

THE ANTIGENICITY OF GST ANTIGEN EXTRACTED FROM CHINESE STRAIN OF *SCHISTOSOMA JAPONICUM*

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Abstract. The GST antigen, similar to Sj26 (Philippine strain), which plays an important role in inducing protective immunity against *Schistosoma japonicum*, can be extracted and purified from adult worms of the Chinese strain of *S. japonicum*. There are two bands at 26 kDa and 28 kDa of GST antigen called the 26-28 kDa GST antigen as identified by SDS-PAGE, and these have GST activities. Mice were immunized with the 26-28 kDa antigen and the specific antibody response in serum was assayed by ELISA, IFA and western blot. The antigenicity of the 26-28 kDa GST antigen in mice was significant. For example, the antigen could stimulate mice to increase the level of serum IgM and IgG; the antibodies in serum of immunized mice could be localized in the antigenic determinants of tegument or body of the worms; specific antibodies against the antigens increased markedly after immunization as measured by ELISA or IFA; the antibody from mice immunized with the 26-28 kDa GST antigen can recognize 26-28 kDa antigenic molecules, identified by immunoblot assay.

INTRODUCTION

With progress in the study of protective immunity in Schistosomiasis, it has been demonstrated that GST antigen extracted from adult worms such as Sj26 or p28 has an important role played in immunity against Philippine strain of *Schistosoma japonicum* and *S. mansoni* (Capron *et al.*, 1987; Balloul *et al.*, 1987; Mitchell *et al.*, 1984, 1985). In order to develop a vaccine against the Chinese strain of *S. japonicum*, isolation and purification of the glutathione S-transferase (GST) antigen and investigation of its antigenicity are important steps. We report here methods for extraction and identification of the GST antigen from the Chinese strain of *S. japonicum* and the antibody response in mice immunized with this antigen.

MATERIALS AND METHODS

Extraction and identification of GST antigen

According to Smith *et al.* (1986), about 12,000 adult worms of *S. japonicum* (Chinese strain) were collected from the portal veins of infected rabbits. Adult worms were washed in washing buffer (containing PMSF and DTT) and homogenized at 0°C. The homogenate was loaded on to a affinity

column with oxygenated and reduced glutathione-agarose matrix, and GST antigen was eluted from the column with high salt buffer and PBS containing GSH, then dialyzed against PBS. Enzyme activity of schistosome GST was determined spectrophotometrically at 340 nm as Habig *et al.* (1974) described. Purity of this extracted antigen was detected by 12.5% SDS-PAGE, according to Laemmli (1970). Protein concentration was determined by Lowry *et al.* (1951) assay.

Immunizing schedule: Each mouse was immunized subcutaneously or by foot pad intradermal injection with 50µg antigen in an equal volume of Freund's complete adjuvant (FCA); 21 days later they were immunized again with 10µg antigen only. 28 days after the first immunization, they were boosted once by iv injection of 10µg antigen per mouse. Then immune sera were collected every week postimmunization (Davern *et al.*, 1987).

Observation of immune response

Enzyme-linked immunoadsorbent assay (ELISA): Routinely the plates were coated with 1% antigen (SEA) soluble egg at 1:500 dilution or with 1µg per well GST antigen solution. Horse radish peroxidase (HRP) - conjugated goat anti-mouse IgG antibodies were used at 1:1,000 dilution.

Immunofluorescence assay (IFA): Schistosome adult worms were obtained from infected rabbits 45 days after infection. The worms were mounted with Tissue-Tek OCT compound using a frozen block procedure and frozen worms were sectioned at 8 μ m with a Tissue-Tek microtome cryostat. Rabbit antisera were used 1:20-1:2,560 dilutions. FITC-conjugated goat anti-mouse IgG antiserum was used at 1:40 dilution.

Two-direction agarose immunodiffusion: Plates were covered with 1.5% agarose. Goat anti-mouse IgM, IgG and IgG subsets (IgG1, IgG2a, IgG2b and IgG3) antibodies were added to center holes respectively, and antiserum of immune mice was added at 1:2 - 1:512 dilutions to pericentral holes.

Western blotting and immuno-blot assay: Adult worm antigens were extracted from adult worms using sample buffer (0.0625M Tris-HCl, pH6.8; 3% SDS; 5% -ME and 10% glycerin). SDS-PAGE was done on 12.5% acrylamide gel slabs. Adult worm antigens were transferred electrophoretically from the separating gel to a nitrocellulose membrane at 4°C, 100V, according to Tsang *et al* (1983). Antisera of immunized mice at 1:100 or 1:200 dilutions and HRP- goat anti-mouse IgG antibodies at 1:500 dilution were used to perform immune detection on Western blots, enzyme substrates 3,3'-diaminobenzidine tetrahydrochloride.

RESULTS

Extraction and identification of GST antigen

The enzyme activity was 96.77 IU/ml. By SDS-PAGE, there were two isoenzyme bands located at 26 kDa and 28 kDa of the GST antigen from Chinese strain of *S. japonicum*, called the 26-28 kDa antigen (Fig 1). The protein concentration of this antigen was 0.66 mg/ml.

Observation of immune response after immunization with the GST antigen

ELISA: Sera from immunized mice were tested by ELISA using two antigens as described in Material and Methods. Certain levels of antibody response in mice immunized with the 26-28 kDa antigen could be observed in ELISA with both antigens. However, the response of anti-26-28 kDa sera to

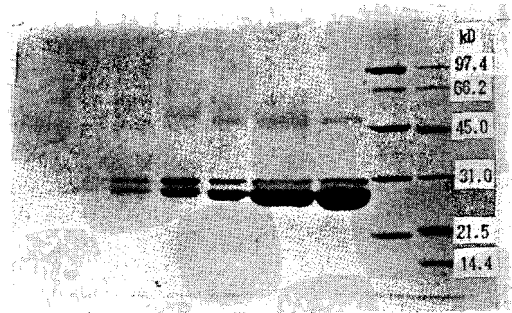


Fig 1—SDS-PAGE (12.5% gel) of 26-28 kDa GST antigen from adult worms of Chinese strain of *S. japonicum*.

the homologous antigen was much higher than that the heterologous antigen SEA, showed by ELISA coating with 26-28 kDa GST antigen (OD : $2.23 \pm 0.83 \sim 0.26 \pm 0.02$ at the dilution of antisera from 1:10 to 1:5,120). The OD value detected by ELISA coating with SEA for antisera from mice immunized with 26-28 kDa antigen was much lower than that 26-28 kDa GST antigen (OD : $0.51 \pm 0.16 \sim 0.50 \pm 0.01$ at the dilution of antisera from 1:10 to 1:5,120). The results suggested that there was no obvious cross reaction between SEA antigen and 26-28 kDa antigen.

IFA: The location of anti-GST antisera on tegument and parenchyma of schistosome adult worms was observed by IFA. The results indicated that mice immunized with 26-28 kDa antigen (Fig 2-3) could produce an antibody response.

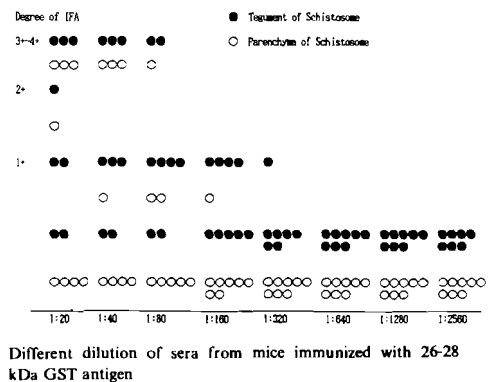


Fig 2—Localization of the antibody from mice immunized with 26-28 kDa GST antigen on tegument and parenchyma of Schistosoma (Chinese strain) by IFA.



Fig 3—The fluorescence stain of antibody from mice immunized with 26-28 kDa GST antigen on tegument and parenchyma of adult worm (Chinese strain).

Two-direction immunodiffusion: Compared with sera from normal mice (control), a slightly increased level of IgM (1:8-1:16) and a significantly increased level of IgG (1:32-1:256), particularly IgG1, were observed in mice immunized with the 26-28 kDa antigen, while only a slightly increased level of IgG3, IgG2a and IgG2b could be shown.

Western blotting and immuno-blot assay: Antibodies from mice immunized with the 26-28 kDa antigen could specifically recognize the 26-28 kDa schistosome antigen, as shown in Fig 4.

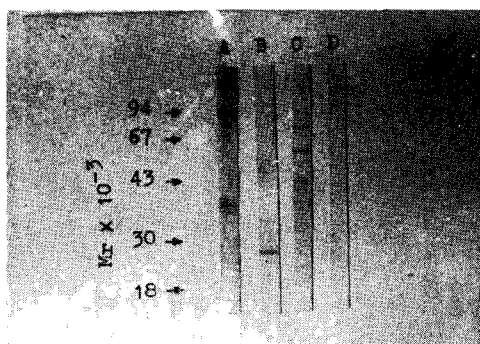


Fig 4—Western blotting of antibody from mice immunized with 26-28 kDa GST antigens.

- A - Serum from mice immunized with 90 kDa antigen
- B - Serum from mice immunized with 26-28 kDa GST antigen
- C - Infected murine serum (IMS)
- D - Normal murine serum (NMS)

DISCUSSION

Sj26, glutathione S-transferases abundant in schistosome adult worms, which has a potential role in inducing protective immunity to *S. japonicum*, was firstly extracted and purified from adult worms of Philippine strain of *S. japonicum* by affinity chromatography. The Sj26 antigen from schistosomes, not from the host, is immunogenic. A significant protective immunity against schistosome infection has been induced in mice immunized with Sj26 antigen (Smith *et al.*, 1986). Two bands of 26-28 kDa GST antigen which we extracted from Chinese strain of *S. japonicum* were observed when the purity of this antigen was investigated by SDS-PAGE. According to Mitchell (personal communication), who was the first to report two coenzyme bands of Sj26 (Philippine strain) GST antigen near 26 kDa, the two bands of 26-28 kDa antigen (Chinese strain) were similar to those of the Sj26 GST (Philippine strain) antigen. This 26-28 kDa antigen from the Chinese strain also has GST activity. So the 26-28 kDa antigen extracted from the Chinese strain of *S. japonicum* and Sj26 extracted by Mitchell from the Philippine strain of *S. japonicum*, as well as Sm28 (p28) and Sm26 extracted by Capron from *S. mansoni*, are similar to each other.

The antigenicity of the 26-28 kDa antigen when it was used to immunize mice was significant. Firstly, this antigen can stimulate mice to increase the level of IgM and IgG, especially IgG1; secondly, the antibodies in sera of immunized mice could be localized specifically in the antigenic determinants of the tegument or parenchyma of the worm; thirdly, the level of specific antibodies against the 26-28 kDa antigen increased markedly after immunization, as measured by ELISA or IFA (ELISA: 1:640, IFA: 1:40-1:160); fourthly, the antibody from mice immunized with the 26-28 kDa antigen could recognize the 26-28 kDa bands.

These preliminary data on the extraction and purification of GST from the adult worm of the Chinese strain of *S. japonicum* and the observation of its antigenicity may be an important step in the search for candidate antigens for development of vaccines against *S. japonicum* using the anti-idiotypic approach with anti-GST monoclonal antibodies. Furthermore, on the base of the amino acid sequence of purified GST antigen of the Chinese strain, comparison of homology between GST antigens

from different geographic strain of *S. japonicum* could be carried out. If the homology is high, it may be possible to make primers or DNA probes to amplify the gene encoding the 26-28 kDa antigen *in vitro* by PCR or to screen the present SjcDNA library and DNA genomic library by hybridization.

ACKNOWLEDGEMENTS

The authors would like to thank Dr A Sher and Dr GF Mitchell for their technical consultation and advice in purification of Schistosome GST antigen. This investigation was part of the project funded by the "863" National Program of Bio-Tech, China.

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