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Studies on Host-Parasite Relationship between *Schistosoma japonicum* and *Oncomelania* Snails
1. Antigenic Communities between the Chinese Strain of *Schistosoma japonicum* Adult Worm and *Oncomelania* Snails

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Key words; *Schistosoma japonicum*, *Oncomelania* snails, Immunoelectrophoresis, host-parasite relationship

Introduction

It is well-known that the different geogr-aphical strains of *Schistosoma japonicum* show different infectivities to various species of *Oncomelania* snails.

The Chinese strain of *S. japonicum* has been reported to have a high degree of infectivity to *Oncomelania hupensis hupensis*, while the other *Oncomelania* snails showed a lesser degree of infectivity. (Iwanaga and Tsuji, 1982 a, b). These findings indicate that there are species specificities in the infectivity of *Oncomelania* snails to geographical strains of *S. japonicum*, and suggest the possible presence of basic physiologic differences among each subspecies of *Oncomelania* snails.

Recently, immunoelectrophoresis has been utilized for stuyding the antigenic communities between parasite and intermediate hosts, and Tsuji and Yokogawa (1972) reported that more common precipitin bands were seen between a parasite and its suitable host.

The present paper deals with antigenic communities between *Oncomelania* snails and the Chinese strain of *S. japonicum* adult worms, as detected by immunoelectrophoresis.

Materials and Methods

Five subspecies of laboratory-reared *Oncomelania* snails were employed in these experiments. The laboratory colonies were obtained as follow: *Oncomelania hupensis hupensis* originating from China, *O. h. nosophora* from Japan, *O. h. quadrasi* from Leyte, *O. h. chiui* from Shihmen, Taiwan and *O. h. formosana* from Ilan, Taiwan.

The *S. japonicum* strain used in the present study originated from infected snails collected in an endemic area, in Shang-hai, China, by Dr. Sarasin of Tropical Institute Basel, Switzerland, and the life cycle was maintained in our laboratory with the use of mice and laboratory colonies of *O. h. hupensis*.

Immunoelectrophoresis was done according to the techniques of Tsuji (1974) on 0.9 % agarose L (Behring-werke AG, Germany) in veronal buffered saline (pH 8.2). As antigens, 0.1 % NaCl extracts of adult worms of *S. japonicum* and *Oncomelania* snails were used.

Oncomelania antigens were also fractionated by gel-filtration on Sephadex G-100 column

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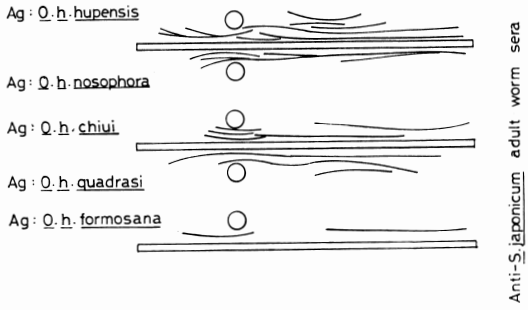

Fig. 1 Immunoelectrophoregrams between antigens of various *Oncomelania* snails and anti-*Schistosoma japonicum* adult worm sera, Chinese strain.

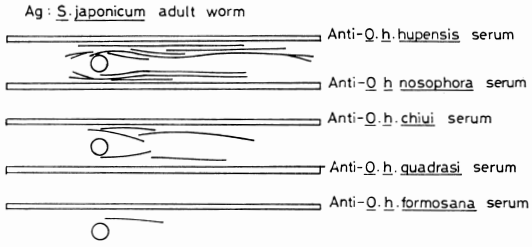

Fig. 2 Immunoelectrophoregrams between antigens of *Schistosoma japonicum* adult worm, Chinese strain and anti-*Oncomelania* snail sera.

chromatography to estimate the molecular weights of the substances responsible for the common antigenicity against anti-*S. japonicum* adult worm serum. For calibration, tyroglobuline (MW. 670,000), gamma-globulin (MW. 158,000), ovalbumin (MW. 45,000), myoglobine (MW. 17,000) and vitamin B 12 (MW. 1,350) were used.

Sephadex G-100 was prepared with phosphate buffer (0.1 M), and poured into a 2.5× 40 cm column. The column was charged with 50 mg of each antigen. Each 4 ml of effluence was collected and analyzed for protein by light absorption at 280 nm.

Results

1. Antigenic communities between anti-*S. japonicum* adult worm serum and crude antigens of *Oncomelania* snails.

As shown in the immunoelectrophoretic diagrams in Fig. 1, anti-*S. japonicum* adult worm sera produced 9 bands against *O. h. hupensis* antigen, similarly, 5 bands against both *O. h. nosophora* and *O. h. chiui* antigens, 3 bands against *O. h. quadrasi* antigen and 2 bands against *O. h. formosana* antigen.

2. Antigenic communities between the antigen of *S. japonicum* adult worms and anti-*Oncomelania* snail sera.

As shown in the immunoelectrophoretic diagrams in Fig. 2, antigens of *S. japonicum* adult worms produced 8 bands against anti-*O. h. hupensis* serum, similarly, 4 bands against anti-*O. h. nosophora* serum, 3 bands against anti-*O. h. chiui* serum, 2 bands against anti-*O. h. quadrasi* serum and 1 band against anti-*O. h. formosana* serum.

3. Antigenic communities between anti-*S. japonicum* adult worm serum and fractionated antigens of *Oncomelania* snails.

In the column chromatography of 0.1 % NaCl extract antigens of *Oncomelania* snails, *O. h. hupensis*, *O. h. nosophora*, *O. h. chiui* and *O. h. formosana* were fractionated to 3 fractions, and 4 fractions with *O. h. quadrasi* at 280 nm by Sephadex G-100. (Fig. 3)

With regard to the antigenic communities between anti-*S. japonicum* adult worm sera and fractionated antigens of 5 subspecies of *Oncomelania* snails, *O. h. hupensis* antigen produced 6 bands in the first fraction, 2 bands in the second fraction, 1 band in the third fraction against anti-*S. japonicum* adult worm sera, similarly, *O. h. nosophora* produced 3 bands in the first fraction, 2 bands in the second fraction, no band in the third fraction, *O. h. quadrasi* produced 3 bands in the first fraction, 2 bands in the second fraction, 1 band in the third fraction, no band in the forth fraction, *O. h. chiui* produced 4 bands in the first fraction, 2 band in the second fraction, 1 band in the third fraction, and *O. h. formosana* produced only 2 bands in the first fraction as shown in Fig. 4.

Discussion

Since the establishment of immunoelectrophoresis by Grabar and Williams (1953), this method has been extensively utilized for immunological aspects of physiological studies of parasites. Using the immunoelectrophoresis

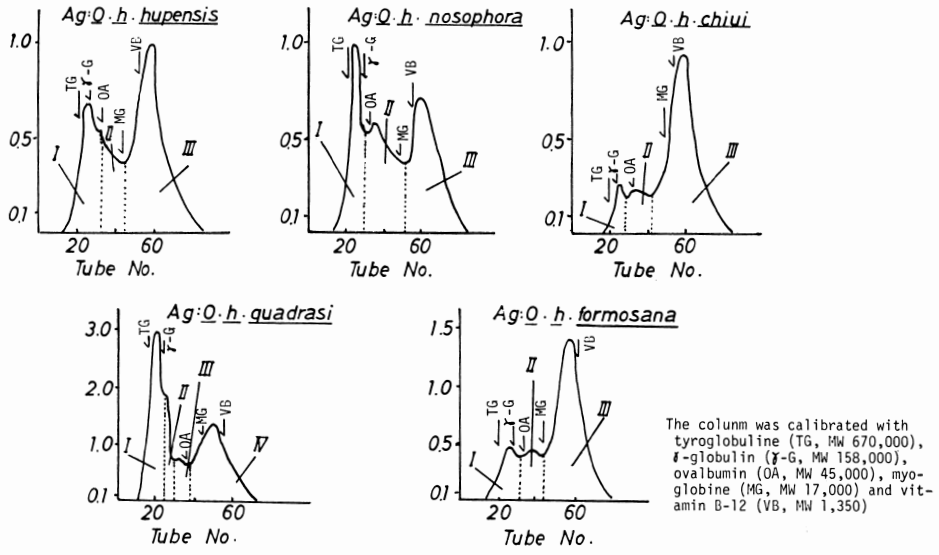

Fig. 3 Column chromatograms for various antigens of *Oncomelania* snails on Sephadex G-100 (O. D. at 280nm).

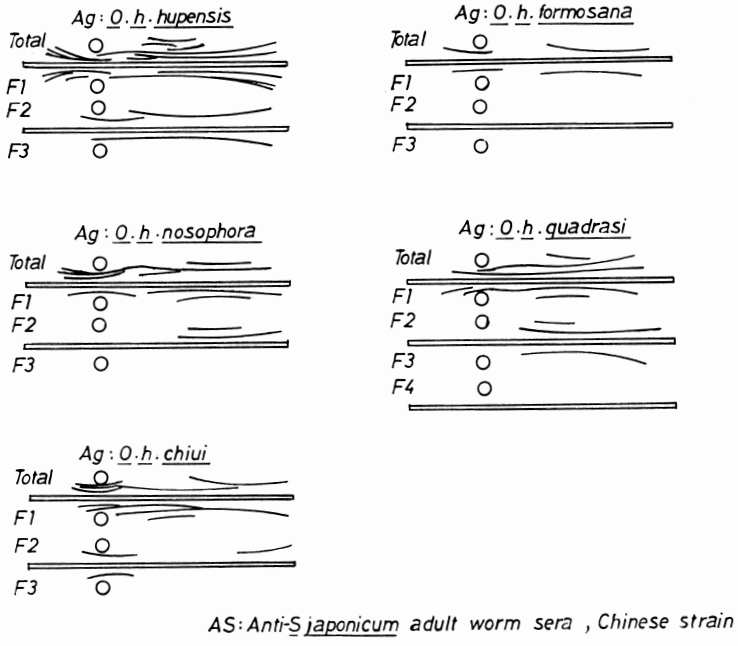

Fig. 4 Immunoelectrophoregrams between anti-*Schistosoma japonicum* adult worm sera and fractionated antigens of *Oncomelania* snails.

techniques, it was demonstrated that in the antigenic communities among several parasites, there exist many common antigens in parasites most nearly allied to each other, but species of parasites produced itself the different specific band immunoelectrophoretically. (Biguet *et al.*, 1962; Capron *et al.*, 1964, 1965b; Tsuji *et al*, 1967; Tsuji, 1975). Furthermore, Capron *et al.* (1965, a) and Tsuji (1975) reported that there were immunological cross-re-

Table 1 Infection rates of *Oncomelania* snails exposed to *Schistosoma japonicum* miracidia, Chinese strain

Snail subspecies (F3-F6)	Infection rate (%)*
Oncomelania hupensis hupensis	59–60
Oncomelania hupensis nosophora	45–49
Oncomelania hupensis chiui	20–21
Oncomelania hupensis quadrasi	0
Oncomelania hupensis formosana	0

* Infection rate as obtained by exposure of snail to 5 miracidia

activities between adult worms and larval stages of parasites.

In the domain of conchology, species discrimination of snails were classified by immunological methods. (Tran Vay Ky *et al.*, 1962; Rosé *et al.*, 1966). In this study, antigenic communities between 5 subspecies of *Oncomelania* snails and the Chinese strain of *S. japonicum* adult worms were assessed by immunoelectrophoresis, and *O. h. hupensis* antigen produced the most bands (9 bands) against anti-*S. japonicum* adult worm serum.

In experimental infection rates of *S. japonicum* miracidia to *Oncomelania* snails in our laboratory, *O. h. hupensis* obtained the highest infection rates (about 60 %) of the 5 subspecies of *Oncomelania* snails (Table. 1). These findings suggested that the most suitable host of the Chinese strain of *S. japonicum* was *O. h. hupensis*. This observation agrees with the report of Iwanaga *et al.* (1983) that *Achatina fulica* which obtained the highest infection rate to *Angiostrongylus cantonensis* produced the most common antigenicity against *A. cantonensis* adult worm.

Although both subspecies of *O. h. nosophora* and *O. h. chiui* antigens produced 5 bands against anti-*S. japonicum* adult worm serum, the infection rates of *S. japonicum* miracidia to *O. h. nosophora* were higher than those of *O. h. chiui*. The infection rates of snails to *S. japonicum* are summarized in Table. 1 under the condition of exposure with 5 miracidia per snail. However, Iwanaga *et al.* (1979) reported that the best infection rates were obtained by using 3 miracidia per snail exposure smaller snails than *O. h. nosophora*. Therefore, to study the infection rate of *S. japonicum* miracidia to *O. h. chiui*, we should use 3 miracidia each.

O. h. quadrasi antigen produced 3 bands, and 2 bands with *O. h. formosana* to anti-*S. japonicum* adult worm serum, but they were not found to be susceptible to *S. japonicum* (Iwanaga and Tsuji, 1982 b). Tsuji *et al* (1978) reported that anti-*S. japonicum* adult worm serum of Yamanashi strain was identified by 2 bands with *O. h. formosana* antigen, and showed a very low infectivity (0.8 %) to this strain of *S. japonicum*. Thus, it appears that both subspecies of *O. h. quadrasi* and *O. h. formosana* are capable of infection with the Chinese strain of *S. japonicum*.

On the other hand, common antigenicity between antigens of *S. japonicum* and anti-*Oncomelania* snail sera, antigens of *S. japonicum* produced 8 bands against anti-*O. h. hupensis* serum, similarly, 4 bands against anti-*O. h. nosophora* serum, 3 bands against anti-*O. h. chiui* serum, 2 bands against anti-*O. h. quadrasi* serum and 1 band against anti-*O. h. formosana* serum. It was observed that more bands were seen between *S. japonicum* and its suitable host, but numbers or immunoelectrophoretic position of precipitin bands between anti-*S. japonicum* adult worm sera and antigens of *Oncomelania* snails were not consistent with those between antigens of *S. japonicum* adult worm and anti-*Oncomelania* snail sera.

The common antigenicity between fractionated snails antigens and anti-*S. japonicum* adult worm serum were shown Fraction 1, Fraction 2 and Fraction 3 except those of *O. h. nosophora* and *O. h. formosana* antigen, but the precipitin bands in Fraction 3 seemed to be an offshoot of Fraction 2, therefore, the common antigenicities of them existed in Fraction 1 and 2. Their molecular weights were calibrated 17,000-670,000 from the marker position (Fig. 3).

Summary

Immunoelectrophoretical studies on antigenic communities were carried out by the use of sera from rabbits immunized with the Chinese strain of *S. japonicum* adult worms and extracts of *Oncomelania* snails and *vice-versa*.

With regard to antigenic communities between *S. japonicum* adult worms and 5 subspecies of *Oncomelania* snails, *S. japonicum* adult worms produced 8 to 9 bands with *O. h. hupensis* and 4 to 5 bands with *O. h. nosophora*, 3 to 5 bands with *O. h. chiui*, 2 to 3 bands with *O. h. quadrasi* and 1 to 2 bands with *O. h. formosana*.

In our laboratory, the infection rate of *S. japonicum* miracidia to *O. h. hupensis* was 59-60 %, similarly, 45-49 % for *O. h. nosophora* and 20-21 % *for O. h. chiui*, but both subspecies of *O. h. quadrasi* and *O. h. formosana* were not found to be susceptible to *S. japonicum* miracidia. It was observed that more bands were seen between *S. japonicum* and suitable hosts.

Oncomelania snails were fractionated by Sephadex G-100, and each fraction antigen was tested with anti-*S. japonicum* adult worm sera by immunoelectrophoresis. As results, *O. h. quadrasi* antigen was fractionated to 4 fractions, similarly, 3 fractions with *O. h. hupensis*, *O. h. nosophora*, *O. h. chiui* and *O. h. formosana* antigen. The common antigenicities between fractionated antigens of 5 subspecies of *Oncomelania* snails and anti-*S. japonicum* adult worm sera mostly existed in Fraction 1 and 2, and estimated to have molecular weights of 17,000-670,000.

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日本住血吸虫と *Oncomelania* 属貝における宿主寄生虫関係に関する研究

1. 中国産日本住血吸虫成虫と *Oncomelania* 属貝における共通抗原性

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中国産日本住血吸虫成虫と5亜種 *Oncomelania* 属貝との間に見られる共通抗原性について，免疫電気泳動法を用いて検討した．その結果，日本住血吸虫成虫は，*Oncomelania hupensis hupensis* との間に8～9本，*O. h. nosophora* との間に4～5本，*O. h. chiui* との間に3～5本，*O. h. quadrasi* との間に2～3本，そして *O. h. formosana* との間に1～2本の沈降帯が認められた．これらの成績を *Oncomelania* 属貝に対する日本住血吸虫の感染性と比較してみると，セルカリアの感染率が高い貝ほど，多くの共通抗原が存在することが判明した．また一方，各種 *Oncomelania* 属貝粗抗原をSephadex G-100 カラムクロマトグラフィにより分画したところ，*O. h. quadrasi* 抗原は4つに分画され，*O. h. hupensis*, *O. h. nosophora*, *O. h. chiui* および *O. h. formosana* は3つに分画された．これら分画抗原と日本住血吸虫抗血清との間に見られる共通抗原の多くは，分画ⅠおよびⅡに存在し，その分子量は17,000～670,000であった．