Cross Reactivity of Schistosome Antigens between *Schistosoma mansoni* and *japonicum*

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KITANI, K., MIURA, R. and IUCHI, M. *Cross Reactivity of Schistosome Antigens between Schistosoma mansoni and japonicum.* Tohoku J. exp. Med., 1979, 127 (2), 151-156 — Fifty six patients with chronic Schistosomiasis japonica were given skin tests with preparations made of *Schistosoma mansoni* and *Schistosoma japonicum*. There was a high, significant positive correlation between the results of both antigens. Among 47 serum samples tested with indirect hemagglutination (IHA) 27 had the antibody titer 1:16 or higher, which was diagnostically significant in combination with positive skin test results. The results indicate a definite cross reactivity of *japonicum* and *mansoni* antigens in patients with Schistosomiasis japonica. — cross reactivity; schistosomiasis japonica; *Schistosoma japonicum*; *Schistosoma mansoni*

Cross reactivity of schistosome antigens between schistosomes of different strains in human schistosomiasis has been reported (Pellegrino et al. 1962; Kagan et al. 1966; Moore et al. 1968). Most studies, however, were conducted on patients infected with *Schistosoma mansoni*, *hematobium* or avian schistosomes (Pellegrino et al. 1962; Kagan et al. 1966; Moore et al. 1968) that were tested with antigens made from other strains of schistosome. Reports on patients with Schistosomiasis japonica from this point of view are relatively few. This report describes the results of a study of immunological reactions in patients with chronic Schistosomiasis japonica tested with preparations made from *Schistosoma mansoni*.

MATERIALS AND METHODS

Fifty six patients (41 males and 15 females) with chronic Schistosomiasis japonica under the medical care of the Kofu City Hospital, Kofu, Japan, were studied. All the patients had a past history of infection with *Schistosoma japonicum*, most of them more than a decade before. They did not have any recent episode of infection. Patients were examined by liver biopsy and parasite ova were found in the liver tissue. However, the vital reaction of all the parasite ova tested by the succinic acid dehydrogenase method (Oda 1959) was negative. Morphological features of the ova in the liver also supported this finding. Pathological examination of the liver revealed a variety of liver histology from normal liver, hepatitis, liver fibrosis to cirrhosis and hepatoma.

Intradermal skin test. Forty one patients (28 males and 13 females) were tested with

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the *mansoni* antigen as well as the *japonicum* antigen for comparison. The antigen from *Schistosoma japonicum* was an acidic protein fraction prepared from adult worms (Ishizaki et al. 1968) according to the method described by Melcher (1943). The nitrogen content was 0.03 mg per ml. The antigen from *Schistosoma mansoni* was generously supplied by the Behringwerke AG, Marburg Lahn, West Germany. The details of preparation were reported elsewhere (Benders et al. 1974). It was made from adult *Schistosoma mansoni* worms and also contained 0.03 mg per ml of nitrogen.

In the volar surface of one forearm, intradermal injections (0.05 ml each of antigen preparation) from the 2 schistosome strains were given about 5 cm apart. A control injection of saline of equal amount was given in the same area in the other forearm. Exactly 15 min after the injection, the maximum and minimum diameters of both the wheal and the erythema were measured. The size of the wheals and erythemas was expressed as the average of these two diameters. Of these 41 patients, sera obtained from 36 patients (24 males and 12 females) were tested with the indirect hemagglutination test (IHA test) described below. The IHA test was also performed on sera obtained from additional 11 patients (8 males and 3 females).

Indirect hemagglutination test (IHA). The IHA reagent was a soluble, purified *Schistosoma mansoni* antigen isolated from adult *Schistosoma mansoni* obtained from artificially infected mice. The antigen was adsorbed on stabilized erythrocytes. According to the general understandings, it is diagnostically significant when titers with complete agglutination are obtained in serum dilutions of 1:16 or higher. An accurate diagnosis could be made with serum antibody titers of 1:64 or higher, if less, only in combination with other serological diagnostic means.

RESULTS

Fig. 1 shows the distribution of the size of the wheals developed by the two different antigens expressed as an average diameter. The size of wheals developed by both antigens are in the same range. The highest incidence was seen in the range from 11 to 12 mm in the *mansoni* test and at 13 mm in the *japonicum* test. The distribution of sizes of the wheal of the *mansoni* antigen is to the left in the graph (1 to 2 mm smaller) when compared with *japonicum* antigen test. The wheal with *mansoni* antigen also has a sharper peak in the range of 11 to 12 mm in the diameter. The average wheal size was 13.95 ± 3.74 mm (mean $\pm$ S.D.) for wheals induced with the *japonicum* antigen and 12.71 ± 4.79 mm for wheals with the *mansoni* antigen ($n=41$, $0.1 > p > 0.05$, paired t test). There was no observable

Fig. 1. Distribution of wheal size by two skin test antigens.

Fig. 2. Comparison of diameter of the wheal (closed circle) and erythema (open circle) between *japonicum* and *mansonii* antigens on patients with chronic Schistosomiasis japonica.

Fig. 3. Incidence of IHA titer in 47 patients with chronic Schistosomiasis japonica.

difference in the distribution pattern of the wheal between males and females for either with the *mansonii* antigen or the *japonicum* antigen. The diameters of wheal and erythema developed by *mansonii* and *japonicum* antigens in the same patient is compared in Fig. 2. The two wheal diameters had a correlation coefficient of 0.74. Linear regression revealed the following relation between the two diameters, $Y=0.60X+4.54$, where Y =*mansonii* diameter and X =*japonicum* diameter.

The correlation of the size of the two erythemas observed in those patients with erythema was significant and showed the same tendency observed in wheals. Among the 17 patients who developed an erythema with the *japonicum* antigen, 16 also developed an erythema with the *mansonii* antigen. All patients with an erythema with the *mansonii* antigen developed an erythema with the *japonicum* antigen. The average erythema size was 20.62 ± 5.83 mm with the *japonicum* antigen and 17.09 ± 6.00 mm with the *mansonii* antigen ($n=16$, $p<0.05$, paired t test).

Fig. 3 shows the distribution pattern of the IHA titer. Among 47 serum

Fig. 4. Relation between IHA titer and skin test wheal size.

samples, 20 had the antibody titer less than 1:8 and 27 had the titer less than 1:16 with the IHA test. The highest titer of 1:256 was found in a serum sample obtained from a 52 year-old male patient with cirrhosis of the liver. However, there was no distinct relationship between the clinical diagnosis or symptoms and IHA titer. Fig. 4 shows the relation between IHA titer and the diameter of the wheal in the skin test. There was no apparent positive correlation between IHA titer and skin test.

DISCUSSION

Patients included in the present study are a special group of patients with *Schistosomiasis japonica*. The area inhabited by these patients is a former endemic area along Kamanashi river in Yamanashi prefecture Japan. However, this area was totally transformed into an urban district almost 10 years ago, and there has been no local source of recent schistosome infection. Consequently, these inhabitants are considered to have been infected more than a decade before, and have had no occasion for super- or re-infection in recent years.

Despite a decade since onset, approximately 95% of the patients with a positive skin test with the *japonicum* antigen had parasite ova in the liver or rectal tissues. However, vital reaction of the ova was negative and there was no fecal elimination of live ova. These patients were diagnosed as having chronic *Schistosomiasis japonica*, but there was no indication for treatment by anti-schistosome drugs. However, the pathological condition of the liver which was originally created by schistosomal infection persisted and was slowly worsening (Iio et al. 1971; Kitani et al. 1972).

The result of the present study using the antigens of two different strains of schistosome has shown that patients with chronic *Schistosomiasis japonica* responded to both antigens in a very similar manner. This agrees with previous studies which demonstrated a cross reactivity of schistosome in patients with

Schistosomiasis *mansoni* (Pelliegrino et al. 1962, Kagan et al. 1966), hematobium (Kagan et al. 1966) or avian schistosomiasis (Moore et al. 1968). In studies on patients infected with *Schistosoma hematobium*, *mansoni*, or both, Kagan et al. (1966) showed that the size of wheals induced by antigens of both *Schistosoma mansoni* and *japonicum* was almost identical. The results of the present study seem to be somewhat at variance in this respect since we found that the wheal induced by the *Schistosoma japonicum* antigen was slightly larger than that of the *Schistosoma mansoni* antigen in patients with Schistosomiasis japonica. This tendency was more clearly seen in the erythema. Kagan et al. (1966) referred to an unpublished work by Yogore et al., whose findings were similar to ours; that is, the reactivity of patients with Schistosomiasis japonica to the *Schistosoma mansoni* antigen was less than to the *Schistosoma japonicum* antigen. More information is necessary to clarify this point.

Considering the small difference in wheal size, the highly significant correlation between the size of the two wheals in the same patient and the 94% coincidence of erythema suggest that these two antigens are very similar in nature.

According to Tsuji et al. (1974), in a group of patients selected by the same criteria as was used for the present study, 85% were positive in the circumoval precipitin test, 47% were positive by the Ouchterlony method for adult worm antigen and 26% were positive by the immunoelectrophoresis method of adult worm antigen preparation. The results of the present study with IHA had a less value for positivity for the circulating antibody titer than the reported values with *japonicum* antigens, if the borderline is drawn at 1:64. But, if a titer of 1:16 or higher is judged to be positive, the percent of positivity becomes comparable to the values reported by Tsuji et al. (1974). Thus, the long persistent elevated antibody titer in sera of patients without live worms reported by Tsuji et al. (1974) appears to be confirmed in the present study with the antigens made from *Schistosoma mansoni*.

Since it was shown that a definite cross reactivity is present between the *mansoni* and *japonicum* strains in patients with Schistosomiasis japonica, antigen preparations made from *mansoni* worms would be diagnostic for epidemiological and public health purposes in areas infected with *Schistosoma japonicum* or in areas of mixed infestation.

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