THE POSIBILITY OF GST ANTIGEN FROM CHINESE STRAIN OF *SCHISTOSOMA JAPONICUM* FOR IMMUNODIAGNOSIS OF SCHISTOSOMIASIS

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Abstract. The GST antigen (called 26-28 kDa antigen) extracted and purified from *Schistosoma japonicum* adult worms was applied to the detection of specific antibodies in sera of infected mice and mice immunized with the above protein antigen by ELISA technique. The 26-28 kDa antigen was better than crude antigens (SEA, SWAP) when used to detect specific antibodies in sera from immunized mice. As with crude antigens (SEA and SWAP), the 26-28 kDa antigen could be used to detect specific antibodies in infected sera, with titers as high as 1:160 - 1:320. There were no false positive reactions and a positivity rate as high as that using SWAP occurred when the 26-28 kDa antigen was used in schistosomiasis patients and normal subjects by intradermal test. It is suggested that the 26-28 kDa antigen may be a suitable candidate for immunodiagnosis of schistosomiasis.

INTRODUCTION

Recently, it has been suggested that Sj26 (p 28) target antigen extracted from schistosome adult worms has an important role in inducing protective immunity in the host (Capron *et al*, 1987; Balloul *et al*, 1987; Mitchell *et al*, 1984, 1985). The extraction of glutathione-S-transferase (GST) antigen from Chinese strain of *Schistosoma japonicum* was performed as we described previously (Liu *et al*, 1989a). As the extracted antigen is relatively pure, giving only two bands at 26 kDa and 28 kDa by SDS-PAGE, and has GST activity, we investigated the suitability of the 26-28 kDa antigen for immunodiagnosis of schistosomiasis. In this report, we have observed by ELISA using this antigen specific antibody in sera of mice immunized with schistosome antigens (26-28 kDa antigen or NTG-attenuated cercariae of *S. japonicum*) or infected with Chinese strain of *S. japonicum*, as well as *in vivo* response to 26-28 kDa antigen in patients with schistosomiasis japonica and normal subjects by intradermal test.

MATERIAL AND METHODS

Antigen

26-28 kDa antigen was obtained from adult worms by affinity chromatography with glutathione agarose column (Balloul *et al*, 1987; Smith *et al*, 1986; Liu *et al*, 1989a). The purity of this antigen was reliable as only two bands were seen with 26 kDa and 28 kDa antigen by 12.5% SDS-PAGE. It's protein concentration was 0.66 mg/ml. GST activity of this antigen was 96.77 IU/ml (Habig *et al*, 1974). Soluble egg antigen (SEA) and schistosome adult worm antigen preparation (SWAP) were extracted routinely. The protein concentrations were 4.7 mg/ml and 2.32 mg/ml, respectively.

Sera

(a) Sera from mice immunized with 26-28 kDa antigen : BALB/C mice were immunized with purified 26-28 kDa antigen in FCA at the dose of 50 μg antigen per mouse, boosted 3 and 4 weeks later with 10 μg of purified 26-28 kDa antigen alone *ip* and *iv* successively. Antisera to 26-28 kDa were collected every week postimmunization. (b) Sera from mice immunized with NTG-attenuated cercariae : C57BL/6 mice were infected percutaneously with 30 cercariae (Chinese strain of *S. japonicum*) attenuated by mutagen NTG (30 μg/ml for 30 minutes), and the antisera were collected from 4 weeks to 7 weeks later (Liu *et al*, 1989b). (c) Serum from infected mice were collected 6 weeks to 8 weeks postinfection from C57BL/6 mice exposed to 25 cercariae (Chinese strain) percutaneously. (d) Control sera were collected from the same batch of normal mice.

ELISA

Plates were coated with 26-28 kDa antigen, 1 μg per well and schistosome crude antigens such as SEA, SWAP at 1:500 dilution. Antisera from immunized or infected mice were diluted from 1:10 to 1:5,120. HRP-conjugated goat anti-mouse IgG antibody at 1:1,000 dilution was used. OD value determined spectrophotometrically at 492 nm was corrected against that of positive antiserum which served as a reference standard. OD 0.2 was taken as the threshold value of positive reaction.

Intradermal test

Two 0.5 cm diameter circlets were impressed on the anterior brachial skin of subjects. 26-28 kDa antigen or SWAP was injected intradermally until it filled in one circlet (about 0.03 ml each). 15 minutes later, the diameter of the papule was measured. A positive reaction was established when the diameter of the papule was larger than 0.8 cm, accompanied by redness and local itching.

RESULTS

ELISA

OD values of antisera from 30 control mice were all under 0.2, which was determined by ELISA with any one of 26-28 kDa, SEA and SWAP. The results of tests for specific antibody in sera of immunized mice by ELISA with 26-28 kDa antigen are shown in Table 1. In ELISA with 26-28 kDa antigen, the OD value of specific antibody in sera of immunized mice was much higher than that with SEA and similar to that with SWAP. For example, when immunized sera were diluted at 1:20, the average OD value was 1.93 ± 0.71 with 26-28 kDa antigen, which was 3.64 times higher than that with SEA and SWAP, and 2.95 times higher at a further dilution of 1:80 (Table 1). The OD value of antisera of mice infected with attenuated cercariae gave no significant difference in ELISA with 26-28 kDa antigen, SEA and SWAP. The average of OD values of antisera (1:20) at 4 weeks were respectively 0.36 ± 0.02, 0.26 ± 0.01 and 0.22 ± 0.01 in ELISA with 26-28 kDa antigen, SEA and SWAP; 0.67 ± 0.04, 0.66 ± 0.02 and 0.49 ± 0.03 at 6 weeks; 1.23 ± 0.30, 0.84 ± 0.01 and 0.68 ± 0.03 at 7 weeks, respectively. Other antisera were collected at 8 weeks from mice infected *pc* with 25 cercariae of Chinese strain of *Schistosoma japonicum*. The maximum dilution of these sera having positive reactions determined by ELISA with 26-28 kDa antigen was 1:160 (Fig 1). Comparatively, 1:5,120 with SEA and 1:160 titers with SWAP were observed. The mean value of OD of the infected sera in ELISA with 26-28 kDa antigen was 0.36 ± 0.03; with SEA, 0.75 ± 0.22; with SWAP, 0.54 ± 0.05.

Intradermal test

The reactions to 26-28 kDa antigen and SWAP in 30 normal subjects from non-endemic areas were all negative by intradermal test. The results of 49 chronic schistosomiasis patients with egg positive reactions in their stools were obtained. The positive rate of the intradermal test in patients to 26-28 kDa antigen was 93.9%, and 87.7% to SWAP. The coincidence rate was 89.8%, while the negative rate with both above antigens was 4.1%.

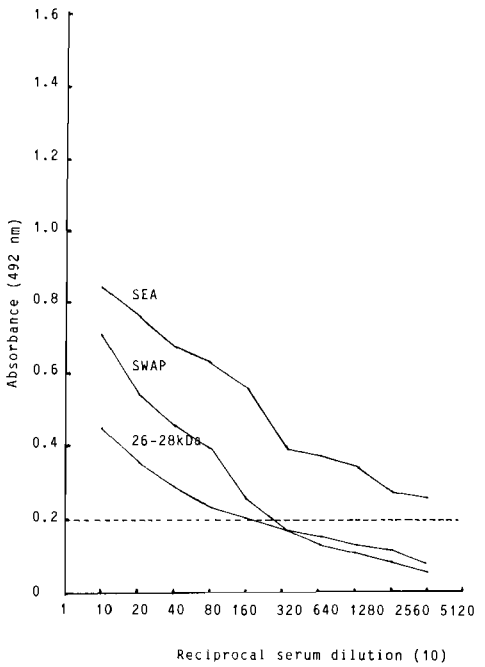

Fig 1—Antibody response in mice infected with Chinese strain of *S. japonicum* against crude antigens (SEA, SWAP), purified 26-28 kDa GST antigens from adult worms of *S. japonicum* by ELISA.

Table 1

Level of specific antibody from mice immunized with 26-28 kDa GST antigen by ELISA.

ELISA coating antigen	No. exp	No. mice immunized	Immunization	OD value of sera from 26-28 kDa GST-immunized mice in ELISA									
				1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120
26-28 kDa	I*	4	26-28 kDa + CFA	2.23± 0.83	1.93± 0.71	1.61± 0.57	1.21± 0.49	0.81± 0.29	0.60± 0.21	0.46± 0.10	0.35± 0.05	0.28± 0.03	0.26± 0.02
26-28 kDa	II*	3	26-28 kDa	0.85± 0.18	0.71± 0.14	0.50± 0.07	0.38± 0.08	0.29± 0.04	0.28± 0.03	0.25± 0.03	0.22± 0.02	0.21± 0.01	0.17± 0.001
SEA	I	4	26-28 kDa + CFA	0.51± 0.16	0.53± 0.23	0.48± 0.13	0.41± 0.09	0.46± 0.16	0.49± 0.16	0.49± 0.14	0.50± 0.16	0.46± 0.05	0.50± 0.01
SEA	II	4	26-28 kDa	0.50± 0.09	0.43± 0.11	0.39± 0.08	0.34± 0.04	0.30± 0.03	0.37± 0.08	0.29± 0.03	0.28± 0.03	0.23± 0.03	0.21± 0.04
SWAP	I	3	26-28 kDa + CFA	2.27± 1.29	2.01± 1.16	1.94± 1.15	1.39± 0.83	0.98± 0.59	0.86± 0.49	0.60± 0.34	0.58± 0.32	0.40± 0.03	0.27± 0.11
SWAP	II	4	26-28 kDa	0.64± 0.18	0.40± 0.11	0.33± 0.07	0.23± 0.05	0.18± 0.03	0.16± 0.03	0.16± 0.02	0.15± 0.02	0.13± 0.01	0.11± 0.01

I* 5-10 week sera after immunization with 26-28 kDa GST antigen + CFA
II** 6-9 week sera after immunization with 26-28 kDa GST antigen

DISCUSSION

The target 26-28 kDa antigen, found and purified in the process of developing a schistosome vaccine, is glutathione S-transferase, which is implicated in the induction of humoral immunity. Purified by affinity chromatography, the above antigen showed only two bands by SDS-PAGE. As the purity and antigenicity of 26-28 kDa antigen have been shown in a previous study, it encouraged us to investigate the posibility of 26-28 kDa antigen being used for immunodiagnosis of schistosomiasis. The preliminary results showed that: (a) No false positive reaction occurred in ELISA with 26-28 kDa antigen in sera from control mice. The OD value was below 0.2, just as occurred with SEA and SWAP did when used routinely in ELISA; (b) When the level of specific antibody in sera of mice immunized with 26-28 kDa antigen was tested, the OD value was 4 times higher in 26-28 kDa-ELISA than in SEA-ELISA and SWAP-ELISA but similar to that with SWAP; (c) The titer of specific antibody in infected sera of mice could reach 1:160 - 1:320 in ELISA with 26-28 kDa antigen. Although the OD value in ELISA was lower than that given by other antigens, 26-28 kDa antigen can be used with the same diagnostic effect as SEA and SWAP routinely did when infected sera were diluted not more than 1:160. However, the kind of specific antibodies detected in ELISA with 26-28 kDa antigen might not be the same as that detected with SEA and SWAP, and the meaning of positive reactions to 26-28 kDa antigen and other antigens might possibly be different. Whether the anti- 26-28 kDa antibodies existed in the infected sera can be investigated by ELISA with 26-28 kDa protein antigen, and the level of this specific antibody might be relevant to the host immunity to schistosome infection. SEA and SWAP, the soluble crude antigens extracted from schistosome eggs or adult worms with complicated antigenic components containing polysaccharide and glycoprotein have been used to test the antibodies to egg or adult worm. Further research on the differences in the role, type and effect of specific antibodies between those recognizing crude egg or worm antigens and purified 26-28 kDa protein antigen could be useful. (d) The intradermal tests with 26-28 kDa antigen were negative in normal subjects. The positive rate in patients with chronic schistosomiasis reached 86.9% and the coincidence rates with SEA and SWAP were respectively 89.8% and 91.3%. The above preliminary results showed that there is a possibility of using 26-28 kDa antigen for immunodiagnosis of schistosomiasis.

In the laboratory, this antigen can be used in detection of specific antibodies in sera of infected mice. The result was also good in intradermal tests with in chronic patients. In order to apply this antigen on a large scale for immunodiagnosis of schistosomiasis, further study should be carried out on cross-reactions between schistosome and other flukes, and the mechanism of change and regulation of antibody response to 26-28 kDa antigen in cured schistosomiasis patients. Furthermore, as it is well known that residents in endemic areas have some immunity to schistosome infection. There is a need to know how to assess the level of immunity, the relationship between immunity and the recurrence or epidemics of schistosomiasis, in relation to schistosomiasis control programs. Whether it is better to detect the level of protective antibodies (*eg* anti-26-28 kDa antibody) to reflect the protective level in the population in the endemic area rather than use 26-28 kDa antigen for immunodiagnosis of schistosomiasis is an important question for further in vestigation.

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REFERENCES

Balloul JM, Grzych JM, Pierce RJ, Capron A. A purified 28,000 dalton protein from *Schistosoma mansoni* worms protects rat and mice against experimental *S. mansoni*. *J Immunol* 1987; 138 : 3448-54.

Capron A, Pierce R, Balloul JM, *et al.* Protective antigens in experimental schistosomiasis. *Acta Tropica* 1987; 44 (Suppl 12) : 63-9.

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

Lanar DE, Pearce EJ, James SJ. Identification of paramyosin as Schistosome antigen recognized by intradermally vaccined mice. *Science* 1986; 234 : 593-9.

Liu SX, Ding LY, Song GC, Chen CY. Antigenicity of 24-26 kDa and 90 kDa 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 : 89-93.

Mitchell GF, Beall JA, Cruise KM, Tiu WU, Garcia EG. Antibody responses to the antigen Sj26 of *Schistosoma japonicum* worms that are recognized by genetically resistant 129/J mice. *Parasite Immunol* 1985; 7 : 165-78.

Mitchell GF, Cruise KM, Garcia EG, Tiu WU. Anti-worm antibody specificities in 129/J mice resistant to infection with *Schistosoma japonicum*. *J Parasitol* 1984; 70 : 983-5.

Smith DB, Davern KM, Board PG, Tiu WU, Garcia EG, Mitchell GF. Mr 26,000 antigen of *Schistosoma japonicum* recognized by resistant WEHI 129/J mice is a parasite glutathione S-transferase. *Proc Natl Acad Sci USA* 1986; 83 : 8703-7.