Brief Report

Vaccination of Goats with 31 kDa and 32 kDa *Schistosoma japonicum* Antigens by DNA Priming and Protein Boosting

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Two *Schistosoma japonicum* vaccine candidate antigens Sj 31 and Sj 32, which have shown particular promise to induce protective immunity in mice, were used to immunize goats by using a DNA priming-protein boosting strategy in present work. DNA vaccine formulations of the two antigens (VRSj31 and VRSj32) were produced and injected intramuscularly twice at a 2-week interval and then recombinant proteins (rSj31 and rSj32) together with Freund Complete Adjuvant (FCA) were used to boost the goats. The experiment was repeated in different batche cercariae. A strong anamnestic antibody response was induced after boost. A significant reduction of liver egg counts and miracidial hatching was showed in both experiments. Significant protections against challenge infection were elicited with 31.6% of percentage reduction for worm recovery in the second experiment and 20.9% in the first experiment, respectively. *Cellular & Molecular Immunology*. 2007;4(2):153-156.

Key Words: subunit vaccine, DNA vaccine, *Schistosoma japonicum*

Introduction

Although great effort, from which the public health benefits, has been done in the past more than five decades, *Schistosoma japonicum* (*S.japonicum*) continually causes 0.9 million people infected and 40 million at risk of infection in China (1). Furthermore, it seems likely that the present control strategy, repeating chemotherapy of all diagnosed and at-risk populations, failed to stop transmission of the infection. The most important reason of the difficulties to control this disease is that it is a zoonosis infecting not only man but also a wide rang of wild and domestic animals, in which, goat is considered as one of the important animal reservoirs of schistosome infection and is responsible for the contamination of the environment with schistosome eggs (2). Therefore, to search an effective vaccine and immunizating protocol to reduce schistosome infection in goats becomes very important.

To develop an vaccine against schistosomiasis, several vaccine candidate antigens have been tested, in which 31 and 32 kDa were considered promising candidate antigens because of their significant levels of protective immunity in mice as single components (3-5). Concomitant with the characterization and testing for protection efficacy of candidate vaccine antigens, many kinds of vaccination modes have been employed to explore alternative ways of presenting antigens (6-9).

In the present study, to develop a vaccine capable of preventing goat from infection, we administered the 31 and 32 kDa DNA vaccines to prime goat and then boosted with subunit vaccine.

Materials and Methods

Experimental animals and parasites

Thirty-six male, 6-10-month-old castrated goats were bred in the non-endemic Yuan Jia He area of Ningxiang County, Hunan Province. All goats had no history of exposure to fascioliasis and none of them was positive for Fasciola eggs by faecal examination. Goats were randomly divided into two groups by body weight, 24 goats were used as group 1 and the others were for group 2. In each group, *S.japonicum* cercariae used for animal infections were obtained from

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Oncomelania hupensis purchased from the Institute of Parasitic Diseases, Jiangsu, China.

DNA and subunit vaccine preparation

Using the method described by Zhang YY (10), the construction and purification of the plasmid DNA encoding the 31 kDa fragment of *S.japonicum* Cathepsin B (Sj31) or 32 kDa (Sj32) were perform. Briefly, full-length gene for Sj32 and partial-length gene for Sj31 (308-755 bp) were amplified with gene specific primers carrying *Sal* I and *Xba* I restriction site, then cloned into the VR1012 vector after digestion with restriction enzymes. Plasmids VRSj31 and VRSj32 were confirmed by sequence analysis and then purified using the Qiagen Plasmid DNA purification Kit as described in the manufacturer's protocol.

The partial-length gene coding Sj31 or full-length gene for Sj32 was amplified by PCR and cloned into pGEX-5X-3 vector (Amershampharmacia Biotech). The reason for using just a portion of the gene of Sj31 is to achieve high level expression of soluble recombinant protein. The recombinant rSj31 or rSj32 fused with *S.japonicum* glutathione S-transferase (GST) was expressed in *E. coli* (ER2566) and purified from the bacterial proteins by solubilising in urea.

DNA and protein immunization protocol

In vaccinated groups, goats were primed intramuscularly in the haunch with 300 μg VRSj31 and 300 μg VRSj32 twice at 2 week-intervals and then boosted subcuteneously with 300 μg rSj31 and 300 μg rSj32 coadministrated with Freund Complete Adjuvant (FCA) at three separate points in the haunch 4 weeks after the second immunization. Goats in control group were primed 600 μg of vector plasmid DNA twice and boosted with FCA.

Parasitological methods

Twenty-eight days after the third immunization, cover slip method was used to experimentally infect goats with 300 cercariae. A different batch of cercariae was used for the second experiment (11). Forty-two days after challenge, the method of Shi FH, et al. was employed to perfuse goats. Briefly, the dorsal aorta was clamped anteriorly, and the worms recovered from the severed portal vein into a sieve under pressure of tapwater infused firstly into the thoracicaorta and then into the inferior vena cava (12). For the miracidial hatching test, 5 g of faeces from the rectum was filtered through nylon sieves of 180 and 300 meshes, then the sediment was placed in a 100 ml flask and water was added to the brim. After 2 h incubation at 25°C, 50 ml of surface water was withdrawn, a drop of iodine added and all the miracidia in the sediment counted after centrifugation. For liver egg counting, five small samples taken from different parts were mixed and 5 g of them was digested in 100 ml of 5% KOH at 37°C for 2 h, then 100 μl aliquots were counted for eggs.

Immunohistochemistry of expression of Sj31or Sj32 in vivo

Muscle samples were taken from the immunizated site 28 days after the second prime and were fixed in 10% formalin after being sheared. The samples were embeded in wax and sectioned. Following washed with PBS, samples were blocked with 1% bovine serum albumin (BSA) for 30 min, and then incubated with immunosera of Sj31 or Sj32 from mice. HRP-conjugated rabbit anti-mouse IgG (Sigma) was used as secondary antibody. The antigens expressed were visualized by addition of diamino-bedzidine (DAB).

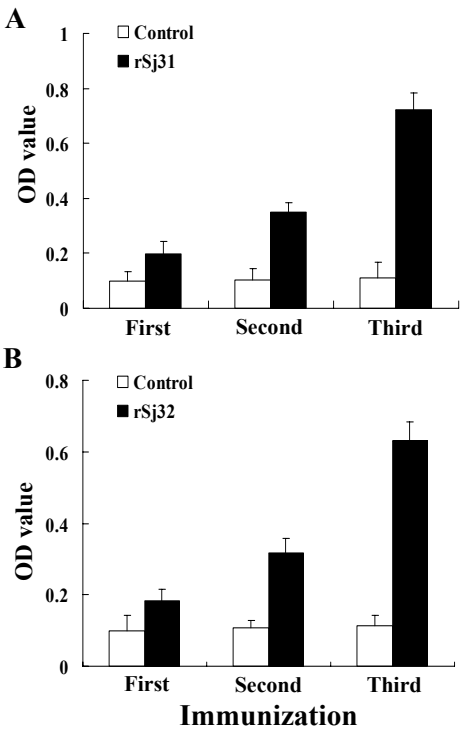

Figure 1. Specific antibodies were induced in goat sera after priming and boosting. Goat sera were collected after each immunization. Serum levels of anti-Sj31 (A) or anti-Sj32 (B) immunoglobulines were detected by ELISA. The data were shown as mean OD value ± SD.

Detection of specific antibodies by ELISA

Sera were collected after each immunization and challenge and stored at -20°C until further use. To detect anti-Sj31 or anti-Sj32 immunoglobulins, plates were coated overnight at 4°C with 100 μl of a 10 μg/ml solution of rSj31 or rSj32 diluted in 0.1 mol/L $NaHCO_3$ (pH 9.6) and then blocked for 2 h at 37°C in blocking buffer (5% skimmed milk). After three washes with PBST (0.05% Tween-20 in PBS), sera were diluted 1:100 in PBS and incubated overnight at 4°C. After washed four times with PBST, the plates were incubated with peroxidase-conjugated rabbit anti-goat IgG (H + L) (KPL, USA) for 1 h at 37°C at a 1:2,000 dilution. After four washes, specific IgG were visualized by the addition of 3,3,5,5-tetramethylbenzidine (TMB). The absorbance was determined at 630 nm. Each serum sample was tested in duplicate.

Statistical analysis

Data analyses were performed with the Statistical Package

Table 1. Worm recoveries, faeces and tissue egg counts

	Vaccinated	Control	%R[a]	p[b]
Group 1				
Worm recovery	128.50 ± 16.60	162.40 ± 48.01	20.9	0.086
LEPG[c]	1418.75 ± 332.15	2704.80 ± 661.78	47.5	0.001
Miradidium count[d]	15.00 ± 13.92	32.80 ± 12.59	54.3	0.01
Group 2				
Worm recovery	131.91 ± 45.39	192.78 ± 39.57	31.6	0.005
LEPG[c]	1646.64 ± 667.09	3410.11 ± 1029.57	51.7	0.001
Miradidium count[d]	15.69 ± 9.27	30.20 ± 12.52	48.3	0.04

[a]percentage reduction compared with control group.
[b]p value in One-ANOVA test compared with control group.
[c]liver eggs per gram.
[d]miradidia per gram faeces.

for the Social Science (SPSS, version 8.0). Data were expressed as mean ± SD. A p value of < 0.05 was considered statistically significant.

Results

Sj31 and Sj32 were expressed in muscle

Ability of VRSj31 and VRSj32 plasmid expressing Sj31 or Sj32 was evaluated after intramuscular injection. Sj31 or Sj32-specific immunohistochemistry showed cytoplasmic expression of Sj31 or Sj32 whereas no signal was observed in samples of control group vaccinated with vector VR1012 (data not shown).

A significant antibody response was induced after priming and boosting

Anti-Sj31 and anti-Sj32 IgG responses were shown by ELISA. Four weeks after the second immunization, goats in vaccinated group generated a significant antibody response in sera as compared to goats in control group. Secondary IgG response was detected 4 weeks after boosting with a single subcutaneous injection of rSj31 and rSj32. A very strong anamnestic response was observed (Figure 1).

Vaccination could induce a significant reduction in faecal miradidium and LEPG

Six weeks post-challenge, all goats were perfused, faecal egg (ceracidial hatching test) and tissue egg counts (liver eggs per gram, LEPG) were carried out (Table 1, group 1). There are significant reductions in faecal miradidium (54.3%) and LEPG (47.5%), when compared to control group. In worm burden there is a 20.9% reduction, however, there was no statistical significance.

To confirm the promise protection illustrated, another experiment was carried out in which group goats were vaccinated using a same vaccination protocol as used in group 1, only different batche cercariae was used. The worm recoveries, tissue and faecal egg counts were carried out and shown in Table 1. A significant reduction of liver egg counts and miracidial hatching was observed in this experiment and a significant level of protection against challenge infection (31.6%) was elicited.

Discussion

For *S.japonicum* immunoprophylaxis, little progress has been made, and the vaccines so far tested having failed to induce more protection against challenge in large scale trials than the goal of WHO. The reason is mainly that the more effective antigen has not been discovered and the mechanisms of immunity remain unclear. At present, the global trend of schistosome vaccinology is directed towards defined antigen vaccines. In the present work, the effects of vaccination of goat with 31 and 32 kDa which have shown partial protective efficacy against S.*japonicum* in mice (3-5, 9) were studied using a priming-boosting immunization strategy which can induce both cellular immunity and humoral immunity. The protective effects were evidenced as reductions in the mean worm and/or egg counts in vaccinated group goats as compared to goats in control. Two experiments were carried out using the same immunization protocol. In the group 1, goats showed significant reduction (47.5%) in liver egg count, 54.3% of miracidia in faecal hatching test and a reduction of 20.9% in worm burden. In the group 2, similar reductions in liver egg count (51.7%) and faecal hatching test (48.3%) were observed, but there was a higher reduction (31.6%) in worm burden which was significant as compared to that of in the the group 1. This may be due to the different batch of cercariae used for the challenge infection in the group 2, in which the worms recovered from the control goats were more. Many *S.japonicum* vaccine candidates previously shown to have vaccine potential in laboratory and/or field experiments in domestic animals (12-15), 31 and 32 kDa were also shown also to be capable of eliciting partial protection against experimental challenge in goat.

DNA vaccination has been an increasingly interesting approach for vaccination because the injected plasmid can directly transfect macrophages and dendritic cells and induce both humoral and cellular immunity (16, 17), but levels of DNA-raised Abs were found to be far lower in comparision

with that of protein-raised (18). In the current study, attempts were made to enhance the levels of Ab response to plasmids encoding antigens. Firstly, DNA vaccine was used to prime twice and then the recombinant subunit vaccine was used to boost. As shown in Figure 1, a strong anamnestic Ab response was induced after subcutaneous boosting. The massive increase of antibody titer following DNA priming might be attributed to a recall of B-cell memory. Some results (8, 19, 20) had shown that DNA priming was more powerful than protein priming in inducing memory Ab response. This indicated that this vaccination protocol seems to be the most relevant one in inducing immunological memory in different models. Usually, DNA immunization is associated to a Th1- biased response with IgG2a isotype predominance whereas protein immunization leads to a specific Th2 response with IgG1 isotype predominance (21), but in some models (8), both protein and DNA priming induced predominantly one kind of Ab response due to the route of immunization and the antigen used. Which response biased in our model is now under trial.

In conclusion, our study confirmed the vaccine potential of 31 kDa and 32 kDa in goats using priming-boosting immunization protocol. This encouraging result suggests that development of vaccines capable of preventing domestic animals from being infected to stop transmission of the infection is feasible.

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