

# PROTECTIVE IMMUNITY INDUCED WITH 23 kDa MEMBRANE PROTEIN DNA VACCINE OF *SCHISTOSOMA JAPONICUM* CHINESE STRAIN IN INFECTED C57BL/6 MICE

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**Abstract.** A 23 kDa membrane protein DNA vaccine for *Schistosoma japonicum* Chinese strain was developed and tested for its protective efficacy and immune responses in infected C57BL/6 mice. The cDNA encoding SjC23 amplified from pUC19-SjC23 were subcloned into an eukaryotic expression vector (pcDNA3.1). Forty-eight female C57BL/6 mice were divided into three groups. Each mouse of group A (control group) was immunized intramuscularly (i.m.) with 100 µg of pcDNA3.1; of group B (SjC23 group) was immunized (i.m.) with 100 µg of pcDNA3.1- SjC23; of group C (SjC23+IL-12) was immunized (i.m.) with a mixture of 100 µg of pcDNA3.1- SjC23, 100 µg of pcDNA3.1- p35 and 100 µg of pcDNA- p40. These were followed by two boosts of the same DNA once every two weeks. All mice were challenged with 45 cercariae of *Schistosoma japonicum* Chinese strain at week 8, and were killed and perfused at week 14. The numbers of recovered worms and hepatic eggs were counted. The expression of SjC23 and p35, p40 in muscle tissue was determined by immunohistochemical method. By culture of spleen cells, the production of IL-2, IL-4, IL-10 and IFN-γ with the stimulation of specific antigen of the recombinant hydrophilic domain of SjC23 (rSjC23-HD) was determined after the last immunization (before challenge). Sera were collected from each group before immunization and two weeks before and after challenge. Anti-SjC23 antibodies were tested by Western blot. The results showed that SjC23 and p35, p40 of mouse IL-12 were expressed on the membrane and in the plasma of the muscle cells of immunized C57BL/6 mice. A rise of IL-2 and IFN-γ in the SjC23 group and SjC23+IL-12 group was observed; No changes were found in IL-4 and IL-10. Detection of anti-SjC23 antibody with Western blot showed that after the third immunization (before challenge) all the serum samples from the control group were negative; 8 of 10 sera from the SjC23 group and 9 of 10 sera from the SjC23+IL-12 group were positive. The worm reduction rates in the SjC23 group and SjC23 +IL-12 group were 26.9% and 35.4% respectively; the liver eggs reduction rates were 22.2% and 28.4%, respectively in comparison to the control group. This indicates that the pcDNA3.1-SjC23 DNA vaccine can induce partial protection against *Schistosoma japonicum* infection in C57BL/6 mice.

## INTRODUCTION

Schistosomiasis is a widespread disease with a major public health problems in endemic countries. More than 600 million people are at risk with about 200 million actually infected in 74 countries in Africa, the Middle East, South America and Southeast Asia (Chitsulo *et al*, 2000). China is an epidemic region of schistosomiasis japonica. There are 108 counties and 57 farms at the county level with schistosomiasis endemic in China, and 0.82 million people with the

disease (Chen *et al*, 2002). Since the floods along the Yangzi River and the government policy for protecting against floods, "Return farmland to lake", for the control of floods, the disease will tend to become more serious (Department of Diseases Control, 2001). For past several decades with the integrated strategy of mass chemotherapy and snail eradication in high-risk areas for control of the disease, great achievements have been made. Reinfection occurred rapidly after the mass chemotherapy was stopped. Large scale, repeated and long term mass chemotherapy is not only cost ineffective but drug resistance to praziquantel is a potential risk. A schistosomiasis vaccine is needed as a long-term complimentary measure to schistosomiasis control (McManus, 2000). The

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Sm23 protein could induce up to a 40-50% protective effect and is one of six candidates recommended by WHO (Bergquist and Colley, 1998). The 23 kDa membrane protein is a potential vaccine candidate for *Schistosoma japonicum* infection (Shi *et al*, 1998). It has been reported that recombinant hydrophilic domain of Sjc23 (rSjc23-HD) can induce 59% worm reduction rate in infected sheep. The DNA vaccine is a novel vaccine, has more advantages, it can induce both cellular (including CTL cytotoxic activity) and humoral immune responses, is easy to prepare and inexpensive. The DNA vaccine has been studied in some infectious diseases including parasite infection (Ramsay *et al*, 1997). In this study, the gene of Sjc23 was cloned into plasmid pcDNA3.1 as a DNA vaccine. The immune protective effect and immune response of the DNA vaccine was tested in infected C57BL/6 mice.

## MATERIALS AND METHODS

### Construction of pcDNA3.1-Sjc23

Based on the published gene sequence of Sjc23 by Zhu *et al* (1997), a pair of primers (p1, p2) were designed. P1: 5'-GCGGATCCATGGCGACTTTGGGTACTG-3', P2: 5'-GCCTCGAGTTAAACATTCTGATAATCG-3', synthesized by the Shanghai Sangon Co. The restriction endonuclease sites of BamH1 and Xho1 (Promega) were designed at the 5' end of p1 and p2 respectively. The full length cDNA of Sjc23 was cloned from pUC19-Sjc23 with PCR and digested with BamH1 and Xho1 and subcloned into an eukaryotic expression vector pcDNA3.1 plasmid to generate the recombinant plasmid pcDNA3.1-Sjc23. The recombinant plasmid was transformed into the *E. coli* strain XL1-blue, and was verified by restriction digest and sequencing.

### Large-scale preparation of DNA vaccines

DNA vaccines of pcDNA3.1-Sjc23, pcDNA3.1-p35, pcDNA3.1-p40 and the control of pcDNA3.1 DNA plasmids for intramuscularly injection were prepared in large scale according to the manufacturer's instruction using Qiagen-Mega -2500 kits (Qiagen, Germany). The purification and concentration were determined and redissolved in 0.9% NaCl at a final concentration of 1 mg/ml.

### Animal experiments

Forty-eight female, 5~6 weeks old C57BL/

6 mice (purchased from the Shanghai Center for Experimental Animal) were divided into A, B, and C groups. Each mouse of group A (control group) was immunized into both quadriceps femoris muscles by intramuscularly injection (i.m.) with 100 µg of pcDNA3.1; of group B (Sjc23 group) was immunized with 100 µg of pcDNA3.1-Sjc23; of group C (Sjc23+IL-12) was immunized with a mixture of 100 µg of pcDNA3.1-Sjc23, 100 µg of pcDNA3.1-p35 and 100 µg of pcDNA-p40 DNA. Followed by two boosts with the same dosage at two weeks intervals. All the mice were challenged with 45 cercariae of *Schistosoma japonicum* Chinese strain at week 8, and were then sacrificed and perfused at week 14. The numbers of recovered adult worms and hepatic eggs were counted. The worm reduction rate and the egg reduction rates were calculated with the following formula:

The worm reduction rate (%) is the mean number of adult worms per mouse in the control group minus the mean number of adult worms per mouse in the experimental group divided by the mean number of adult worms in the control group;

The egg reduction rate (%) is the mean number of eggs per mouse in the control group minus the mean number of eggs per mouse in the experimental group divided by mean number of eggs per mouse in the control group.

### Expression of Sjc23 and the p35, and p40 subunits of IL-12 in the muscle tissue of mice

At ten days after the first immunization, two mice of every group were boosted again with the same doses and same methods. Four days later, the mice were sacrificed and the frozen slices of the immunized muscle tissue of the mice were prepared. An immunohistochemical method was used to detect the expression of Sjc23 and the subunit of IL-12 in the muscle tissue of mice. For detection of Sjc23, heavily infected rabbit serum was added and used as the first antibody, incubated at 4°C overnight, and goat-anti-rabbit IgG-HRP as the second antibody, displaying the color with the DAB, and observing the results with a microscope. For detection of p35 and p40, goat anti-p35 antibody and goat anti-p40 antibody (Santa Cruz) were added and used as the first antibody, incubated at 4°C overnight, using the anti-ABC system (Santa Cruz) to give a response

and display color, observing the results with a microscope.

### Cytokine detection

Spleens were collected from 2 experimental mice of each group at two weeks after the last immunization (before challenge). A single cell suspension was made and adjusted to a concentration of  $6 \times 10^6$  cells/ml. One hundred microliters of the cell suspension of  $6 \times 10^5$  cells was added to each well of a 96 wells flat-bottom plate. One hundred microliters of recombinant hydrophilic domain of Sjc23 (rSjc23-HD) was added to wells in triplicate at 20  $\mu$ g/ml, making the final protein concentration of 10  $\mu$ g/ml. One hundred microliters of 10  $\mu$ g ConA was added and used as a positive control and 100  $\mu$ l of 10% FCS RPMI 1640 as a negative control. The cells were incubated at 37°C in 5% CO<sub>2</sub> for 72 hours. Over the incubation, the supernatants were collected. The concentration of cytokine IL-2, IL-4, IL-10 and IFN- $\gamma$  were determined using a sandwich ELISA kit (Endogen) according to the manufacture's instructions for the kit. Results were expressed in pg/ml by reference standard recombinant mouse IL-2, IL-4, IL-10 and IFN- $\gamma$ .

### Antibody detection

Two weeks after immunization, 10 sera from every group, and two weeks after challenge 5 sera from every group were used to detect anti- Sjc23 antibodies by Western blot.

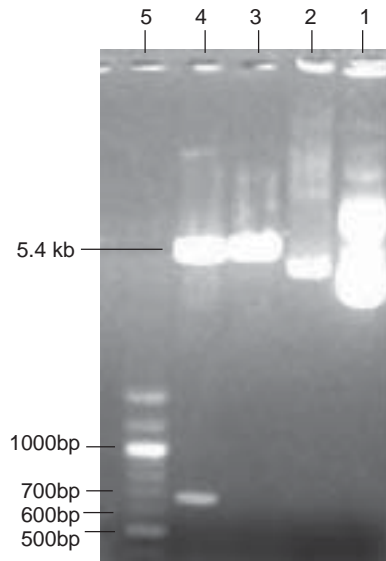
### Statistical analysis

The Student's *t*-test was used in the study. Values of  $p < 0.05$  were considered to be significant.

## RESULTS

### Determination of pcDNA3.1-Sjc23 and preparation of DNA vaccines

The recombinant plasmid pcDNA3.1-Sjc23 was digested with the restriction endonucleases of BamH1 and Xho1, and 1% agarose gel electrophoresis was run. The products consisted of the predicted length (Fig 1). The gene sequencing further verified the construction of the recombinant plasmid—pcDNA3.1-Sjc23 was correct. pcDNA3.1-Sjc23, pcDNA3.1-p35, pcDNA 3.1-p40 and pcDNA3.1 DNA plasmids were prepared in large scale according to the manufacture's instruction using Qiagen-Mega –2500 kits (Qiagen,



Lane 1: pcDNA3.1 plasmid, lane 2: recombinant pcDNA3.1-Sjc23 plasmid, lane 3: restriction analysis of pcDNA3.1, lane 4: restriction analysis of pcDNA3.1-Sjc23, lane 5: DNA standard markers.

Fig 1—Restriction enzyme analysis of recombinant plasmid pcDNA3.1-Sjc23.

Germany). The OD 260/280 value of the DNA vaccines prepared was between 1.8 and 2.0.

### Expression of Sjc23 and p35 and p40 subunits of IL-12 in the muscle tissue of mice

For detection of expression of Sjc23, the specific brown stain on the membrane and in the plasma of quadriceps femoris muscle cells in the Sjc23 group and Sjc23+IL-12 group could be found, while in pcDNA3.1 group could not be found.

For detection of expression of the p35 and p40, the specific brown stain on the membrane and in the plasma of quadriceps femoris muscle cells in Sjc23+IL-12 group could be found, while in the pcDNA3.1 group and Sjc23 group could not be found.

### Cytokine detection

Stimulated by HD (rSjc23-HD) the Th2-associated cytokines IL-4 and IL-10 showed no differences among the three groups, but in the Sjc23 and Sjc23+IL-12 group compared with those in pcDNA3.1 group as shown in Table 1, the obvious rising of Th1-associated cytokine IL-2 and IFN- $\gamma$  was detected.

### Antibody detection

After the third immunization (before challenge) all the serum samples from the control group were negative; 8 of 10 sera from the SjC23 group and 9 of 10 sera from the SjC23+IL-12 group were positive. After challenge, all the sera from the three groups were positive.

### Immune protective efficacy

For the recovered worms and eggs see Table 2.

By Student *t*-test analysis, the worm reduction rates of the SjC23 and SjC23 group plus IL-12 group were significantly higher than that in the control group ( $p < 0.01$ ), the egg reduction rates were also significantly higher than in the control group ( $p < 0.01$  or  $p < 0.05$ ). The worm reduction rates of SjC23 plus IL-12 group were also significantly higher than in the SjC23 group ( $p < 0.05$ ).

## DISCUSSION

The 23kDa surface antigen protein of the schistosome, expressed during all stages of its lifecycle, is a member of the transmembrane-4-superfamily (TM4SF) of proteins, characterized by four hydrophobic membrane-spanning domains and two extracellular hydrophilic domains (Harn *et al*, 1985; Reynolds *et al*, 1992). The large

extracellular hydrophilic domains of Sm23 have been shown to contain several B cell and T cell epitopes and to be highly immunogenic (Reynolds *et al*, 1992). A study with *Schistosoma japonicum* has shown that there are similarities between SjC23 and Sm23 (Reynolds *et al*, 1992). It has been reported that Sj23 is a potential vaccine candidate for schistosomiasis japonica. The DNA vaccine has more advantages than the subunit peptide vaccine. Waine *et al* (1999) reported that the gene of the 23 kDa membrane protein for *Schistosoma japonicum* Philippines strains was cloned into the VR 1022 plasmid to be used as a DNA vaccine, and the anti-Sj23 antibody could be detected in the immunized mice after the vaccine, but no protective effects could be seen. Li and Shi (1999) reported that a 33% reduction in the worm rate could be observed in mice immunized with the Sj23 DNA vaccine.

In this study, the gene of 23 kDa membrane protein for *Schistosoma japonicum* Chinese strain was cloned into an eukaryotic expression vector pcDNA3.1 as a DNA vaccine. A 26.9% worm reduction rate could be induced in infected C57BL/6 mice by immunization with SjC23 DNA vaccine. Co-immunization with the pcDNA3.1-p35 and pcDNA3.1-p40 plasmid DNA (IL-12) could significantly improve the worm reduction

Table 1  
The detection results for IL-2 and IFN- $\gamma$  after immunization.

Groups	IL-2 pg/ml		IFN- $\gamma$ pg/ml	
	ConA	HD <sup>a</sup>	ConA	HD
Control group	0	2.1	7,265.9	1,028.7
SjC23 group	16.1	34.8	2,403.0	1,451.6
SjC23+IL12 group	46.5	65.2	5,692.0	2,191.6

<sup>a</sup>HD: recombinant hydrophilic domain of SjC23 (rSjC23-HD).

Table 2  
Protection of SjC23 DNA vaccines in C57BL/6 mice.

Groups	No. of mice	Mean no. of adult worms	Worm reduction rate (%)	p value	Mean no. of eggs per mouse	Egg reduction rate (%)	p value
Control group	10	30.8 $\pm$ 5.9	-	-	157,333 $\pm$ 31,480	-	-
SjC23 group	6 <sup>a</sup>	22.5 $\pm$ 1.9	26.9	<0.01	122,445 $\pm$ 30,085	22.2	<0.05
SjC23+IL12 group	10	19.9 $\pm$ 1.3	35.4	<0.01	112,700 $\pm$ 33,027	28.4	<0.01

<sup>a</sup>Part of mice death among the test.

rate to 35.4% ( $p < 0.05$ ). This indicates that the SjC23 DNA vaccine can induce a partial protective effect. Co-immunization with IL-12 as an adjuvant in DNA vaccinations seem to increase the protective efficacy, it may stimulate NK cells to secrete IFN- $\gamma$ , and stimulate Th0 shift to Th1, and activate CTL cytotoxicity activity (Scott and Trinchierir, 1997). The results of cytokine detection showed that IL-2 and IFN- $\gamma$  in the SjC23 and SjC23+IL-12 group were significantly increased compared with the pcDNA3.1 group after immunization, but IL-4 and IL-10 levels were not changed by immunization. IL-2 and IFN- $\gamma$  were secreted by CD4<sup>+</sup>Th1 with stimulation of special antigens. The results in this study indicated that pcDNA3.1-SjC23 DNA vaccine induced a Th1 T cell response in infected C57BL/6 mice.

The results of antibody detection showed that although the antibody response in most of the vaccinated mice in the SjC23 and SjC23 +IL-12 group Western; could be detected by Western blot technique, it was not strong since they were negative by ELISA technique.

The study indicates that the SjC23 DNA vaccine can induce partial anti-infection protective effects. Probably T cell immunity plays a major role in the protective immunity of the SjC DNA vaccine.

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