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Sera of *Schistosoma japonicum*-infected patients cross-react with diagnostic 31/32 kD proteins of *S. mansoni*

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SUMMARY

Schistosoma mansoni adult worm antigens were tested for cross-reactions with sera obtained from patients infected with *S. japonicum*. The sera consistently recognized a doublet of bands, in immunoblots, which had molecular weights of approximately 31 and 32 kilodaltons (kD). This reaction was found to be markedly reduced with sera of patients who had received chemotherapy and who had a low risk of reinfection. Sera obtained from uninfected persons or from patients infected with other parasites never reacted with the antigen doublet. *Schistosoma japonicum*-infected mice produced antibodies during prepatency which predominantly recognized antigens of this molecular weight range in immunoblots performed with *S. mansoni* or *S. japonicum* proteins. Sera from *S. mansoni*-infected patients with a high specificity for the diagnostic *S. mansoni*-antigen cross-reacted with a corresponding component also in *S. japonicum* worms. Immunofluorescence assays performed with sera of schistosomiasis japonica patients confirmed earlier results localizing the diagnostic 31/32 kD antigens in the gut of *S. mansoni*. These cross-reacting 31/32 kD *S. mansoni* protein antigens may be applied for the immunodiagnosis of schistosomiasis japonica.

Keywords immunoblots immunodiagnosis *Schistosoma japonicum* *Schistosoma mansoni* schistosome antigens

INTRODUCTION

Diagnosis of schistosomiasis japonica by the demonstration of eggs in the patients' stool is not only laborious but also lacks sensitivity (Bang *et al.*, 1945; Yogore, Lewert & Blas, 1983; Lewert, 1984; Watt *et al.*, 1986). Serological assays are less time-consuming and more sensitive. They are therefore particularly necessary for diagnosis in areas, where control of schistosomiasis has led to low prevalence and intensity of infections. Available immunodiagnostic tests are based on antigen mixtures obtained from eggs or adult worms (reviewed by Mott & Dixon, 1982; Kagan, 1982; Mott, 1984). The use of defined single antigens is likely to increase the specificity of immunodiagnostic tests and to facilitate their standardization. Antigens that might be potentially useful in a purified form have been described (reviewed by Mitchell & Cruise, 1986), but to our knowledge none have been tested on a larger scale. One reason might be the lack of availability in sufficient quantities, a problem that could possibly be resolved by recombinant DNA technology applied to the production of protein antigens. *A Schistosoma japonicum*-antigen, Sj26, with a suspected potential for vaccination (Mitchell *et al.*, 1985) has now been cloned and identified as glutathion S-transferase

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(Smith *et al.*, 1986), but its possible use for serodiagnosis remains to be established. Another protein antigen of S. *japonicum*, Sj 23, has a diagnostic potential, but was characterized only from a Philippine, but not Chinese, parasite strain (Cruise *et al.*, 1983).

An *S. mansoni*-protein which is consistently recognized by sera from *S. mansoni*-infected patients, but not from uninfected persons or of individuals with other parasitic infections, has recently been described. IgG-antibodies against this 31 kD-antigen were demonstrable already during prepatency in mice and man. Due to the high specificity and sensitivity of the reaction, a diagnostic potential for this 31 kD-protein had been proposed (Ruppel *et al.*, 1985a, b).

The reaction of sera from animals and patients infected with *S. japonicum* has now been investigated with *S. mansoni*-antigens. The data suggest that immunodiagnosis of schistosomiasis japonica is possible by using 31/32 kD *S. mansoni*-worm antigens. Preliminary results have been published elsewhere (Ruppel *et al.*, 1986).

MATERIALS AND METHODS

Parasites. We used adult worms of a Puerto Rican strain of *S. mansoni* and of a Chinese strain of *S. japonicum* isolated near Wuhan in Hubei Province. Cercariae were obtained from *Biomphalaria glabrata* and *Oncomelania hupensis*, respectively, and applied percutaneously to male NMRI mice. Parasites were collected by perfusion 5 weeks (*S. japonicum*) or 7 weeks (*S. mansoni*) later, dissolved in sample buffer (0·125 M Tris-HCl pH 6·8, 10% glycerol, 5% 2-mercaptoethanol, 3% SDS), boiled for 2 min and sonicated. In all experiments, male and female worms were used in equal numbers.

Patients and sera. Sera were obtained from schistosomiasis patients with parasitologically proven infections. The *S. mansoni*-infected patients have been described earlier (Ruppel *et al.*, 1985a). The sera used here were obtained 48 days after infection of patient 12 and 4 months after infection of patients 16 and 17, respectively.

Three groups of patients infected with *S. japonicum* were from the Hubei Province. Patients of group A ($n=15$) were from Han Young and Dong Shi Hu and blood was obtained in autumn, shortly after the transmission season. All patients passed *S. japonicum* eggs in their stools at the time blood was taken. All patients were positive in the indirect haemagglutination assay (IHA; 1:10 to 1:1280) and circumoval precipitin test (COPT; 8 to 45%). Their age ranged from 9 to 64 years.

Patients of group B were from Han Young, and blood was taken in spring, before onset of the transmission season. All patients had excreted *S. japonicum* eggs either less than 1 year (patients B1–B8) or 1 month (B9–B20) before obtaining blood. All had received nithiocyamine chemotherapy (Shanghai Medical Company, Peoples' Republic of China; 2 mg/kg/day for 3 days) about 1·5 years before the time of collecting blood samples. Therefore, all patients of group B had probably been repeatedly infected. All had positive reactions in the IHA ($>1:20$) and COPT ($>5\%$). Their ages ranged from 11 to 53 years with the majority being adolescent. Both sexes were included.

Patients of Group C ($n=26$) were from Xiong-Kou in Chan-Jian county, a village where schistosomiasis is now partially eradicated. All patients of group C had a known history of *S. japonicum*-infection during the last 8 years and had received chemotherapy at various times after 1980. As they work in a factory, they were exposed to a low risk of reinfection. At the same time, when blood was obtained from these patients (spring 1986), the miracidial hatching assay, IHA and COPT were performed. All tests were negative except for patient C24 who was positive in all tests, C4, C13, C14, C15 and C19 who were weakly positive in IHA, and C15 who was weakly positive in COPT.

Thirty-one control persons were also from Hubei. They had no known history of schistosomiasis, did not excrete schistosome eggs and had negative reactions in the IHA ($<1:10$) and COPT ($<3\%$).

Sera were lyophilyzed for transport, reconstituted with water, divided into samples and kept at -20° or -40°C until use.

Electrophoresis and immunoblotting. Polyacrylamide gradient (10–14·5%) slab gels, electrophoretic transfer of proteins from the gel onto nitrocellulose (NC) sheets (Schleicher & Schüll; BA85, pore size 0·45 μm) and immunoblots were performed with individual patient sera essentially as

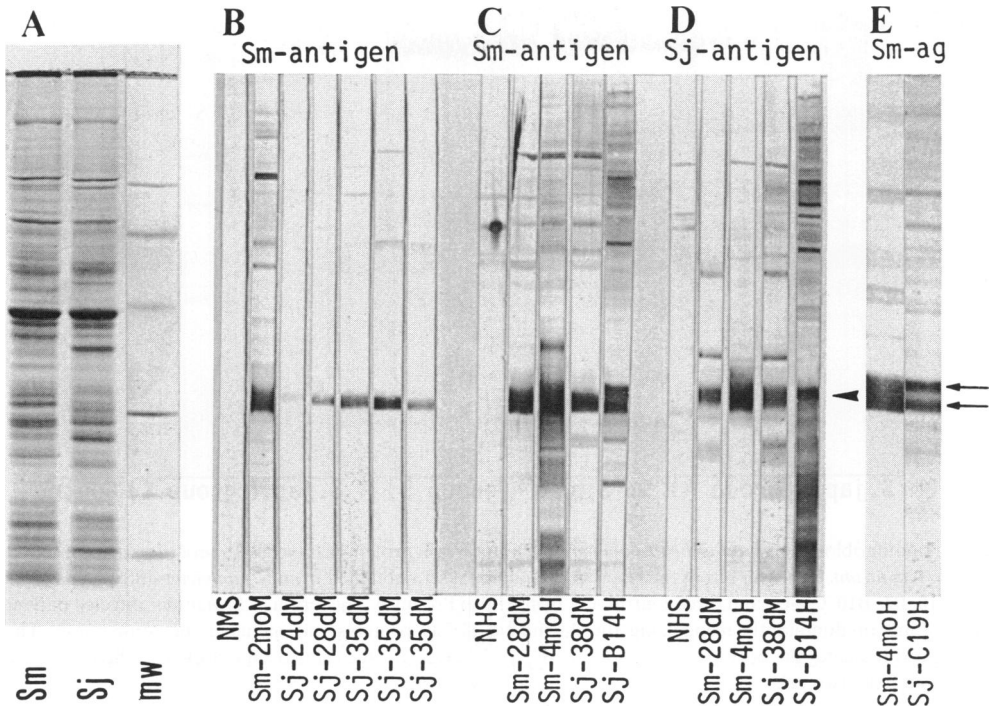

Fig. 1. Recognition in immunoblots of *Schistosoma mansoni* and *S. japonicum* adult worm proteins by murine and human sera obtained after heavy infection with either schistosome species. (A) Total *S. mansoni* (Sm) and *S. japonicum* (Sj) worms were separated by SDS-PAGE and stained for protein; molecular weight (mw) markers were (from top to bottom) phosphorylase b (94 kD), bovine serum albumin (67), ovalbumin (43), carbonic anhydrase (30), soybean trypsin inhibitor (20, 1) and a-lactalbumin (14, 4). (B) *S. mansoni*-proteins were tested in immunoblots with normal mouse serum (NMS), a pool of sera from mice infected for about 2 months with *S. mansoni* (Sm-2moM), or individual sera of mice obtained 24 days or more after infection with about 300 cercariae of *S. japonicum* (Sj-24dM, Sj-28dM, Sj-35dM). (C) *S. mansoni*-proteins were tested in immunoblots with normal human serum (NHS), sera of mice obtained 28 or 38 days after infection with about 300 cercariae of *S. mansoni* (Sm-28dM) or *S. japonicum* (Sj-38dM), serum of *S. mansoni*-patient no 17 obtained 4 months after infection (Sm-4moH) or serum of *S. japonicum*-infected patient no B14 (Sj-B14H). (D) *S. japonicum*-proteins tested in immunoblots with the same sera as in C. (E) *S. mansoni*-proteins were tested in immunoblots with serum of *S. mansoni*-patient no 16 obtained 4 months after infection (Sm-4moH) or serum of *S. japonicum*-infected patient no C19 (Sj-C19H). E is magnified compared to A–D.

The arrowhead indicates the 31 Kd-position in A-D. The arrows indicate the 31/32 kD doublet in E. The results are combined from four gels.

described (Ruppel *et al.*, 1985a,b). Protein binding sites of the NC were saturated with 0·05% Tween 20 (Batteiger, Newhall & Jones, 1982) in Tris-buffered saline (TBS; 0·01 M Tris-HCl pH 7·4, 0·9% NaCl), and sera as well as horseradish peroxidase-conjugated goat antibodies to human IgG (Tago, Burlingame, CA, USA; gamma chain specific, affinity purified) were diluted (1:150 and 1:500, respectively) in Tween-TBS.

Immunofluorescence. Assays were performed with cryostat sections (8 μm) of adult *S. mansoni* male worms as previously described (Ruppel *et al.*, 1985a).

RESULTS

The protein pattern of adult *S. japonicum* as revealed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate was similar to the one of *S. mansoni* proteins (Fig. 1A).

Sera of mice obtained about 4 weeks after infection with *S. japonicum* reacted in immunoblots predominantly with one *S. mansoni* component (Fig. 1B). The position of the heterologous reaction

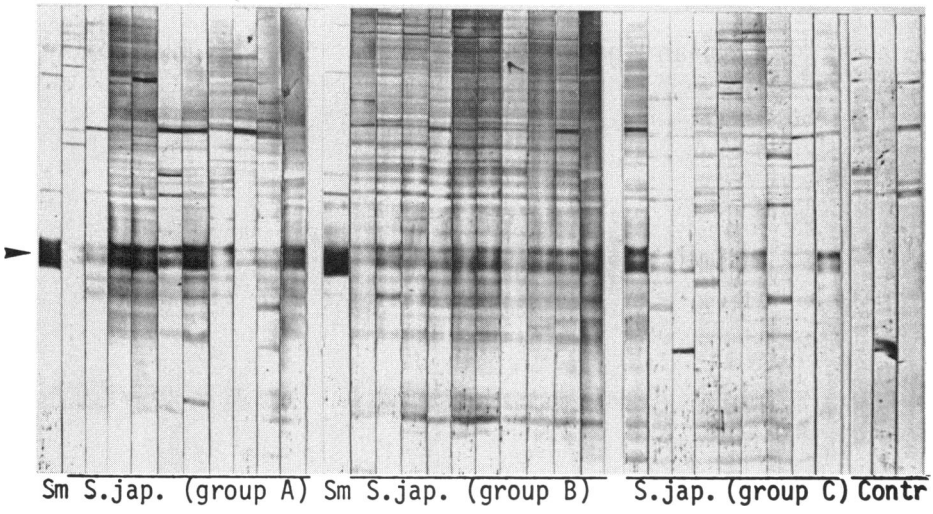

Fig. 2. Immunoblot analysis of *Schistosoma mansoni* adult worm proteins assayed with sera of patients infected with *S. japonicum*. Lanes represent the reaction of individual sera obtained from *S. japonicum*-infected patients A1–A10, B1–B10, C1–C9 and three control sera from Hubei Province. The serum of *S. mansoni*-infected patient no 12 (Sm) predominantly recognizing the diagnostic 31 kD proteins was included for comparison. The arrowhead indicates the position of the doublet of 31/32 kD diagnostic schistosome proteins. The results are combined from two gels.

coincided with the 31 kD position of the homologous reaction in the *S. mansoni* system. Immunodominant 31 kD antigens of *S. mansoni* were recognized by human sera obtained after infection with either schistosome species (Fig. 1C). These sera of humans and mice also recognized a 31 kD component among adult *S. japonicum* proteins (Fig. 1D). The data suggest the presence of similar or identical proteins which share high antigenicity and a molecular weight of about 31 kD in both schistosome species.

Sera from patients with a parasitologically proven history of *S. japonicum* infection were tested for the presence of antibodies recognizing proteins of adult *S. mansoni* in immunoblots. The reactivities in the 31/32 kD region of two selected sera from patients with *S. mansoni* and *S. japonicum*-infections, respectively, are compared in Fig. 1E. The reactions obtained with a series of representative sera from *S. japonicum*-patients are shown in Fig. 2. The following conclusions can be drawn:

(1) All of the sera from *S. japonicum* patients tested (groups A and B) cross-reacted in immunoblots with several *S. mansoni* antigens;

(2) the pattern of recognized bands was individually variable, but all sera cross-reacted with a doublet of *S. mansoni* antigens, except those of patients A17 and C3, which reacted only with the lower band (results not shown);

(3) the doublet bands were in the same position as the reaction of sera with high specificity against diagnostic *S. mansoni* antigens and had molecular weights of 31 and 32 kD, respectively;

(4) the earlier treatment of patients with nithiocyamine followed by reinfection (group B) had no apparent influence on their antibody reaction against the 31/32 kD *S. mansoni* antigens;

(5) chemotherapy in the absence of reinfection (group C) resulted in a lower reaction of the patient sera with 31/32 kD proteins;

(6) uninfected control persons (group D) never reacted with 31/32 kD *S. mansoni* antigens, although low or sporadic reactivity occurred with other components.

Additional sera of Chinese patients infected with other parasites were tested with respect to their reaction with the 31/32 kD *S. mansoni* components (Table 1). False positive or false negative reactions were not observed with any of the sera available for this study.

Table 1. Recognition in immunoblots of the 31/32 kD doublet of *Schistosoma mansoni* polypeptides by sera of Chinese patients infected with *S. japonicum* or other parasites

Infection	No of sera	Positive reactions	Negative reactions
Schistosoma japonicum (groups A, B)*	35	35†	0
Trichinella spiralis	5	0	5
Cysticercus of *Taenia solium*	5	0	5
Wuchereria bancrofti	5	0	5
Opistorchis viverrini‡	17	0	17
None	31	0	31

* Patients of group C have not been included, since they had received chemotherapy several years ago and their risk of re-infection was low.

† The sera of two patients recognized only one component of the doublet.

‡ Opistorchiasis-patients were from Thailand. The data obtained with these sera are reproduced here from an earlier report (Ruppel *et al.*, 1985a, Table 1).

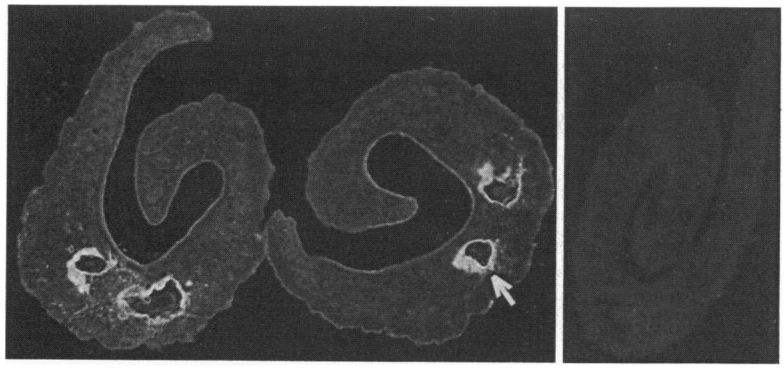

Fig. 3. Immunofluorescence assay performed with sections of adult *Schistosoma mansoni* worms and serum of (left) a *S. japonicum*-infected patient (B12) or (right) a healthy control person. The sera were diluted 1:40. In left, fluorescence was observed predominantly at the parasite gut (arrow) and was weak at the tegument. Parenchyma-associated fluorescence was not observed with this serum. No fluorescence was detectable in the control section (right).

The reactivity of sera from *S. japonicum* patients with sections of adult *S. mansoni* was investigated by immunofluorescence. All patients of groups A and B had antibodies binding to the gut with titres varying from 1:40 to 1:1280. An example of this heterologous reaction is shown in Fig. 3. In group C, however, titres were much lower, and 13 patients had titres of 1:20 or less, while titres above 1:160 were observed only in three patients. No immunofluorescent reactions were observed with control sera. Taken together, the data obtained with all patient sera showed a positive correlation (correlation coefficient $r^2 = 0{\cdot}55$) between the titre of gut associated immunofluorescence and the intensity of staining of the 31/32 kD doublet graded on an arbitrary scale from 0 to 5+.

DISCUSSION

This study demonstrates the diagnostic potential of 31/32 kD *S. mansoni*-antigens to detect antibodies specific for infections with *S. japonicum*. The antigens were recognized by sera of *S. japonicum*-infected patients as a doublet of two closely adjacent bands in immunoblots. Less than 10% of the patient sera reacted only with one of the two components. The reactions were different in this aspect from those observed with *S. mansoni*-infection sera, which mostly reacted as a single, but broad band (Ruppel *et al.*, 1985a). It is conceivable that the broad band in the 31/32 kD region consists of several closely adjacent components and that two of them cross-react with *S. japonicum*-patient sera. If *S. mansoni*-patient sera recognized additional bands, the clarity with which the two cross-reacting components appear with *S. japonicum*-sera might be obscured in the homologous *S. mansoni*-system. In any event, the data demonstrate that more than one diagnostic polypeptides exist in the 31/32 kD molecular weight range, where only one protein was earlier assumed to be present (Ruppel *et al.*, 1985a).

Cross-reactions between well-defined *S. mansoni*-antigens and *S. japonicum*-infection sera have been studied with egg antigens (Mott & Dixon, 1982; Mott, 1984; Norden & Strand, 1984a). In particular, a 31 kD egg componment has been reported as a candidate for immunodiagnosis, but its reactivity was tested with sera of only two *S. japonicum*-patients (Hillyer, Nieves-Frau & Vasquez, 1986). This 31 kD egg antigen is likely to be different from the 31 kD component described by ourselves, which is present also in male worms (Ruppel *et al.*, 1985a). Among adult worm glycoproteins (Tsang *et al.*, 1983; Norden & Strand, 1984b), a 29·3 kD component of *S. mansoni* cross-reacted with *S. japonicum*-patient sera (Tsang *et al.*, 1984). This antigen migrated as a single band and appears to have a molecular weight different from the 31/32 kD doublet described here.

The 31/32 kD doublet of *S. mansoni*-antigens cross-reacted consistently with *S. japonicum*-infected patient sera, but never with sera obtained from uninfected persons or from patients with other parasitic diseases including other trematode infections. This extends our previous observations on the specificity for schistosomiasis of the reaction with these antigens. The data suggest the possible use of these proteins for immunodiagnosis of schistosomiasis japonica. The point appears to be particularly relevant, since immunodiagnosis of this disease is still based on rather crude antigen preparations (Mott & Dixon, 1982; Mott, 1984). Although a defined *S. japonicum* worm protein (Cruise *et al.*, 1983) and an egg glycoprotein (Tracy, Domingo & Mahmoud, 1985) have been proposed for serodiagnostic purposes, they have to our knowledge not yet been used in the field. Current research in our laboratories is directed towards that goal using recombinant 31/32 kD schistosome antigens (M. Q. Klinkert, A. Ruppel & E. Beck, unpublished).

The reaction between *S. japonicum* patient sera and antigens on cryostat sections of *S. mansoni* has been observed earlier, and the cross-reactive *S. mansoni* antigens appeared to be of somatic rather than gut-derived nature (Mott & Dixon, 1982). In contrast to the low reactivity of gut antigen recorded in that study (54·2% of egg-positive persons), we observed gut associated fluorescence with all of our 35 patient sera, while many of them recognized somatic antigens only weakly. The reasons for these discrepancies might include egg excretion rates of the patients or technical details and require further studies.

A number of gut-associated antigens have been described for *S. mansoni* (Kelsoe & Weller, 1978; Nash, 1978; Carlier *et al.*, 1980; Deelder *et al.*, 1980; Nash, Lunde & Cheever, 1981; Nash & Deelder, 1985; Chappell & Dresden, 1986). The intestinal origin of the 31/32 kD diagnostic proteins was proposed earlier (Ruppel *et al.*, 1985a) and has recently been proven by studies with monoclonal antibodies (Ruppel, Breternitz & Burger, 1987). Antibodies binding in immunofluorescence assays to the gut have been associated in particular with recently acquired *S. mansoni*-infections of humans (van Helden *et al.*, 1975; Deelder & Kornelis, 1981) and experimental *S. japonicum*-infections of rabbits (Beisler *et al.*, 1984). Antibodies against the 31/32 kD proteins of both schistosome species are likely to contribute to the immunofluorescence patterns observed in these studies and may, indeed, appear already during prepatency (Ruppel *et al.*, 1985a,b; this report). However, our studies with both *S. japonicum*- and *S. mansoni*-infected patient sera demonstrate that antibodies against the 31/32 kD proteins are present not only in recently acquired, but also in long standing schistosome infections.

In this communication we considered only cross-reactions between *S. japonicum*-infection sera and *S. mansoni*-antigens with diagnostic potential. Preliminary observations suggest the existence of 31/32 kD proteins with related epitopes in both *S. japonicum* and *S. haematobium*. We have not yet purified these proteins in order to study their possible biological functions. However, proteolytic schistosome enzymes share with the 31/32 kD diagnostic proteins the localization in the schistosome gut (Timms & Bueding, 1959; Kasschau, Robinson & Dresden, 1986), the approximate molecular weight (Sauer & Senft, 1972; Deelder, Reinders & Rotmans, 1977; Chappell & Dresden, 1986) and a limited degree of molecular heterogeneity (Chappell & Dresden, 1986; Lindquist *et al.*, 1986). Whether or not the diagnostic 31/32 kD proteins are identical with proteolytic enzymes, remains to be established.

In conclusion, we demonstrated that immunodiagnosis of *S. japonicum*-infections is possible with defined schistosome worm proteins. We suggest that antigens of *S. mansoni* with a molecular weight of about 31 and/or 32 kD may be useful for the development of a serodiagnostic test for schistosomiasis japonica.

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