0.05% collagenase (C-0330, Sigma) in PBS containing 0.1% glucose at 37°C for 3 h. It was sieved on 20 and 150 mesh and washed with 1.0% NaCl solution, suspended in PBS and homogenized with a Teflon homogenizer in an ice-chilled water bath. The mixture was stirred overnight at 4°C and centrifuged at $100,000 \times g$ for 1 h. The supernatant was used as SEA. J1 and J2 were purified from the crude SEA by a combination of repeated chromatography of the DE52 (Whatman) anion-exchange column and the Sephacryl S-200 (Pharmacia, Uppsala, Sweden) gel column as described previously (Owhashi *et al.*, 1987a).

Sera

Schistosoma japonicum patients' sera were obtained from an endemic area of the Sorsogon Province, Philippines. As a negative control, 20 sera of healthy Japanese were used. Anti-allergen sera were obtained from female BALB/c mice (10 weeks old) which were immunized intraperitoneally with $1 \mu\text{g}$ allergen in 1 mg $\text{Al}(\text{OH})_3$ adjuvant at weekly intervals. Five weeks later, the mice were bled. Sera from five mice of the same group were pooled and used for PCA.

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) for detecting specific IgE levels was carried out as follows; allergens were coupled to CNBr-activated paper discs as described previously (Owhashi & Ishii, 1981). The discs were then incubated with 20 times diluted individual patients' sera for 3 h at room temperature. After washing three times with saline containing 0.05% Tween 20, the

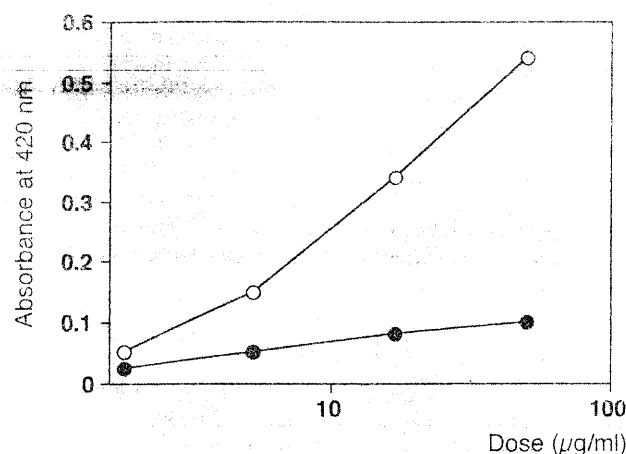


Fig. 1. Specific IgE antibody levels to cercarial extract in the sera of *Schistosoma japonicum* patients. Various concentrations of crude cercarial extract (○) or crude SEA (●) were examined for allergenicity determined by IgE-ELISA using 40 times diluted pooled *S. japonicum* patient's serum.

discs were incubated with 40 times diluted b-galactosidase-labelled anti-human IgF (Kyushu Kainos Co., Fukuoka, Japan) at 25°C for 20 h, and then washed four times with 2.0 ml of saline containing 0.05% Tween20. The discs were then incubated with 0.1 ml of substrate solution (0.1% *o*-nitrophenyl-galactoside, 1.5mM MgCl_2 and 0.7% 2-mercaptoethanol in 0.05 M PB) at 37°C for 1 h. The enzyme reaction was stopped by adding 0.3 ml of 0.1 M Na_2CO_3 . The optical density of the substrate solution was measured at 420 nm. ELISA for detecting specific IgG levels was carried out according to the method of Tanaka *et al.* (1983) with slight modifications in the procedure. In brief, each well of polystyrene micro-ELISA plates (Dynatech, M129A) was sensitized by incubating 0.1 ml of semi-purified cercarial allergen ($1 \mu\text{g/ml}$) in carbonate buffer at 4°C overnight. After three successive washings with saline containing 0.1% Tween 20, 0.1 ml of serum, diluted 1:200 was added to the each well. After incubation at 37°C for 1 h, the wells were washed three times and then 0.1 ml of horseradish peroxidase-labelled anti-human IgG rabbit serum (gamma chain specific, Miles-Yeda), diluted 1:2000, was added to each well. The plates were incubated at 37°C for 1 h. After washing, enzyme reaction was performed at 25°C for 1 h, using 0.2 ml of *o*-phenylenediamine (Sigma) as a substrate. The reaction was stopped by adding 25 μl of 8 N H_2SO_4 , and the absorbance of each well was read by the Titertek Multi-scan at a wavelength of 492 nm.

Gel chromatography

Gel chromatography of the crude extract of *S. japonicum* cercariae (Ce-ext) on Sephadex G-200 (Pharmacia, Uppsala, Sweden) was performed at 4°C using a 1.6×66 cm column equilibrated with PBS. Elution was performed with PBS at a flow rate of 4 ml/h and 2.2 ml fractions were collected. gentin (Boehringer Mannheim, Germany), BSA (Sigma) and α -chymo-trypsinogen (Sigma) were used as standards for calibration of the Sephadex G-200 column.

Affinity chromatography

Affinity chromatography of the semi-purified cercarial allergen (JAC) on Con A-Sepharose 4B (Pharmacia) was carried out using a column (0.7 × 12 cm) equilibrated with Con A buffer (0.1 M acetate buffer, pH 6.0, containing 0.5 M NaCl, 1 mM CaCl₂, 1 mM MgCl₂ and 1 mM MnCl₂). Elution was performed at a flow rate of 3.2 ml/h at 4°C, and 2.0 ml fractions were collected. Bound materials were competitively eluted with 0.2 M methyl- α -D-glucoside in Con A buffer. The effluent and the eluate were dialysed against PBS for ELISA.

PCA reaction

PCA reaction was carried out according to the method of Mota & Wong (1969) with slight modifications in the procedure (Owhashi *et al.*, 1987a). In short, male Sprague-Dawley rats weighing 350–400 g were given intradermal injections in a shaved area on the back with 0.05 ml of 100 times serially diluted serum. Four hours later, 1.0 ml of 1% Evans blue saline solution was injected intravenously, followed by a challenge of intradermal injection with 0.05 ml of semi-purified cercariae allergen or purified egg allergen (2 μ g/ml) on the same point of the back. Bluening of the sites was observed 30 min after the challenge from inside the skin and the dilution causing bluing of more than 8 mm in diameter was considered as the PCA endpoint titre. The threshold was determined from the size of bluing of normal serum as control.

Results

To compare IgE response to cercarial allergen with that to egg allergen in *S. japonicum* infection, specific IgE antibody level to Ce-ext or SEA was examined by ELISA

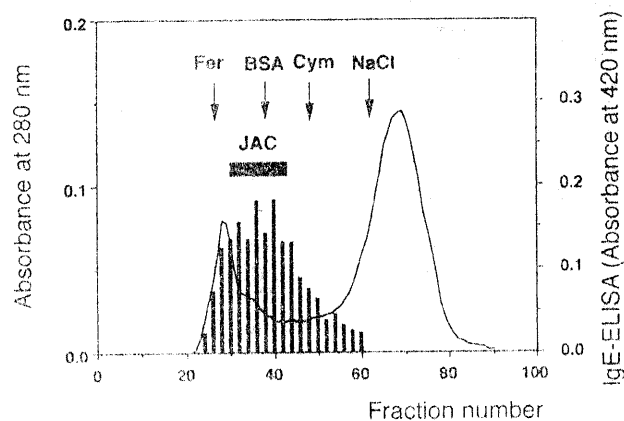


Fig. 2. Gel chromatography of the crude extract of *Schistosoma japonicum* cercariae (Ce-ext) on Sephadex G-200. Absorbance at 280 nm (—) and the result of IgE-ELISA (■) using pooled sera of *S. japonicum* patients of each fraction are shown. The allergenic fraction (JAC) was used as semi-purified cercarial allergen of *S. japonicum* (see figs 3–6). Arrows indicate standards used for calibration of the Sephadex G-200 columns: Fer (ferritin); BSA (bovine serum albumin); Cym (α -chymotrypsinogen); NaCl (sodium chloride).

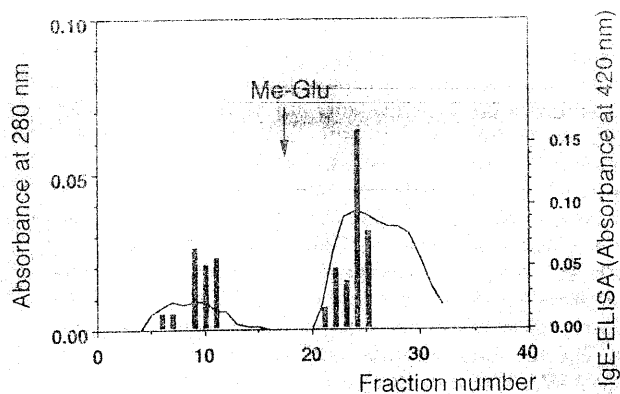


Fig. 3. Affinity chromatography of JAC on Con A-Sepharose. Absorbance at 280 nm (—) and the result of IgE-ELISA (■) of each fraction are shown; Me-Glu (methyl- α -D-glucoside).

using pooled *S. japonicum* patients' sera ($n = 20$). As shown in fig. 1, IgE antibody to Ce-ext was much higher than that to SEA. To enrich the allergenic components, crude Ce-ext (6 mg, 2 ml) was fractionated by gel chromatography on Sephadex G-200. As shown in fig. 2, two major peaks of absorbance at 280 nm were detected. Each fraction was directly used for coupling antigen to CNBr-activated paper discs, and the paper discs were examined for IgE-ELISA using pooled *S. japonicum* patient serum. The peak of allergenic activity was observed between the two major peaks of absorbance at 280 nm. The active fractions were pooled and used as semi-purified allergen of *S. japonicum* cercariae (JAC). The apparent molecular weight of JAC was estimated approximately as 60–100 kDa. We previously showed that the allergenic (Owhashi & Ishii, 1981; Owhashi *et al.*, 1987a) or antigenic (Ishii & Owhashi, 1982) components from *S. japonicum* eggs are consistently glycoproteins that bind to Con A-Sepharose. To characterize the properties of JAC, 2 mg of JAC (1.5 ml) was applied to Con A-Sepharose column, and the bound materials were subsequently eluted with methyl- α -D-glucoside. After dialysis against PBS, each fraction was examined for IgE-ELISA. As shown in fig. 3, allergenic components of JAC were essentially bound to Con A-Sepharose, indicating its glycoprotein nature. The allergenic activity was completely lost by heating at 100°C for 30 min when determined by IgE-ELISA. On the other hand, JAC retained approximately 50% activity after heating at 56°C for 30 min. To examine the cross-reactivity of JAC with the purified *S. japonicum* egg allergens (J1, J2), three groups of mice were immunized with JAC, J1 or J2 using A1(OH)₃ as adjuvant. PCA titre of the pooled serum from each group was measured by using JAC, J1 or J2 as a challenge antigen. As shown in fig. 4, anti-JAC, anti-J1 or anti-J2 serum was highly specific to the corresponding antigen. The titre of anti-J1 or anti-J2 antibody was higher than that of anti-JAC. When IgE-ELISA of patients' sera was performed using JAC, J1 or J2 as an antigen, the correlation between anti-JAC and anti-J1 ($r = 0.27$), or between anti-JAC and anti-J2 ($r = 0.12$) was low (fig. 5). In contrast, the correlation between anti-J1 and anti-J2 was high ($r = 0.78$). When specific IgE antibody levels to JAC of individual patients were compared with

the specific IgG antibody levels to JAC, a high correlation was not observed between them (fig. 6).

Discussion

The main aim of this study is to characterize the major allergen of *S. japonicum* cercariae, and to compare it with the purified allergenic components from *S. japonicum* eggs (Owhashi *et al.*, 1987a). We have little information about it, probably because the amount of available *S. japonicum* cercariae was too little to accomplish this study. Another problem might be the heterogeneity of the allergenic components. The soluble egg antigen (SEA) of *S. japonicum* (Owhashi & Ishii, 1981) or *S. mansoni* (Pierce *et al.*, 1983) also contains heterogeneous allergenic components. Among them, we successfully isolated two major allergenic components (J1, J2) from the crude SEA by a combination of DE52 anion-exchange chromatography and gel chromatography on Sephacryl S-200. In the present study, we used Sephadex G-200 gel chromatography for the partial purification of allergenic components from cercarial extract because of the limited amount of the *S. japonicum* cercariae. Both J1 and J2 bound to Con A, and were sensitive to periodate treatment, indicating their glycoprotein nature (Owhashi *et al.*, 1987a). J1 was stable to heating at 100°C, whereas J2 was sensitive to heating. The present study showed that cercarial allergen also exhibits glycoprotein nature. However, the apparent molecular weight of JAC is different from J1 (260 kDa) or J2 (46 kDa) (Owhashi *et al.*, 1987a). JAC is similar to J2, but different from J1 in terms of the stability against heating. Specific IgE antibody levels of individual patients to JAC were not correlated with specific IgG antibody levels to JAC (fig. 6). Similarly, we previously demonstrated that specific IgE antibody levels to J1 or J2 are not correlated with specific IgG antibody levels to the respective antigen (Owhashi *et al.*, 1987b). Depletion of IgG antibody from the sera did not affect the result of IgE-ELISA, indicating that the blocking effect of IgG antibodies in IgE-ELISA is slight in schistosomiasis japonica (Owhashi *et al.*, 1987b). Furthermore, we also showed that the results of the

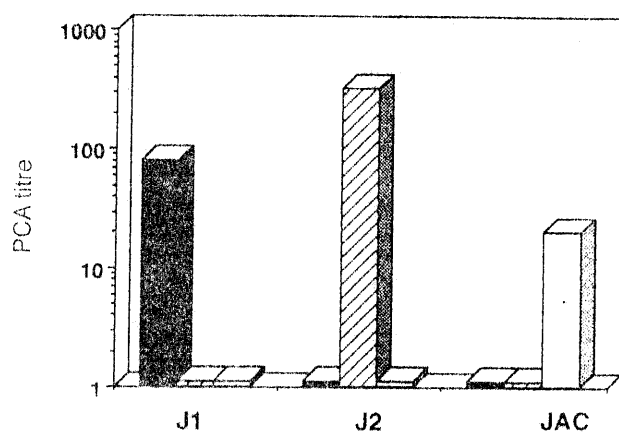


Fig. 4. Cross-reactivity of cercarial allergen with egg allergen. PCA titre of anti-J1 (■), anti-J2 serum (▨) or anti-JAC (□) raised in BALB/c mice was examined using 2 µg/ml of JAC, J1 or J2 as challenge antigen.

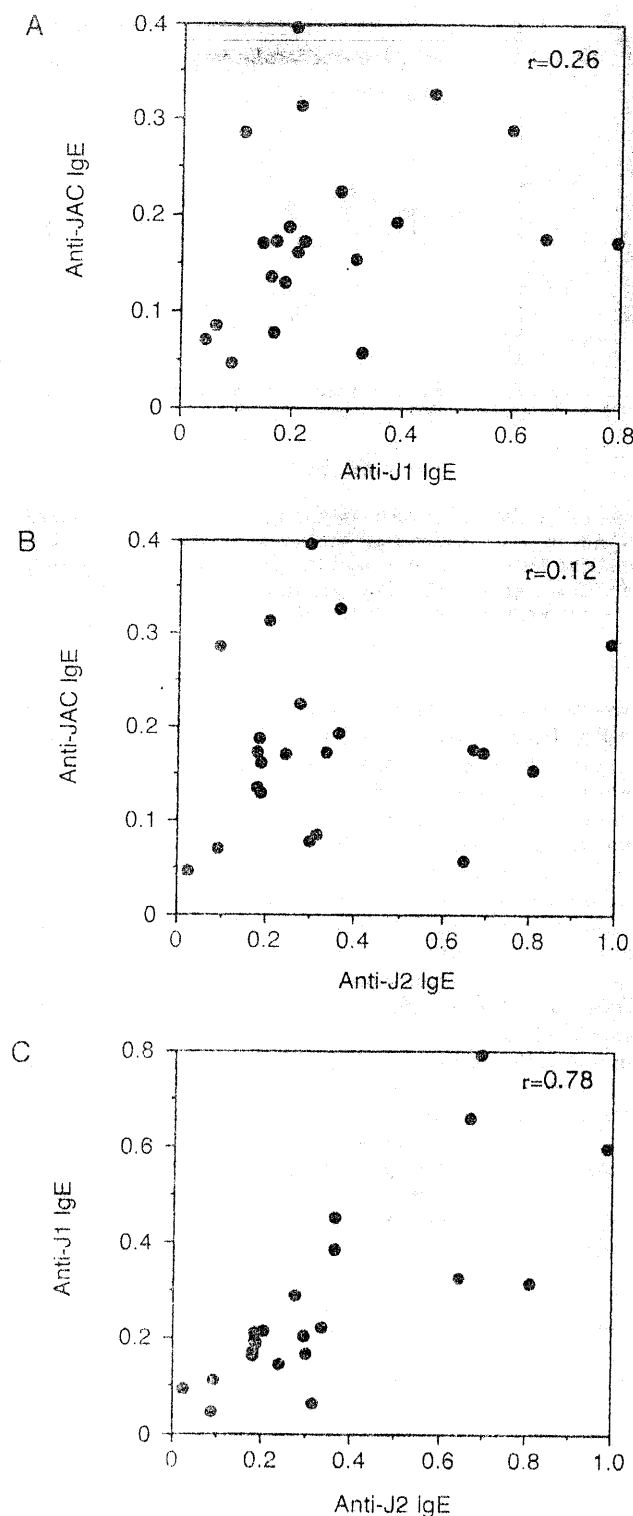


Fig. 5. Correlation between anti-JAC and anti-J1 (A), anti-JAC and anti-J2 (B), or anti-J1 and anti-J2 (C) IgE antibody levels in the sera of patients. Specific IgE antibody *Schistosoma japonicum* levels were measured by IgE-ELISA using JAC (25 µg), J1 (12.5 µg) or J2 (25 µg) as antigens. Uninfected control sera represented 0.03 ± 0.01 for JAC, 0.03 ± 0.01 for J1 and 0.04 ± 0.01 for J2.

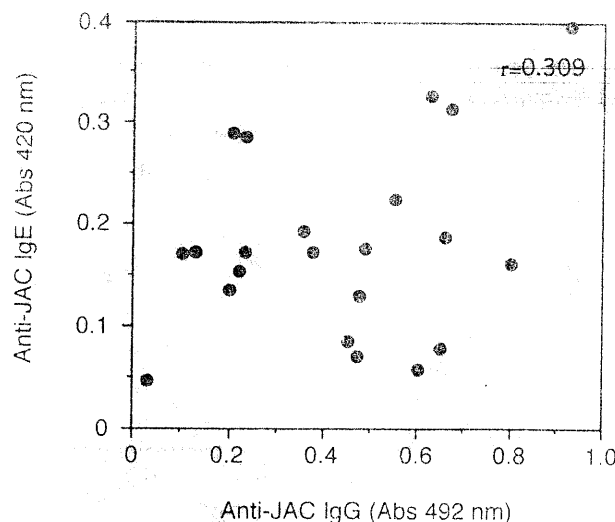


Fig. 6. Correlation between specific IgE and IgG levels to JAC in the sera of *Schistosoma japonicum* patients. Specific IgE antibody levels were measured by IgE-ELISA using 25 µg/ml of JAC, and IgG-ELISA using 1 µg/ml of JAC. Uninfected control sera represented 0.04 ± 0.01 for IgG-ELISA and 0.03 ± 0.01 for IgE-ELISA.

circumoval precipitin test were not correlated with those of RAST using crude SEA (Horii *et al.*, 1990). These facts suggest that class switching of antibody production is regulated by various factors, such as the stage or severity of infection and the genetic background of the host, but is not directly related to the nature of antigens. The sensitivity of IgE-ELISA was low in comparison with IgG-ELISA (fig. 5). Furthermore, IgE levels to JAC were not correlated with IgG levels to SEA ($r = 0.13$, data not shown) which are generally accepted as one of the parameters for *S. japonicum*-infection (Mott *et al.*, 1987). These facts suggest that the usefulness of IgE-ELISA for immunodiagnosis may be limited in schistosomiasis japonica. Related to this, Lunde *et al.* (1979) showed that the ratio of specific IgG antibodies to cercarial and adult worm antigens could discriminate between acute or early and chronic schistosomiasis. Similarly, acutely infected patients showed a higher specific IgE antibody to SEA than chronically infected patients (Lunde & Ottesen, 1980). Taken together, the significant level of specific IgE antibody to cercarial allergen may suggest the history of acute infection.

Many investigators have shown the existence of IgE-dependent killing of schistosomula by eosinophils, macrophages, neutrophils or platelets (Anwar *et al.*, 1980; Capron *et al.*, 1981; Joseph *et al.*, 1983; Kojima *et al.*, 1985; Verwaerde *et al.*, 1987). Furthermore, a mast cell mediator such as ECF-A or histamine enhances the eosinophil-mediated cytotoxicity of schistosomula (Anwar *et al.*, 1980). These facts suggest that immediate-type hypersensitivity to cercarial components would be important for the protective immunity against invading schistosomula. Therefore JAC would be one of the potentially protective antigens against *S. japonicum* infection. Unfortunately, the total amount of available cercariae from *Oncomelania* is limited because of the small

size of the snail and the difficulty of their breeding. Molecular cloning of cDNA encoding JAC may be one of the effective ways to determine the molecular structure of peptide moiety of JAC, and to obtain a sufficient amount of the allergen for further studies.

References

- Anwar, A.R.E., McKean, J.R., Smithers, S.R. & Kay, A.B. (1980) Human eosinophil- and neutrophil-mediated killing of schistosomula of *Schistosoma mansoni* *in vitro*. I. Enhancement of complement-dependent damage by mast cell-derived mediators and formyl methionyl peptides. *Journal of Immunology* 124, 1122-1129.
- Capron, M., Bazin, H., Joseph, M. & Capron, A. (1981) Evidence for IgE-dependent cytotoxicity by rat eosinophils. *Journal of Immunology* 126, 1764-1768.
- Horii, Y., Ishii, A. & Owhashi, M. (1985) *In vitro* and *in vivo* induction of neutrophil and eosinophil chemotactic responses by *Schistosoma japonicum* cercaria. *American Journal of Tropical Medicine and Hygiene* 34, 513-518.
- Horii, Y., Owhashi, M. & Ishii, A. (1990) Mechanisms of eosinophil accumulation around eggs of *Schistosoma japonicum*: role of two purified components, allergen and eosinophil chemotactic factor, from soluble egg antigens measured on sensitized guinea pig skin. *Parasitology Research* 76, 602-605.
- Ishii, A. & Owhashi, M. (1982) Enzyme-linked-immunosorbent assay with egg antigens of *Schistosoma japonicum*. *Zeitschrift für Parasitenkunde* 67, 279-287.
- Ishii, A., Owhashi, M. & Hashiguchi, J. (1982) *Schistosoma japonicum*: Immediate hypersensitivity reactions to egg antigen in human and rodents. *Experimental Parasitology* 54, 104-112.
- Joseph, M., Auriault, C., Capron, A., Vomg, H. & Viens, P. (1983) A new function for platelets: IgE-dependent killing of schistosomes. *Nature* 303, 810-812.
- Kojima, S., Yamamoto, N., Kanazawa, T. & Ovary, Z. (1985) Monoclonal IgE-dependent eosinophil cytotoxicity to haptenated schistosomula of *Schistosoma japonicum*: Enhancement of the cytotoxicity and expression of Fc receptors for IgE by *Nippostrongylus brasiliensis* infection. *Journal of Immunology* 134, 2719-2722.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* 193, 265-275.
- Lunde, M.N., Ottesen, E.A. & Cheever, A. (1979) Serological differences between acute and chronic schistosomiasis detected by enzyme-linked immunosorbent assay. *American Journal of Tropical Medicine and Hygiene* 28, 87-91.
- Lunde, M.N. & Ottesen, E.A. (1980) Enzyme-linked immunosorbent assay (ELISA) for detecting IgM and IgE antibodies in human schistosomiasis. *American Journal of Tropical Medicine and Hygiene* 29, 82-85.
- Mota, I. & Wong, D. (1969) Homologous and heterogenous passive cutaneous anaphylactic activity of mouse antisera during the course of immunization. *Life Science* 8, 813-820.
- Mott, K.E., Dixon, H., Carter, C.E., Garcia, E., Ishii, A., Matsuda, H., Mitchell, G., Owhashi, M., Tanaka, H. & Tsang, V.C. (1987) Collaborative study on antigens for immunodiagnosis of *Schistosoma japonicum* infection. *Bulletin of the World Health Organization*, 65, 233-244.

- Owhashi, M. & Ishii, A. (1981) Fractionation and characterization of allergens extracted from eggs of *Schistosoma japonicum*. *International Archives of Allergy and Applied Immunology* 64, 146–156.
- Owhashi, M., Horii, Y., Ishii, A. & Nawa, Y. (1987a) Isolation of two immunogenically different allergens from *Schistosoma japonicum* eggs. *International Archives of Allergy and Applied Immunology* 83, 149–154.
- Owhashi, M., Horii, Y., Imai, J., Ishii, A. & Nawa, Y. (1987b) Reactivity of two major allergens isolated from *Schistosoma japonicum* eggs against IgE and IgG antibodies in human serum. *International Archives of Allergy and Applied Immunology* 83, 290–295.
- Owhashi, M., Nawa, Y. & Watanabe, N. (1989) Granulomatous response in congenitally IgE-deficient SJA/9 mice infected with *Schistosoma japonicum*. *International Archives of Allergy and Applied Immunology* 90, 310–312.
- Owhashi, M., Horii, Y., Ikeda, T., Maruyama, H., Abe, T. & Nawa, Y. (1990) Importance of mast-cell-derived eosinophil chemotactic factor A on granuloma formation in murine schistosomiasis japonica: Evaluation using mast-cell-deficient W/W^v mice. *International Archives of Allergy and Applied Immunology* 92, 64–68.
- Pierce, R.J., Verwaerde, C., Damonville, M., LePresle, T. & Capron, A. (1983) Allergens of *Schistosoma mansoni*. I. Comparison of the IgE response in human and experimental infections towards characterized allergens from adult worm products. *International Archives of Allergy and Applied Immunology* 72, 219–226.
- Tanaka, H., Matsuda, H., Blas, B.L., Nosenas, J.S., Hayashi, M., Iuchi, M., Nakao, M. & Santos, A.T.Jr (1983) Evaluation of micro-ELISA for schistosomiasis japonica using crude egg antigen. *Japanese Journal of Experimental Medicine* 53, 147–154.
- Tracy, J.W. & Mahmoud, A.A.F. (1982) Isolation of *Schistosoma japonicum* egg glycoprotein antigens which sensitize mice to lung granuloma formation and elicit an immediate hypersensitivity response. *American Journal of Tropical Medicine and Hygiene* 31, 1201–1212.
- Verwaerde, C., Joseph, M., Capron, M., Pierce, R.J., Damonville, M., Velge, F., Auriault, C. & Capron, A. (1987) Functional properties of a rat monoclonal IgE antibody specific for *Schistosoma mansoni*. *Journal of Immunology* 138, 4441–4446.
- Warren, K.S., Boros, D.L., Hang, L.M. & Mahmoud, A.A.F. (1975) The *Schistosoma japonicum* egg granuloma. *American Journal of Pathology* 80, 279–290.

(Accepted 26 February 1997)

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