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Studies on Antigenic Communities between the Yamanashi and Chinese Strains of *Schistosoma japonicum* Eggs and *Oncomelania* Snails by Immunoelectrophoresis

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ABSTRACT

Antigenic communities were studied with sera from rabbits immunized with the Yamanashi and Chinese strains of *Schistosoma japonicum* eggs, and the antigens of five subspecies of *Oncomelania* snails, by immunoelectrophoresis.

With regard to antigenic communities defined by the Yamanashi strain of *S. japonicum* eggs and *Oncomelania* snails, the schistosome egg produced 5 to 6 bands with *Oncomelania hupensis nosophora*, 3 to 4 bands with *O.h.hupensis*, 3 bands with *O.h.chiui*, 2 bands with *O.h.quadrasi* and 1 band with *O.h.formosana*. Similarly, the antigenic communities defiend by the Chinese strain of *S.japonicum* eggs and *Oncomelania* snails, the schistosome egg produced 5 bands with *O.h.hupensis*, 4 bands with *O.h.nosophora*, 2 bands with *O.h.chiui*, 1 band with *O.h.quadrasi* and 0 or 1 band with *O.h.formosana*. Our results showed that more bands were seen between the *S.japonicum* egg and suitable intermediate hosts, the same as when *S.japonicum* adult worm antigens were used.

Antigens in common between the fractionated *Oncomelania* and both strains of *S.japonicum* eggs were found mainly in Fractions 1 and 2, and were estimated to have molecular weights of 17,000-670,000.

Key words: *Schistosoma japonicum, Oncomelania snails, Immunoelectrophoresis, Host-parasite relationship*

Several investigators have shown that different geographical strains of *Schistosoma japonicum* possess different infectivity to various species of *Oncomelania* snails[5,6,8,10].

In our previous reports on the antigenic communities between the Chinese and Yamanashi strains of *S.japonicum* adult worms and *Oncomelania* snails, we demonstrated by immunoelectrophoresis that more precipitin bands appeared between *S.japonicum* and its most suitable intermediate host[9,14].

This findings supported our hypothesis that antigens in common were one of the factors defining the different susceptibilities of various species of *Oncomelania* snails to geographic strains of *S.japonicum*. On the other hand, Jackson and Moor (1976)[16] reported that sera from *S.haematobium* infected individuals had higher antibody titers to the antigens of suitable intermediate hosts than did non-infected individuals, and Marrero and Hillyer (1985)[17] found that sera from humans infected with *S.mansoni* cross-reacted with *Biomphalaria glabrata* soluble antigen.

The antigenic communities between a parasite and its intermediate hosts have already been investigated by several researchers[4,14,23], and it seems this type of study will prove useful not only in ecological studies but also in the diagnosis of parasitic diseases.

This paper describes some of the antigenic relationship between five subspecies of *Oncomelania* snails and the Yamanashi and Chinese strains of *S.japonicum* eggs.

MATERIALS and METHODS

The Yamanashi and Chinese strains of *S.japonicum* used in the present study originated from Yamanashi (Japan) and Shanghai (China) respectively. These parasites were maintained in this laboratory using mice and laboratory colonies of *Oncomelania hupensis nosophora* and *O.h.hupensis*. Five subspecies of *Oncomelania* snails employed

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were laboratory-reared snails in our laboratory according to the method of Iwanaga and Tsuji (1972)[11].

The preparation of crude egg antigen was as follows; the intestines of mice infected with *S.japonicum* were minced and digested with 0.1% trypsine in 1/15 N-Na_2HPO_4 at 37°C for 3 hours and centrifuged at 3,000 r.p.m for 5 minutes. The sediment washed in 0.6% NaCl solution and digested again with 0.1% trypsine in 1/15 N-Na_2HPO_4 at 37°C for 2 hours. After centrifugation, the sediment was washed in 0.6% NaCl solution, and digested with 0.05% collagenase in 0.05M phosphate buffered saline (PBS. PH7.0) at 37°C for 3 hours. This solution was centrifuged at 3,000 r.p.m for 5 minutes and the sediment containing eggs was washed in 0.1% NaCl solution. The sediment resuspended in 0.1% NaCl solution was sieved through 150 meshes and homoginized by glass homoginizer. The solution was extracted overnight at 4°C and was then sonicated in an Ultrasonic disruptor (UR-200P). The sonicate was centrifuged at 15,000 r.p.m for 1 hour and the supernatant was dialysed, then lyophilized. The dried material was used as the crude antigen. For *Oncomelania* snails, the antigens were extracted in 0.1% NaCl solution. Antisera used were rabbit anti-sera immunized with *S.japonicum* eggs and *Oncomelania* snail antigens (Tsuji, 1975)[21].

Immunoelectrophoresis was performed according to the technique of Tsuji (1974)[20] on 0.9% agarose L (Behring-werke AG, Germany) in veronal buffered saline (PH 8.2).

Antigens derived from *Oncomelania* snails were also fractionated by gel-filtration on Sephadex G-100 column chromatography to estimate the molecular weight of the substances responsible for the common antigenicity against anti-*S.japonicum* egg sera by the technique of Iwanaga and Tsuji (1985)[14].

RESULTS

1. Antigenic communities between the Yamanashi strain of the *S.japonicum* egg and *Oncomelania* snails.

As shown in the immunoelectrophoretic diagrams in Fig. 1, anti-*S.japonicum* egg sera (anti-SjE) produced 6 bands against *O.h.nosophora* antigen, 4 bands against *O.h.hupensis* antigen, 3 bands against *O.h.chiui* antigen, 2 bands against *O.h.quadrasi* antigen and 1 band against *O.h.formosana* antigen.

The reverse, using anti-snail sera against *S.japonicum* egg antigen, showed 5 bands with anti-*O.h.nosophora* serum, 3 bands with both anti-*O.h.hupensis* and *O.h.chiui* sera, 2 bands with anti-*O.h.quadrasi* serum and 1 band with anti-*O.h.formosana* serum.

2. Antigenic communities between the Chinese strain of the *S.japonicum* egg and *Oncomelania* snails.

As shown in the immunoelectrophoretic diagrams in Fig. 2, anti-SjE produced 5 bands against *O.h.hupensis* antigen, 4 bands against *O.h.nosophora* antigen, 2 bands against *O.h.chiui* antigen and 1 band against both *O.h.quadrasi* and *O.h.formosana* antigens.

The anti-snail sera, tested against SjE, yielded identical results except that no bands appeared with the *O.h.formosana* antiserum.

3. Antigenic communities between the Yamanashi and Chinese strains of *S.japonicum* eggs and fractionated antigens of *Oncomelania* snails.

Gel filtration of various *Oncomelania* extracts

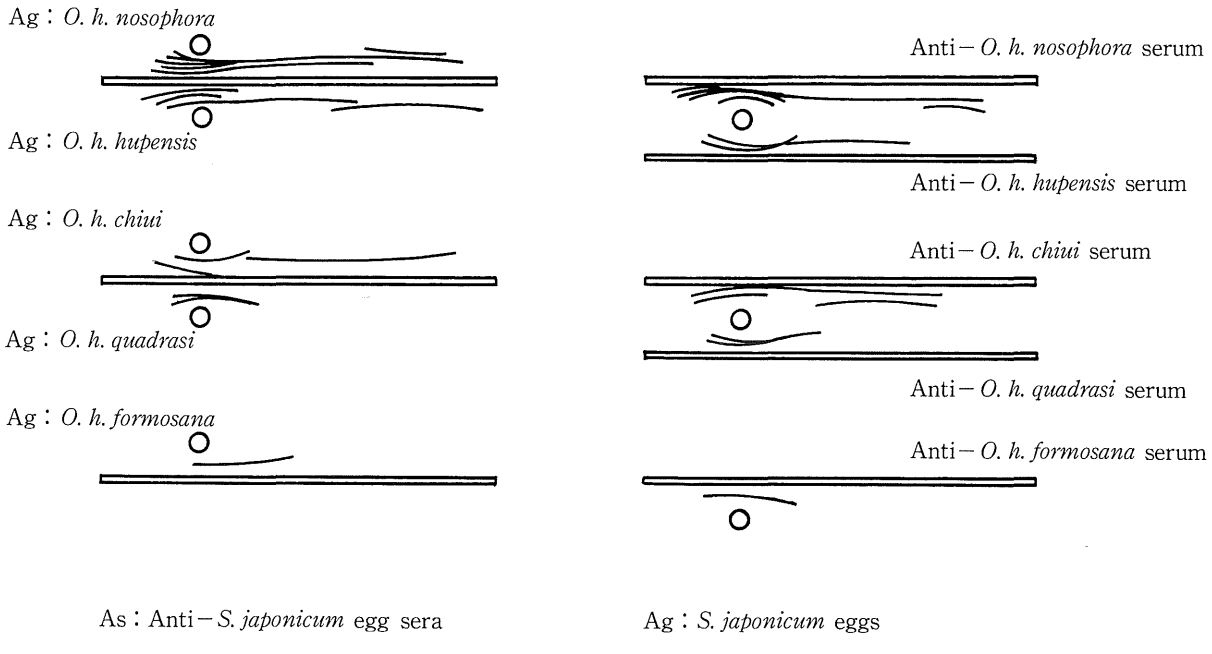

Fig. 1. Immunoelectrophoregrams between various *Oncomelania* snails and the Yamanashi strain of *Schistosoma japonicum* egg

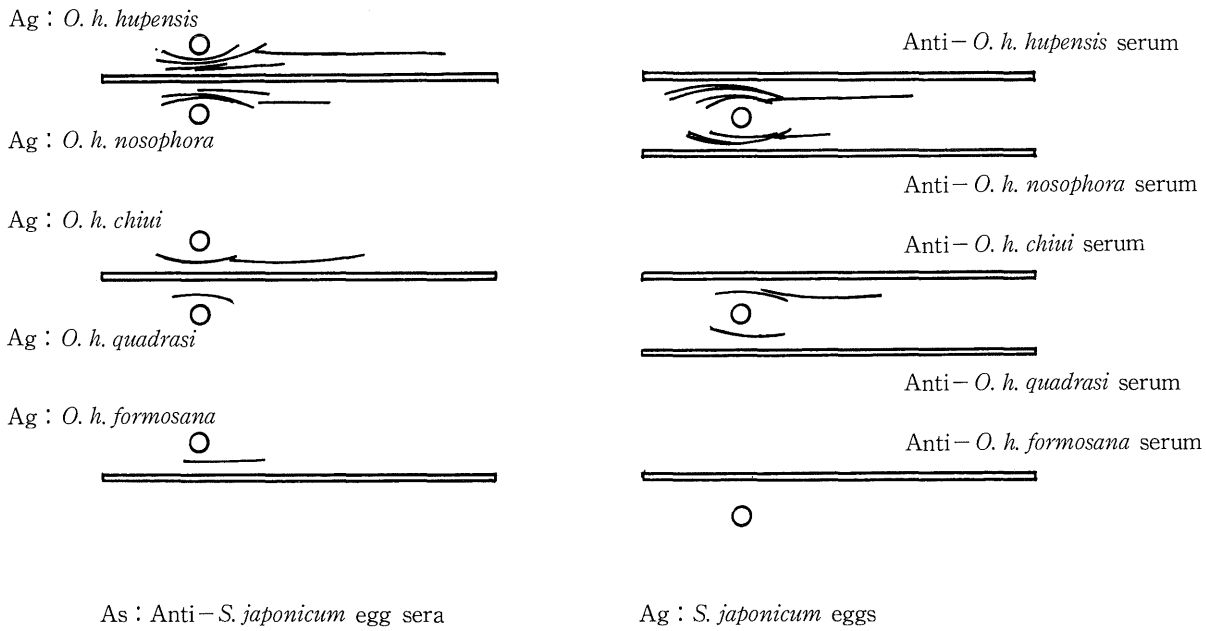

Fig. 2. Immunoelectrophoregrams between various *Oncomelania* snails and the Chinese strain of *Schistosoma japonicum* egg

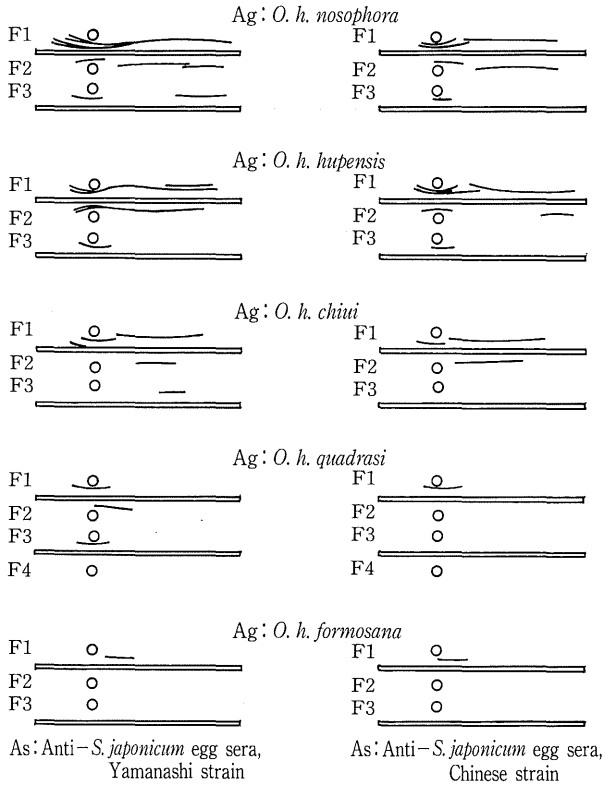

Fig. 3. Immunoelectrophoregrams between fractionated antigens of *Oncomelania* snails and anti-*Schistosoma japonicum* egg sera

yielded 4 factions with *O.h.quadrasi* and 3 factions with the other[14].

With regard to the antigenic communities between anti-Yamanashi SjE and the fractionated five subspecies of *Oncomelania, O.h.nosophora* produced 4 bands with the first fraction, 3 bands with the second fraction and 2 bands with the third fraction. *O.h.hupensis* antigen produced 3 bands with the first, 2 bands with the second and 1 band with the third fractions. *O.h.chiui* antigen produced 3 bands with the first and 1 band with both the second and the third fractions. *O.h.quadrasi* antigen produced 1 band with the fractions 1, 2 and 3, but the fourth fraction did not produced any band. *O.h.formosana* antigen produced 1 band with the first fraction only.

On the other hand, antigenic communities between the anti-Chinese SjE and the antigens of *Oncomelania* resulted in : *O.h.nosophora* antigen; 3 bands with the first fraction, 2 bands with the second fraction, and 1 band with the third fraction. *O.h.hupensis* antigen: 4 bands with the first fraction, 2 bands with the second fraction, and 1 band with the third fraction. *O.h.chiui* antigen: 2 bands with the first fraction, 1 band with the second fraction, and no bands with the third fraction. Both the *O.h.quadrasi* and *O.h.formosana* antigens produced only 1 band each with the first fraction.

DISCUSSION

During the past few decades, many studies on the physiology and morphology of parasites and snails were performed via immunoelectrophoresis[1,3,18,20]. Furthermore, this method has been utilized for studing the antigenic relationships between adult and larval parasites[2,21], and between parasites and their hosts[3,14,15,22].

In this study, the antigenic communities between five subspecies of *Oncomelania* snails and the Yamanashi and Chinese strains of the *S.japonicum* eggs were assessed by immunoelectrophoresis.

As a result, it was found that *O.h.nosophora*

produced the most bands (5 to 6) with Yamanashi strain of SjE and 3 to 4 bands against *O.h.hupensis*, 3 bands against *O.h.chiui*, 2 bands against *O.h.quadrasi* and 1 band against *O.h.formosana*. The experimental infection rates of the Yamanashi strain of *S.japonicum* in *Oncomelania* snails showed that *O.h.nosophora* had the highest infection rate of the five subspecies. the rate of infection of the other subspecies of *Oncomelania* decreased corresponding to the number of precipitin bands[7,8].

On the other hand, *O.h.hupensis* produced the most bands (5) against the Chinese strain of SjE. Compared with the infection rate of the Chinese strain of *S.japonicum* in *Oncomelania*, it was observed that the most bands were seen with the highest infection rate[12,13].

The number of bands resulting from the *S.japonicum* egg antigen was less than those appearing with *S.japonicum* adult worm antigen[9,15]. But it seems that a number of bands observed among *Oncomelania* and adult worm or egg of *S.japonicum* are including same precipitin bands. Further investigation must include absorption procedures to enable a clear distinction to be made between the various bands.

On the precipitin bands between fractionated *Oncomelania* snail antigens and both strains of anti-*S.japonicum* egg, the precipitin bands of fraction 3 are related to fraction 2, therefore, the common bands of them mostly existed in fraction 1 and 2, their molecular weight were calibrated as 17,000-670,000 and their vales agreed with that of common bands observed between *Oncomelania* snails and *S.japonicum* adult worm reported by Iwanaga and Tsuji (1985)[14], and Iwanaga et al (1986)[19].

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