Immunoblot Analysis of Schistosome Antigens: Trial of Identification of Stage−specific and Species−specific Antigens[1)]

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Abstract: The experiment was designed to identify the stage−specific and species−specific immunoreactive components in Schistosomiasis mansoni. Cercarial secretion materials (CSM), whole cercarial and adult worm antigens of *S. mansoni* were separated by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and assayed in immunoblot for reaction with mouse sera obtained at given intervals after infection. The striking difference in the appearance of antibodies reacting with CSM, cercarial antigens and adult worm antigens was observed during the course of infection. When the cercarial antigens were used, as early as 1 week postinfection, IgG antibodies reacted prominently with the components in the region of 34, 28.5 and 26 kDa of molecular weight. The immunoreactivity of these components to the sera remained unchanged up to 4 weeks postinfection. CSM reacted with IgM antibodies as well, but the molecular weights of immunoreactive components differed from those of cercarial antigens during the first 4 weeks postinfection. From week 1 to week 4 postinfection, none of the adult worm antigens reacted with the sera. At week 8, 2 components of 31 and 26.5 kDa of adult worm antigens reacted strongly with IgG antibodies. Other additional major immunoreactive components also reacted with the sera at this stage were 40 kDa of cercarial antigens and 90 kDa of CSM. At week 12, the adult worm antigens of 34 and 60 kDa molecular weights were recognized by the sera. These results suggest that comparison of antibodies reacting with different developmental stages of schistosome antigens clearly distinguish the stage of schistosome infection. *S. mansoni* species−specific antigens were examined by comparing the immunoreactivity of *S. mansoni* adult worm antigens with *S. mansoni* and *S. haematobium* infected human and hamster sera. The immunogens of 45 and 31 kDa of *S. mansoni* adult worm antigens reacted strongly with sera of hamsters infected with *S. mansoni*, but did not with sera of animals infected with *S. haematobium*. *S. haematobium* adult worm antigens, however, contained two components of 45 and 31 kDa which gave slight reaction with *S. mansoni* infected sera. The 45 kDa component of *S. mansoni* adult worm antigens reacted strongly with 11 out of 12 sera collected from patients infected with *S. mansoni*. This antigen also reacted with none of the 9 sera from patients infected with *S. haematobium*. The 31 kDa

Received for publication, July 20, 1987.
Contribution No. 1985 from the Institute of Tropical Medicine, Nagasaki University
1) Presented at the 56th Annual Meeting of The Japanese Society of Parasitology, Yokohama, April 1987.

component of *S. mansoni* adult worm antigens, however, reacted with 7 out of 12 sera collected from the patients infected with *S. mansoni* and with 4 sera from the 9 patients infected with *S. haematobium*. The use of this 45 kDa antigen will possibly improve the diagnosis of *S. mansoni* infection in the areas where both *S. mansoni* and *S. haematobium* coexist.

Key words: Immunobolt, Schistosome antigens, Stage−specificity, Species−specificity

INTRODUCTION

Currently, the improvement in the specificity of immunodiagnostic tests for schistosomiasis has been achieved by the availability of purified antigens (Mott and Dixon, 1982). Most of the serological tests, however, are limited in that they cannot distinguish properly the species of parasitizing schistosome, stage of infection (pre−patent, active infection and previous experience of infection etc.), intensity of infection or the disease status. So far some papers dealt with partial success in differentiating acute from chronic schistosomiasis by antibodies responses to specific schistosome antigens (Helden *et al.*, 1975; Lunde *et al.*, 1979; Kanamura *et al.*, 1979; Suzuki *et al.*, 1979; Nash *et al.*, 1983; Dunne *et al.*, 1984; Norden and Strand, 1985). The test systems used, however, are relatively complicated and/or require sophisticated techniques for the preparation of antigens.

Recently the immunoblot analysis of antigen has shown a great promise in the immunodiagnosis of infectious diseases. The technique is simple and may be used under the field condition. Rupple *et al.* (1985) and Hillyer *et al.* (1986) have succeeded in detecting the *S. mansoni* polypeptide of 31 kDa which may have potential diagnostic value. The present paper was designed to identify *S. mansoni* stage−specific and species−specific immunoreactive components in experimental and human schistosomiases by using the immunoblot analysis.

MATERIALS AND METHODS

Parasites:

S. mansoni and *S. haematobium* used in this study were from Kenya. *S. mansoni* has been maintained in snails *Biomphalaria pfeifferi* and inbred GN hamsters for 6 years in our laboratory. The hamsters were infected with *S. haematobium* cercariae shed from snails *Bulinus globosus* which had been previously infected with miracidia hatched from eggs collected from patients with urinary schistosomiasis in Kenya.

Preparation of Antigens:

1) *S. mansoni* and *S. haematobium* adult worm antigens

S. mansoni adult worms were obtained from inbred GN hamsters 8−10 weeks following percutaneous infection with 300 cercariae by perfusion with warm citrated

saline (0.75% sodium citrate in saline). *S. haematobium* adult worms were recovered from inbred GN hamsters 20 weeks after percutaneous infection by perfusion same as above. The worms were washed three times in PBS (pH 7.4) and stored −70℃ until use.

The adult worm antigens of each species were prepared by the method of Ruppel *et al.* (1985). Briefly, adult worms were suspended in electrophoresis sample buffer (0.5M Tris−HCl buffer pH 6.8, 10% glycerol, 5% 2−mercaptoethanol, 3% SDS and traces of bromphenolblue), sonicated and boiled for 2 min. The suspension was centrifuged at 3000 x g for 30 min. The supernatant was used as adult worm antigens.

2) *S. mansoni* cercarial antigens

S. mansoni cercariae were collected from infected snails *Biomphalaria pfeifferi* and treated as the adult worms were done.

3) Cercarial secretion materials (CSM) of *S. mansoni*

CSM were collected by the method of Stirewalt (1978). Briefly, cercariae were collected from infected snails and suspension of cercariae was filtered through a nylon plankton net with a pore size of 10μm (Swiss Silk Bolting Cloth, Switzerland) to make the cercarial density 3000 per 1ml of water. Then, the cercariae were delivered to a petri dish to which linoleic acid (100μg/㎠) had been applied. The dish was floated on a 41℃ water bath for 45 min. The cercarial suspension was filtered through a nuclepore filter (Nuclepore Corp.) with a pore size of 12μm to remove schistosomula. The filtrate was concentrated by ultrafiltration on a PM 10 membrane (Amicon Corp.) and stored at −70℃ until use.

Protein concentration of each antigens was determined by the method of Lowry *et al.* (1951).

Serum samples:

1) Laboratory animal sera

Twenty five male, 8−week old BALB/C mice were infected percutaneously with 500 *S. mansoni* cercariae. They were divided into 5 groups of 5 at random. Each group was sacrificed for collecting blood at 1, 2, 4, 8 and 12 weeks postinfection. Sera from each group were pooled. Control sera were obtained from noninfected male BALB/C mice.

Five male GN hamsters were infected with 300 cercariae of *S. mansoni* and they were bled 16 weeks later and the sera were pooled. Five male GN hamsters infected with unknown number of *S. haematobium* cercariae 20 weeks previously were bled and their sera were pooled. Control sera were obtained from noninfected male GN hamsters.

All sera were stored at −70℃ until use.

2) Human sera

Human sera were collected from 12 patients with parasitologically proven *S. mansoni* infection and from 9 patients with parasitologically proven *S. haematobiun* infection. Egg counts of all patients were not known. All sera were obtained from people in Taveta, Coast province in Kenya and stored −30℃ until use.

Control sera were collected from noninfected 2 Japanese volunteers and stored at −30℃ until use.

Immunoblot analysis:

Immunoblot analysis was performed as described by Tsang *et al.* (1983). After separation on 12% SDS−polyacrylamide slab gels (Laemmli, 1970), proteins were electrophoretically transferred to nitrocellulose membrane (BIO−RAD, No. 162−0115). The nitrocellulose membrane was cut into strips blocked by incubation for 30 min in 10mM Tris−HCl buffer (pH 7.4) containing 0.85% sodium chloride and 5% BSA (Fraction−V, Nakarai Chemicals Ltd.). Then, the strips were incubated for 24 hours at 4℃ with the test sera or control sera. After a further washing in 0.02% Tween 20 and 0.85% sodium chloride in 10mM Tris−HCl buffer (3 times each of 5 min), the strips were incubated with the second antibody (Peroxidase conjugated anti−human IgG, anti−mouse IgG and IgM and anti−hamster IgG raised in goats, Cappel, Cooper Biomedical) for 30 min. Unbounded antibody was removed by washing in 0.02% Tween 20 and 0.85% sodium chloride in 10mM Tris−HCl buffer (3 times each of 5 min) before addition of the substrate solution (60mg of 4−chloro−1−naphtol, 20ml of ice−cold methanol, 60μl of ice−cold hydrogen peroxide and 100ml of 10mM Tris−HCl buffer pH 7.4 containing 0.85 % sodium chloride). Colour develops usually within 5 min. The reaction was stopped by rinsing the strips in tap water.

RESULTS

1) Identification of *S. mansoni* immunoreactive components recognized by sera of mice infected with *S. mansoni* during the course of infection

To identify stage−specific immunoreactive components, the appearance of antibodies reacting in immunoblots with schistosome antigens was followed in mice for the period between 1 week to 12 weeks postinfection. In our study, 3 different antigens, adult worm antigens, cercarial antigens and CSM were used.

When the adult worm antigens prepared by the method of Ruppel *et al.* (1985) were analysed by using anti−mouse IgG, no components reacted with sera obtained from 1 to 4 weeks postinfection. At week 8 postinfection, 2 components of 31 and 26.5 kDa reacted strongly and several components reacted slightly with sera of infected mice. At week 12, the antigen of 26.5 kDa showed no more reaction, and antigen of 31 kDa was very prominent. Another two additional components of 34 and 60 kDa were also recognized. Other components remained to react as slightly as they did at week 8 postinfection. No reaction was observed with control sera. All of the immunoreactive components were not the major bands stained by Coomassie blue (Fig. 1).

When the cercarial antigens were analysed by using anti−mouse IgG, the sera obtained at week 1 postinfection reacted relatively prominently to the components in the region of 34, 28.5 and 26 kDa and slightly to some other components. From week 2 through 4, the pattern of reaction remained unchanged and no additional components were visualized. At week 8 postinfection, the antigen of 40 kDa became prominent and some additional antigens such as component of 67 kDa were recognized. At week 12, the

antigen of 40 kDa was more prominent. The reaction with anti−mouse IgM was also identical in terms of number of antigens visualized and their immunoreactivities. Control sera did not show any reaction except 16 kDa component (Fig. 2).

When the CSM were analysed by using anti−mouse IgM, three faint reactions were recognized in the region of 49, 40 and 27.5 kDa from week 1 to 4 postinfection. At week 8, an additional component of 90 kDa reacted with the sera. At week 12, the antigen of 49 kDa reacted more strongly and an additional component of 67 kDa was also recognized. The reaction with anti−mouse IgG was less prominent, but no striking difference from the reaction with anti-mouse IgM was observed. No reaction was observed with control sera (Fig. 3). Analysis of the CSM and the cercarial extract prepared by the method of Ruppel *et al.* (1985) showed that the two antigens share the two antigenic components, 40 and 67 kDa, which showed the qualitative similarity in immunoreactivity with the sera of infected mice.

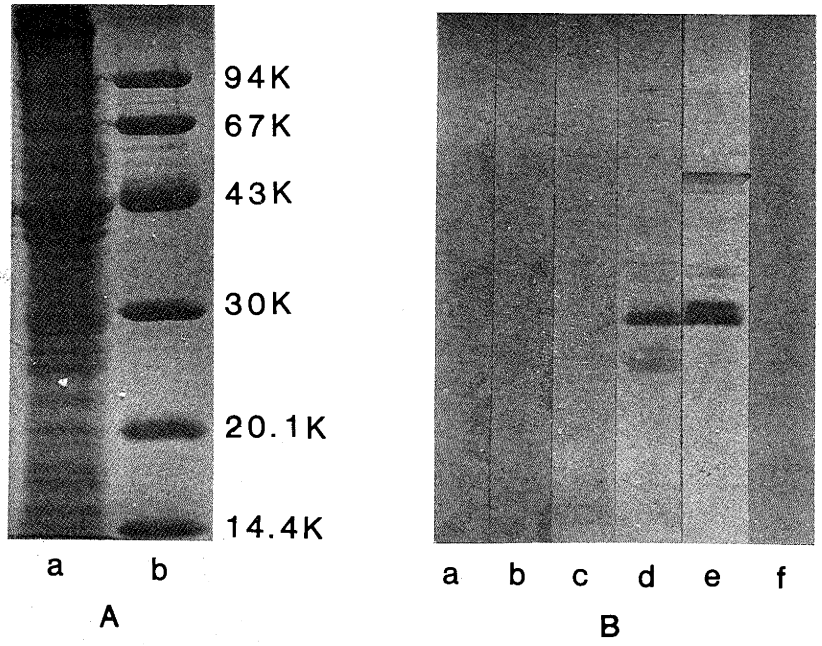

Fig. 1. Immunoblot analysis of stage−specific humoral immune response of murine schistosomiasis against electrophoresis sample buffer extracted adult worm antigens.
A. a) SDS−PAGE profile of electrophoresis sample buffer extracted adult worm antigens stained with 0.1% Coomassie blue. b) Molecular weight markers.
B. Patterns of antigens recognized with sera of a) 1 week, b) 2 weeks, c) 4 weeks, d) 8 weeks and e) 12 weeks after infection and f) control.
Seven μl of the adult worm antigens (67.9μg of antigenic proteins) were applied to each lane. Test and control sera were diluted 1:50 with 10mM Tris−HCl buffer (pH 7.4) containing 0.85% sodium chrolide. Immune coplexes were detected with a 1:200 dilution of peroxidase−conjugated anti−mouse IgG goat serum with 10mM Tris−HCl buffer (pH 7.4) containing 0.85% sodium chloride.

2) Qualitative assessment of antibodies responses to *S. mansoni* and *S. haematobium* adult worm antigens

The study was designed to identify species-specific different reactivity of *S. mansoni* and *S. haematobium* infected sera to their respective homologous and heterologous antigens. The antigens and sera used in this study were *S. mansoni* and *S. haematobium* adult worm antigens extracted by the method of Ruppel *et al.* (1985) and sera of infected hamsters and humans.

Fig. 4 shows the immunoreactivities of sera of infected hamsters to homologous and heterologous antigens. Although the cross reactivity between the two species were observed, we could find the striking different immunoreactivities to antigens between *S. mansoni* and *S. haematobium* infected sera. The sera of hamsters infected with *S. mansoni* reacted to the components of 31 and 45 kDa of both *S. mansoni* and *S. haematobium*

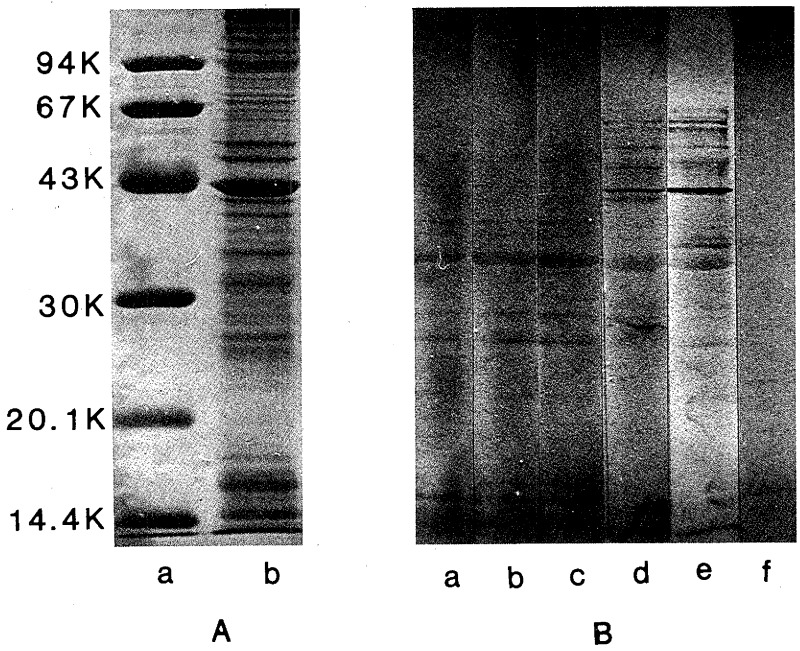

Fig. 2. Immunoblot analysis of stage-specific humoral immune response of murine schistosomiasis against electrophoresis sample buffer extracted cercarial antigens.
A. a) Molecular weight markers. b) SDS-PAGE profile of cercarial antigens stained with 0.1% Coomassie blue.
B. Patterns of antigens recognized with sera of a) 1 week, b) 2 weeks, c) 4 weeks, d) 8 weeks and e) 12 weeks after infection and f) control.
Fifteen μl of the cercarial antigens (30 μg of antigenic proteins) were applied to each lane. Test and control sera were diluted 1:50 with 10mM Tris-HCl buffer (pH 7.4) containing 0.85% sodium chloride. Immune complexes were detected with a 1:200 dilution of peroxidase-conjugated anti-mouse IgG goat serum with 10mM Tris-HCl buffer (pH 7.4) containing 0.85% sodium chloride.

adult worm antigens (Fig. 4 A a), B a)). The sera of hamsters infected with *S. haematobium* did not react to these antigens (Fig. 4 A b) B b)). While the sera of hamsters infected with *S. haematobium* reacted to the component of 33 kDa of both *S. mansoni* and *S. haematobium* adult worm antigens (Fig. 4 A b) B b)). But the sera of animals infecetd with *S. mansoni* did not react to the antigen (Fig. 4 A a) B a)).

Immunoreactivities of sera from humans infected with *S. mansoni* and *S. haematobium* to the *S. mansoni* adult worm antigens prepared by the method of Ruppel *et al.* (1985) were examined. Again the extreme cross reactivity between the *S. mansoni* and *S. haematobium* infection sera were recognized. Although the slight individual variation in intensity and quality of reaction was observed, the notable finding was that the antigen of 45 kDa was reactive only to the sera of *S. mansoni* infection, 11 out of 12 patients with *S. mansoni* infection were immunoreactive (Fig. 5 A) and none of 9 patients with *S. haematobium* infction were reactive (Fig. 5 B). While the antigen of 31 kDa,

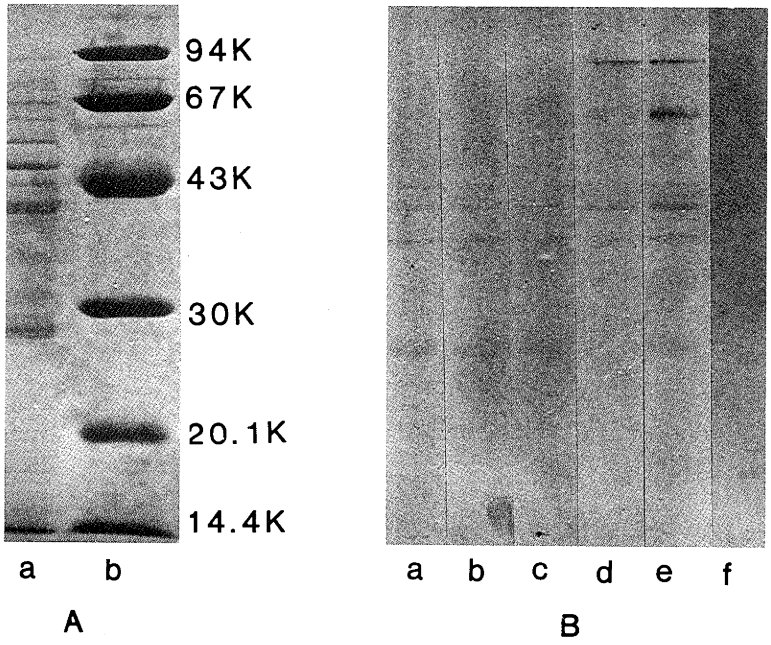

Fig. 3. Immunoblot analysis of stage−specific humoral immune response against cercarial secretion materials (CSM).
A. a) SDS−PAGE profile of CSM stained with 0.1% Coomassie blue. b) Molecular weight markers.
B. Patterns of antigens recognized with sera of a) 1 week, b) 2 weeks, c) 4 weeks, d) 8 weeks and e) 12 weeks after infection and f) control.
Twenty μl of CSM (20 μg of antigenic proteins) were applied to each lane. Test and control sera were diluted 1:50 with 10mM Tris−HCl buffer (pH 7.4) containing 0.85% sodium chloride. Immune complexes were detected with a 1:200 dilution of peroxidase−conjugated anti−mouse IgM goat serum with 10 mM Tris−HCl buffer (pH 7.4) containing 0.85% sodium chloride.

which was valuable for differentiation of species in the hamster model, was observed in 7 out of 12 patients with *S. mansoni* infection and in 4 out of 9 patients with *S. haematobium* infection. The antigen of 33 kDa, which was reactive only to *S. haematobium* infection sera in hamster model, was not visualized with any type of the sera tested. There were no other striking differences in immunoreactivities to the separated antigens between the sera of patients with *S. mansoni* and *S. haematobium* infection.

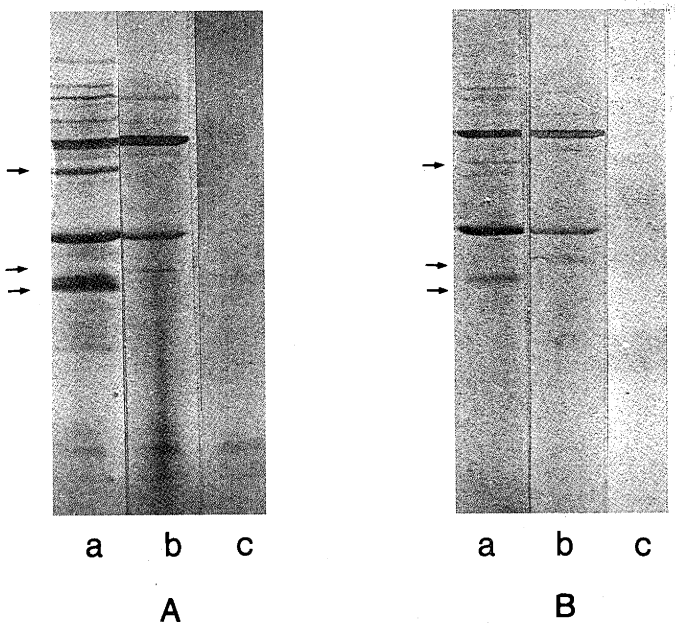

Fig. 4. Reactivity of hamster sera infected either *S. mansoni* or *S. haematobium* against adult worm antigens.
A. a) *S. mansoni* infected sera. b) *S. haematobium* infected sera c) control. Electrophoresis sample buffer extracted *S. mansoni* adult worm antigens were used as test antigens. Seven μl of the adult worm antigens (67.9μg of antigenic proteins) were applied to each lane.
B. a) *S. mansoni* infected sera. b) *S. haematobium* infected sera c) control. Electrophoresis sample buffer extracted *S. haematobium* adult worm antigens were used as test antigens. Twenty−five μl of the adult worm antigens (50 μg of antigenic proteins) were applied to each lane. Test and control sera were diluted 1:50 with 10mM Tris−HCl buffer (pH 7.4) containing 0.85% sodium chloride. Immune complexes were detected with a 1:200 dilution of peroxidase−conjugated anti−hamster IgG with 10mM Tris−HCl buffer (pH 7.4) containing 0.85% sodium chloride.
The positions of the antigens of 45, 33 and 31 kDa are indicated by, respectively, upper, middle and lower arrows.

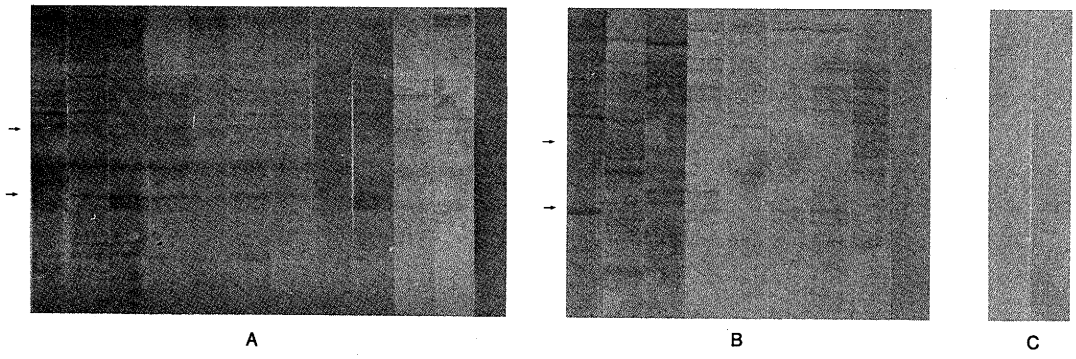

Fig. 5. Reactivity of human sera infected either *S. mansoni* or *S. haematobium* against electrophoresis sample buffer extracted *S. mansoni* adult worm antigens.
A. Sera from patients infected with *S. mansoni*.
The 45 kDa antigen was recognized all sera except 1st lane from the right side.
B. Sera from patients infected with *S. haematobium*.
C. Control sera.
Seven μl of the adult worm antigens (67.9μg of antigenic proteins) were applied to each lane. Test and control sera were diluted 1:100 with 10mM Tris−HCl buffer (pH 7.4) containing 0.85% sodium chloride. Immune complexes were detected with a 1:1000 dilution of peroxidase−conjugated anti−human IgG goat serum with 10mM Tris−HCl buffer (pH 7.4) containing 0.85% sodium chloride. The positions of the antigens of 45 and 31 kDa are indicated by, respectively, upper and lower arrows.

DISCUSSION

To distinguish the acute and chronic infection of schistosomiasis by immunodiagnostic method, parasitologists have tested several combination of detecting assay systems and variable antigens (Oliver−Gonzales *et al.*, 1955; Van Halder *et al.*, 1975; Kanamura *et al.*, 1979; Lunde *et al.*, 1979, 1980; Deelder *et al.*, 1981; Feldmeier *et al.*, 1983). A limited number of studies, however, have been directed toward identifying the specific antigenic molecules responsible for the induction of the initial immune response and the subsequent change from acute to chronic infection (Norden and Strand, 1985). The present study was firstly designed to identify *S. mansoni* stage−specific antigens in murine schistosomiasis by using immunoblot analysis of antigens. Since the different stages of schistosomes stimulate the host immune system according to the course of infection, CSM, whole cercarial and adult worm antigens were used in the present study. The striking difference was observed in the appearance of antibodies reacting with CSM, cercarial and adult worm antigens during the course of infection. Among the major immunoreactive components recognized in the present study, we couldn't detect any antigens with similar molecular weight between cercarial and adult worm antigens. CSM and cercarial antigens share the components of 67 and 40 kDa. So the present paper

suggests that comparison of reaction of antibodies with cercarial and adult worm antigens may distinguish the stages of infection. The sera of animals 1 week postinfection reacted with the components of 34, 28.5 and 26 kDa of the cercarial antigens, but did not react with any components of adult worm antigens. While the sera of animals infected 8 and 12 weeks previously reacted strongly with the components of 31 and 60 kDa of adult worm antigens. However, none of them cannot be identified as the *S. mansoni* stage−specific antigens except 26.5 kDa component of adult worm antigens. The 26.5 kDa component was detected only at week 8 postinfection, whereas the other components retained various degrees of reactivity during the subsequent course of infection.

The present study is comparable to the experiments of Norden and Strand (1985) who described the change of immunoreactive glycoproteins recognized by the sera of mice and humans infected with *S. mansoni* during the course of infection. Although molecular weights of immunoreactive components in the present study differed from those of glycoproteins of Norden and Strand (1985), the present observation that the number of immunoreactive components recognized by sera increased as the infection progress was identical to their observation. They, however, failed to identify any glycoproteins precipitated by sera from week 0 through week 4 after infection, even though they used ^{125}I−labeled cercarial glycoproteins as well.

Hillyer *et al.* (1980) reported that low concentration of SDS increased the solubilization of *Fasciola hepatica* antigens. So the present study used SDS for the extraction of antigens from cercariae and adult worms following the method of Ruppel *et al.* (1985). The major immunoreactive components of *S. mansoni* adult worm antigens reported by Ruppel *et al.* (1985), 31 and 67 kDa, were identified in the present study; 67 kDa protein of Ruppel *et al.* (1985) probably corresponds to the 60 kDa component in the present study, because slight difference in calculated molecular weight may be due to the difference of acrylamide gels used for different experiments. The immunoreactivities of the antigens extracted by SDS with sera were quite different from the antigens extracted by phosphate buffer saline (Sato, unpublished data). Antigens prepared by using different detergent rather than SDS or from different stages of schistosomes (schistosomula and eggs) in their life cycle might lead to the detection of more reliable and sensitive stage−specific antigens for immunodiagnosis of schistosomiasis.

It is well known that the three major species of human schistosomes, *S. japonicum, S. mansoni* and *S. haematobium*, exhibit extensive serological cross reactivity. While little is known about the genus−specific or species−specific immunoreactive proteins of schistosomes. Recently Tsang *et al.* (1984) showed distinct and reciprocating species−specificity between *S. mansoni* and *S. japonicum* microsomal antigens; the degree of species−specificity was not absolute though. And Norden and Strand (1984) found species−specific immunoreactive glycoprotein in *S. japonicum* adult worms, but they failed to recognize the species−specificity in immunoreactive glycoprotein between *S. mansoni* and *S. haematobium*. So it seems that the African species, *S. mansoni* and *S. haematobium*, are antigenically more similar to each other than to the Oriental species,

S. japonicum.

The present study in part was designed to identify species−specific immunoreactive components between *S. mansoni* and *S. haematobium* in the schistosome−hamster model. We could find the distinguished difference in immunoreactivities of three components of adult worm antigens to sera from hamsters with homologous and heterologous infection. Antigen compounds of 45 and 31 kDa of *S. mansoni* adult worm antigens reacted only to the sera of animals infected with *S. mansoni*. And antigen component of 33 kDa of *S. haematobium* adult worm reacted only to sera of animals infected with *S. haematobium*. However, these components may not be identified as species−specific *S. mansoni* or *S. haematobium* antigens, because, *S. haematobium* adult worm antigens also contained the components of 45 and 31 kDa which reacted with *S. mansoni* infected sera and *S. mansoni* adult worm antigens contained the component of 33 kDa which reacted to *S. haematobium* infected sera, although the reaction of these components with sera was relatively weak. This phenomenon could have some explanations. In this experiment, we used hamsters as experimental animals because of their susceptibility to both *S. mansoni* and *S. haematobium*. However, their susceptibility to *S. haematobium* is relatively lower compared with their susceptibility to *S. mansoni*. So the difference of immunoreactivity between *S. mansoni* infected sera and *S. haematobium* infected sera may be due to the difference of intensity of infection and/or duration of infection. Another possibility is that the differences of immunoreactivities in the 2 infections depend on the difference of location of the three antigens in each species, that is, the 45 and 31 kDa antigens are exposed on the surface and the 33 kDa antigen is hidden in the case of *S. mansoni* adult worms. In the case of *S. haematobium* adult worms, only the 33 kDa antigen is exposed and the 45 and 31 kDa antigens are hidden.

The disease status such as intensity of infection, duration of infection or infection with other parasites of patients examined in our study were not known. Our experiment, however, clearly showed that the immunoreactivities of *S. mansoni* patients' sera to the 45 kDa antigen differed from those of *S. haematobium* patients' sera. Eleven out of 12 samples of the formers showed positive reaction to it, while none of 9 of the latters did. The difference is less likely to be due to the cross−reactivity by other parasitic infections, because all the patients examined in our study were from an small village in Kenya. So far we do not have the schistosome antigen with high specificity and sensitivity by which *S. mansoni* and *S. haematobium* infection can be differenciated. The detection of 45 kDa antigen in immunoblot may be valuable for differential diagnosis of schistosomiasis in areas where *S. mansoni* and *S. haematobium* coexist, although we do not know the characteristics of this antigen; whether this antigen can be detected in adult worm of *S. haematobium* in the schistosome − human model.

Ruppel *et al.* (1985) reported that the polypeptide of 31 kDa of *S. mansoni* adult worm antigens had potential diagnostic value, however, they didn't examine whether the 31 kDa component reacted with *S. haematobium* patients' sera. In our study, though we followed their method for the preparation of antigens, the reaction in the region of 31

kDa was observed in only 7 out of 12 patients with *S. mansoni* infection and in 4 out of 9 patients with *S. haematobium* infection.

More recently proteins translated *in vitro* from schistosome adult worm mRNA have been assessed for their antigenic specificities compared to different stages, strain and species of the parasite (Knight *et al.*, 1984; Taylor *et al.*, 1984; Blanton *et al.*, 1986). Blanton *et al.* (1986) reported that many antigens were shared between *S. mansoni* and *S. haematobium*, but mRNA translation products contained two antigens which appeared species-specific for *S. mansoni* (47 and 37 kDa). The more interesting results reported by them are the fact that mRNA extracted from *S. mansoni* coded for a protein (39 kDa) which was specific for *S. haematobium*. The present study also reported that the schistosome adult worm antigens contained a component which reacted only with heterologous sera. The possible explanations to this interesting phenomenon have already been given in the discussion.

ACKNOWLEDGEMENTS

I am grateful to Dr. Masahiko Ehara, Miss Mikako Ishibashi and Mr. Mitsumasa Miura for their suggestions and technical assistance in this study. Appreciation is also expressed to Dr. Khalid M. Hassan, Dr. Masaaki Shimada and Prof. Yoshiki Aoki for thier helpful criticism and suggestions in the preparation of the manuscript.

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イムノブロット法を用いた住血吸虫抗原の解析：stage－specific 抗原及び species－specific 抗原の検索

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イムノブロット法を用いて，マンソン住血吸虫感染 BALB/C マウスより経時的に採集した血清（感染後 1，2，4，8，12週）と，マンソン住血吸虫各種抗原（セルカリア分泌物，セルカリア及び成虫粗抗原）との反応を調べマンソン住血吸虫の stage－specific 抗原について検討した。各種抗原を SDS－ポリアクリルアミドゲル電気泳動で分離した後，ニトロセルロース膜に転写し，被検血清との反応後，酵素抗体法で各種抗原に反応した抗体を検出した。その結果，マンソン住血吸虫感染 BALB/C マウスでは，各種抗原に対する感染血清の反応が，各感染時期（感染初期，産卵開始期及び慢性期）で特徴的なパターンを示すことがわかった。しかし，ある感染時期に特異的な抗原は成虫由来の分子量 26.5kDa の抗原を除いて見つけることはできなかった。

また，本研究においては同じくイムノブロット法により，マンソン及びビルハルツ住血吸虫感染血清（GN ハムスター，ヒト）とマンソン，ビルハルツ成虫粗抗原とを用いて，住血吸虫の species－specific 抗原についても調べてみた。マンソン住血吸虫成虫粗抗原を使って，ハムスター感染血清との反応を行なったところ，分子量 45kDa と 31kDa の2つの抗原に対しては，マンソン住血吸虫感染血清のみが反応し，分子量 33kDa の抗原に対しては，ビルハルツ住血吸虫感染血清のみが反応した。ビルハルツ住血吸虫粗抗原を用いた場合でも同じ結果が得られた。次に，マンソン住血吸虫成虫粗抗原を使って，ヒト患者血清との反応を調べてみると，マンソン感染患者12例中11例が，45kDa 抗原に反応したのに対し，ビルハルツ感染患者9例のいずれもこの抗原に対しては反応しなかった。31kDa 抗原に対しては，交叉反応があり，33kDa の成分は，どのヒト患者血清とも反応しなかった。以上の結果より，分子量 45kDa の抗原は，マンソン・ビルハルツ両住血吸虫症の混合流行地において，マンソン住血吸虫症の特異的な免疫診断に応用できるかもしれない。

熱帯医学 第29巻 第3号 139－152頁，1987年9月