COMPARATIVE STUDY ON ANTIGENICITY AND IMMUNOGENICITY OF 26-28 kDa ANTIGEN AND RECOMBINANT SJ26 (RSJ26) OF *SCHISTOSOMA JAPONICUM*

Liu Shu-Xian, Song Guan-Chen, Ding Li-Yun, Xu Yu-Xin, Cai Zhi-Hong and Wu Gong-Ze

Department of Immunology, Institute of Parasitic Diseases, Chinese Academy of Preventive Medicine, Shanghai 200025, People's Republic of China

Abstract. This paper reports a comparison of the recombinant Sj26 (rSj26) antigen derived from the Philippine strain and the 26-28 kDa antigen isolated and purified from the Chinese strain of *Schistosoma japonicum* with respect to their antigenicity and immunogenicity. The results showed that there were obvious cross reactions between rSj26 and 26-28 kDa antigen when rSj26 antigen was tested against specific antibodies in sera of mice infected with the Chinese strain of *S. japonicum* or the 26-28 kDa antigen was tested against specific anti-rSj26 antibodies by ELISA, IFA and Western blotting. Both the 26-28 kDa and the rSj26 antigen had weak cross reactions with SEA antigen. The worm reduction rate after challenging with Chinese strain cercariae in mice immunized with rSj26 was 26-32%, similar to that in mice immunized with 26-28 kDa antigen. It is suggested that rSj26 antigen can induce a certain level of specific protective immunity in the host against infection by the Chinese strain of *S. japonicum* cercariae.

INTRODUCTION

Schistosoma japonicum Sj26 antigen is acknowledged as one of the potential candidates for a schistosome vaccine. It was first observed in a study on 129/J mice naturally resistant to *S. japonicum*, then it was extracted and purified from *S. japonicum* (Philippine strain). This antigen is glutathion S-transferase, abundant in schistosomes. The gene encoding Sj26 antigen has been cloned and expressed (Mitchell, 1989). We have successfully extracted and purified native 26-28 kDa target antigen from *S. japonicum* (Chinese strain) by affinity chromatography. It's protein bands are of similar molecular mass to Sj26 antigen on SDS-PAGE (Liu *et al*, 1989a). On sequence analysis of 25 N-terminal amino acids, the 26-28 kDa antigen showed more than 70% homology with that of Sj26 antigen (Liu 1992). For this reason we obtained from Dr Mitchell's laboratory *E. coli* containing the plasmid (pSj5) that directs synthesis of native Sj26 antigen, and purified the rSj26 antigen by affinity chromatography (Smith *et al*, 1988; Liu *et al*, 1991). In this paper, the comparative study of antigenicity and immunogenicity of 26-28 kDa antigen and the recombinant Sj26 antigen of *S. japonicum* is reported.

MATERIALS AND METHODS

Animals

Inbred BALB/C mice, 18-25g were used.

Antigens of *Schistosoma japonicum*

The 26-28 kDa GST antigen purified from adult worm of *S. japonicum* (Chinese strain) by affinity chromatography on a glutathione-agarose matrix. The protein concentration of this antigen was 0.66 mg/ml, determined by Lowry assay (Liu *et al*, 1989a). GST activity was 22.4-96.7 IU/ml, determined spectrophotometrically at 340nm as per Habig *et al* (1974). Recombinant Sj26 (rSj26) antigen was obtained from expressed *E. coli* containing the gene encoding rSj26 antigen by affinity chromatography on immobilized glutathione. The protein concentration was 1.8 mg/ml (Liu *et al*, 1991). GST activity was 27.4-48.0 IU/ml; 1% SEA antigens were soluble egg antigens extracted routinely from eggs of *S. japonicum* (Chinese strain). The protein concentration was 4.7 mg/ml.

Sera

Sera from BALB/C mice infected percutaneously

with 25 cercariae of Chinese strain of *S. japonicum* were collected from 6 weeks to 8 weeks after infection. Antisera against rSj26 antigen were obtained in BALB/C mice immunized subcutaneously or by foot pad injection with 50 μg of purified rSj26 antigen in Freund's complete adjuvant and boosted *ip* at 2 weeks and *iv* 3 weeks later with 10μg purified rSj26 antigen, respectively. The immune sera were collected every week after boosting. Antisera to 26-28 kDa antigen were obtained or collected in the same manner.

Methods for comparison of antigenicity

ELISA: Plates were coated at a dose of 1 μg of 26-28 kDa antigen or rSj26 antigen per well, or coated with SEA at 1:500 dilution routinely. The above sera were all diluted from 1:10 to 1:5,120. Horse radish peroxidase-labeled goat anti-mouse IgG antibody was used at 1:1,000 dilution. Substrate was O-Phenylenediamine at a concentration of 0.4 mg/ml.

IFA of frozen adult worm section: A 45-day adult worm of *S. japonicum* (Chinese strain) frozen in OCT was sectioned at 8 μm thickness with a cryostat. The sections were incubated with the antisera to 26-28 kDa antigen or antisera to rSj26 antigen, then with FITC-conjugated goat anti-mouse IgG antibody at 1:40 dilution. Immunofluorescence in tissues such as tegument, parenchyma and gut were observed using a fluorescence microscope.

Western blotting and immunoblot assay: SDS-PAGE was performed on 12.5% gels, according to Tsang *et al* (1983). The rSj26 and SEA antigens in gels were transfered respectively to nitrocellulose membranes at 4°C, 100V and 0.6A, and then the membranes were incubated with infected mice antisera (1:20), or sera from mice immunized with 26-28 kDa antigen or rSj26 antigen overnight at 4°C, respectively. HRP-labeled goat anti-mouse IgG antibody was diluted at 1:500.

***In vitro* micro-lymphocyte proliferative assay:** Briefly, splenic lymphocytes from infected mice, immunized mice and normal mice (mice control) were incubated in triplicate with 26-28 kDa antigen or rSj26 antigen (10 μg/well) and in wells without antigen (control) for 5 days. 16 hours before harvesting, the lymphocytes in each well were pulsed with 1 μci ^{3}H-thymidine and cpm counted by liquid scintillation spectrometer (Beckman LS 100). The lymphocyte proliferative response was calculated by stimulation index (SI).

Method for comparison of protective immunity

Mice immunized with 26-28 kDa antigen or rSj26 antigen and normal mice (challenge control) were infected simultaneously with 30 Chinese strain of *S. japonicum* cercariae. Mice were sacrificed at 6 weeks after infection, perfused and the number of adult worms counted. The worm reduction rate was calculated according to the following formula (Minard *et al*, 1978; Dean *et al*, 1978; Liu *et al*, 1989b).

$$\text{worm reduction rate (\%)} = 1 - \frac{IC-I}{C} \times 100$$

where I = the number of worms in immunized mice without challenge infection
IC = the number of worms in immunized mice with challenge infection
C = the number of worms in mice only with challenge infection

RESULTS AND DISCUSSION

Cross-reaction in antigenicity and immunogenicity between 26-28 kDa antigen and rSj26 antigen was observed as follows:

(a) rSj26 antigen could be recognized by specific antibodies in sera of mice infected with *S. japonicum* (Chinese strain). The levels of antigen-antibody reaction between 26-28 kDa antigen (from Chinese strain) or rSj26 (from Philippine strain) and specific antibodies from mice infected with Chinese strain of *S. japonicum* were very similar. The titers in both were 1:320, determined by ELISA. The rSj26 antigen also could be recognized by anti-26-28 kDa antibodies in sera of mice immunized with 26-28 kDa antigen; the titer was 1:160-320. The 26-28 kDa antigen had significant cross-reaction with anti-rSj26 antibodies in sera of mice immunized with rSj26 antigen, the level of reaction being similar to that between 26-28 kDa antigen and specific antibodies in sera of mice immunized with 26-28 kDa antigen; the titer was 1:1,280 - 1:5,120 (Fig 1).

(b) Either of the specific antibodies induced by 26-28 kDa antigen or that induced by rSj26 antigen

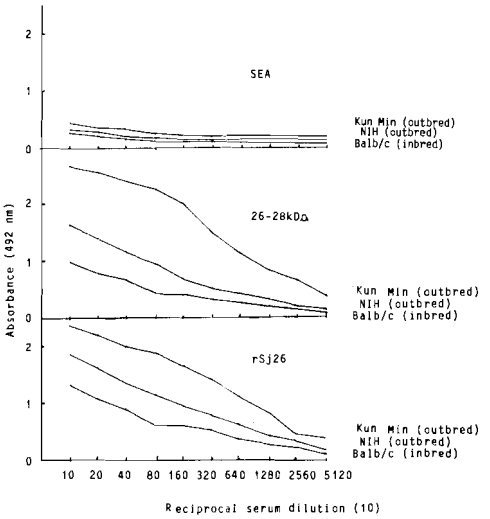

Fig 1—Antibody level (anti-rSj26 antibody) in mice immunized with recombinant rSj26 of Philippines, Mindoro strain of *S. japonicum* purified from *E. coli* by ELISA coating with rSj26, 26-28 kDa and SEA.

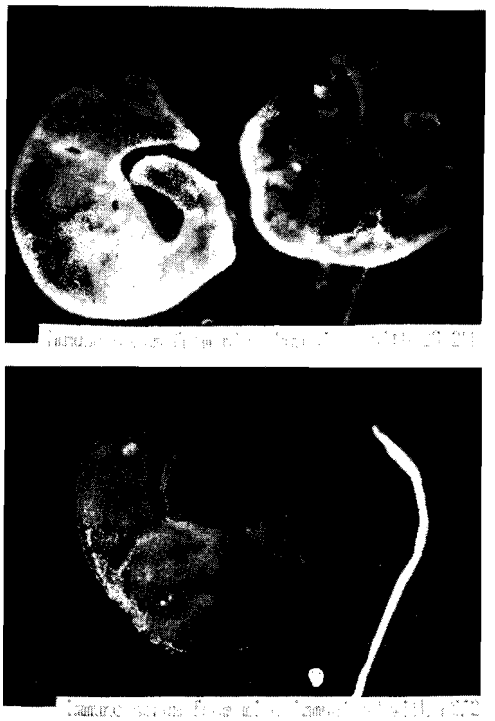

Fig 2—IFA localization of specific antibodies from mice immunized with 26-28 kDa or Sj26 antigens in tegument, body or gut of adult worm of *Schistosoma japonicum* (Chinese strain).

had immunoreaction to tegument, parenchyma and gut of adult worms of *S. japonicum*, observed on frozen worm section by IFA. The immunofluorescent staining of the section was strong (Fig 2).

(c) The result of Western blotting showed that rSj26 antigen could be recognized by not only specific antibody in sera immunized with rSj26 antigen, but also by sera from mice infected with *S. japonicum* (Chinese strain) or immunized with 26-28 kDa antigen. All showed a strongly stained protein band in the 26 kDa region (Fig 3).

(d) Both rSj26 antigen and 26-28 kDa antigen could be recognized by anti-anti-idiotype antibody (Ab3) obtained from sera of mice immunized with purified monoclonal antibody IE2 to *Schistosoma japonicum* (Chinese strain). The titers were respectively 1:1,600 - 1:6,400 for rSj26 antigen and 1:1,600 - 1:3,200 for 26-28 kDa antigen by ELISA.

(e) The sensitized specific lymphocytes of spleen of mice infected with *S. japonicum* were incubated *in vitro* with rSj26 antigen or 26-28 kDa antigen for 5 days. ^{3}H-TdR was pulsed 16 hours

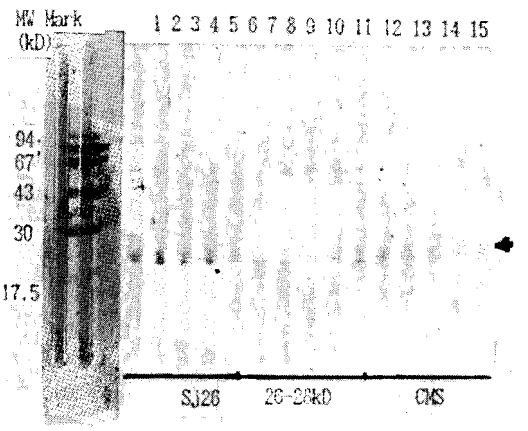

Fig 3—Recognition of specific antibodies from mice immunized with 26-28 kDa or infected with *Schistosoma japonicum* (Chinese strain) on rSj26 antigen after Western blotting.

before harvesting. The result of the *in vitro* micro-proliferative assay showed that the stimulated index of both antigens (26-28 kDa, rSj26) were in the range of 3-5. It suggested that both antigens could activate the lymphocytes sensitized by the infection of *S. japonicum* (Chinese strain) to some extent.

Cross-reaction among 26-28 kDa antigen, rSj26 antigen and egg antigen

When mice were immunized with rSj26 antigen, the anti-rSj26 antibody in the sera could react to soluble egg antigen (SEA) of *S. japonicum* (Chinese strain). The titer was relatively low, only 1:160 by ELISA.

The result of Western blotting showed that the specific antibodies in the sera of mice immunized with 26-28 kDa antigen of adult worms of *S. japonicum* (Chinese strain) or rSj26 antigen (Philippine strain) had cross-reactions with SEA of *S. japonicum* (Chinese strain) and could recognize the antigenic determinants of SEA in the range of 97-120 kDa (Fig 4).

Cross-reaction in protective immunity between 26-28 kDa antigen and rSj26 antigen

The experiment on protective immunity was repeated three times in a total of 84 mice. Thirty-seven mice were immunized with rSj26 and 30 mice with 26-28 kDa antigen. Seventeen normal

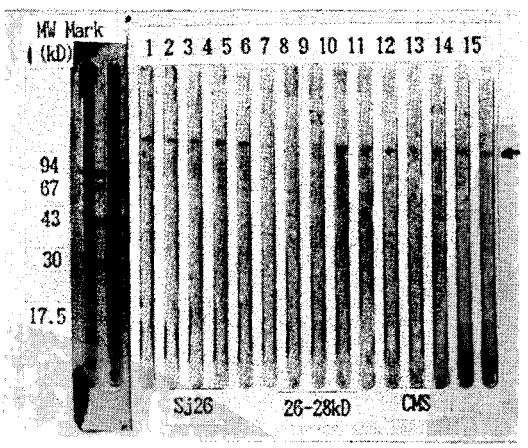

Fig 4—Recognition of specific antibodies from mice immunized with 26-28 kDa or rSj26 antigen on SEA after Western blothing.

mice served as challenge controls for both immunized groups, which were infected with *S. japonicum* (Chinese strain) cercariae at the same time as were the immune mice. At 6 weeks after challenged infection with Chinese strain cercariae, the worm reduction rate of the group immunized with rSj26 antigen was 26.5-32.5% and 26.2-30.4% for 26-28 kDa antigen group.

The above results suggested that, although 26-28 kDa antigen and rSj26 antigen were obtained from different geographic strains of *S. japonicum*, there were a significant cross-antigenicity and a certain degree of cross immuno-genicity. The gene encoding Sj26 antigen had already been cloned and efficiently expressed in the laboratory of Dr Mitchell (Smith *et al*, 1988; Saint, *et al*, 1986). Dr Mitchell proposd that the 26-28 kDa antigen which we extracted and purified from adult worms of *S. japonicum* (Chinese strain) and Sj26 antigen from the Philippine strain of *S. japonicum* both would be glutathione S-transferase isoenzymes. From the comparative study of the two antigens they are similar in molecular size in the location of protein bands of SDS-PAGE and the sequence of amino acids, but also cross-react with respect to antigenicity and immunogenicity. An anti-Sj26 monoclonal antibody has been developed by Davern *et al* (1990) and has been applied to testing for circulating antigen in sera of patients for immunodiagnosis of schistosomiasis or to monitor the effectiveness of chemotherapy. The preliminary data on cross-antigenicity reported in this paper suggests the possibility of using rSj26 antigen instead of native 26-28 kDa antigen for the immunodiagnosis of schistosomiasis. In addition, Sj26 antigen, as one of the candidate antigens for anti-schistosome vaccine development, cross-reacts with 26-28 kDa antigen in protective immunity, and so could be useful in the development of DNA probes or primers to screen the cDNA library of *S. japonicum* (Chinese strain) in order to facilitate cloning and expression of the 26-28 kDa GST gene.

ACKNOWLEDGEMENTS

The authors would like to thank Dr Sher A for his technical advice in isolation and purification of schistosome GST and Dr Mitchell for his consultation and kind supply of *E. coli* containing plasmid pSj5. This investigation was part of a

project funded by the grant 863 from the National Program of Bio-Tech, China.

REFERENCES

Davern KM, Tiu WU, Samaras N, *et al. Schistosoma japonicum:* monoclonal antibodies to the Mr 26,000 schistosome glutathione S-transferase (Sj26) in an assay for circulating antigen in infected individuals. *Exp Parasitol* 1990; 70 : 293-304.

Dean DA, Minard P, Stirewalt MA, Vannier WE, Murrell KD. Resistance of mice to secondary infection, I. Comparison of bisexual and unisexual initial infections. *Am J Trop Med Hyg* 1978; 27 : 951-6.

Habig WH, Pabst MJ, Jacoby WB. Glutathione S-transferase. *J Biol Chem* 1974; 249 : 7130-9.

Liu SX, Ding LY, Song GC, Chen CY. Antigenicity of 24-26 kD and 90 kD protein "target antigen" in adult *Schistosoma japonicum* worms. *Shanghai J Immunol* 1989a; 9 : 339-42.

Liu SX, Song GC, Nie JH, Lois WC. Further study on the immunity induced by mutagenic NTG attenuated cercariae of *Schistosoma japonicum. Chin J Parasitol Parasit Dis* 1989b; 7 : 89-94.

Liu SX, Ding LY, Song GC, Lin ZC, Xu XX. The extraction, purification and prelimilnary application of *Schistosoma japonicum* recombinant Sj26 antigen. *Chin J Parasit Dis Control* 1991; 4 : 201-4.

Liu SX. An overview of molecular parasitology in China. *Chin Med J* 1992, 105 : 91-6.

Minard P, Dean DA, Jacofson RH, Uannier WE, Murrell KD. Immunization of mice with cobalt-60. *Am J Trop Med Hyg* 1978; 27 : 74-86.

Mitchell GF. Glutathione S-transferase potential components of anti-schistosome vaccines? *Parasitol Today* 1989; 5 : 34-7.

Saint RB, Beall JA, Grumont RJ, Mitchell GF, Carcia EG. Expression of *Schistosoma japonicum* antigens in *Escherichia coli. Mol Biochem Parasitol,* 1986; 18 : 333-42.

Smith DB, Johnson K. Single-step purification of polypeptides expressed in *Escherichia coli* as fusion with glutathione S-transferase. *Gene* 1988; 67 : 31-40.

Smith DB, Rubira MR, Simpson RJ, *et al.* Expression of an enzymatically active parasite molecule in *Escherichia coli: Schistosoma japonicum* glutathione S-transferase. *Mol Biochem Parasitol* 1988; 27 : 249-56.

Tsang VCW, Peralta JM, Simons AR. Enzyme-linked immunoelectrotransfer blot techniques (EITB) for studying the specificities of antigens and antibodies separated by gel electrophoresis. *Methods Enzymol* 1983; 337-91.