

Vaccination against *Schistosoma japonicum* Infection by DNA Vaccine Encoding Sj22.7 Antigen

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Abstract To observe the *in vitro* expression of DNA vaccine pcDNA3-Sj22.7 and its immunological effect in mice, the recombinant plasmid pcDNA3-Sj22.7 was used to transfect HeLa cells with liposome-mediated method and the expression of Sj22.7 mRNA and protein was examined using reverse transcription-polymerase chain reaction, sodium dodecylsulfate-polyacrylamide gel electrophoresis and Western blot. Then, the ability of pcDNA3-Sj22.7 to protect against *Schistosoma japonicum* challenge infections was analyzed according to worm reduction rate and egg reduction rate after vaccination of mice. The serum levels of specific IgG antibody and T lymphocyte proliferation response were also determined. After the challenge infection, Sj22.7-driven interferon (IFN)- γ and interleukin (IL)-4 was also quantified. Results showed that pcDNA3-Sj22.7 could express Sj22.7 mRNA and protein *in vitro*. Immunization resulted in a worm reduction rate of 29.70%, egg reduction rate of 47.25% (liver) and 51.73% (intestine), and egg reduction rate of 25.90% (eggs per female), suggesting induction of significant anti-fecundity in the pcDNA3-Sj22.7 group. Enzyme-linked immunosorbent assay and Western blot analysis indicated that immunized mice generated specific IgG against Sj22.7. T lymphocytes from mice immunized with pcDNA3-Sj22.7 showed a significant proliferation response to rSj22.7. The culture of spleen cells showed that secretion of IFN- γ increased but IL-4 decreased. The results indicate that DNA vaccination by pcDNA3-Sj22.7 is sufficient to elicit significant levels of protective immunity against *S. japonicum* infection. The DNA vaccine could induce significant cellular and humoral immune response, and display predominant T helper cell type 1 type immune responses, which contribute to the protective immunity against challenge infection in mice.

Key words *Schistosoma japonicum*; DNA vaccine; Sj22.7; gene expression; immune protection; immune response

Schistosomiasis is, after malaria, the second most important parasitic disease in tropic areas. It affects more than 200 million people and causes more than 500,000 deaths each year [1–4]. A vaccine against this parasitic

infection is desirable to prevent infection. Vaccination can be targeted towards either the prevention of infection or the reduction of parasite fecundity. A reduction in worm numbers is the “gold standard” for anti-schistosome vaccine development. However, as schistosome eggs are responsible for both pathology and transmission, a vaccine targeted on parasite fecundity and egg viability seems to be entirely relevant. Recently, a significant effort has been made to develop a protective vaccine against schistosome infections, and several vaccine candidates and related studies have been identified [5–7]. As the efficacy of any

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of these vaccines against schistosomiasis remains uncertain, the identification and characterization of new anti-schistosome vaccine molecules remains a priority. The development of vaccine remains to be an important long term and challenging goal in the control of schistosomiasis [8].

DNA vaccine is an attractive and novel immunization strategy against a wide range of infectious diseases and tumors. Injection of plasmid DNA as vaccine was first shown to be effective using influenza as a model, where the results showed that DNA encoding nucleoprotein induced cytotoxic T lymphocytes and cross-strain protection of mice [9]. The effectiveness of DNA vaccines against viruses, parasites and cancer cells has been shown in animal models [10]. It has been shown that DNA immunization induces both antigen-specific cellular and humoral immune responses [11]. Nucleic acid vaccination against schistosomiasis has lately been investigated using a panel of plasmids encoding schistosome antigenic proteins such as Sjc26GST, Sj79 [12,13], *Schistosoma japonicum* paramyosin [5,14] and *Schistosoma mansoni* 23, 28 GST [15].

In the previous study, we identified a gene encoding a pairing-associated protein identical to the Sj22.7 adult worm antigen using two-dimensional gel electrophoresis and mass spectrometry [16]. Several investigators have suggested that the 22.7 kDa-antigens of *S. japonicum* adult worms are important vaccine candidates [17]. However, no direct vaccination/challenge experiments using Sj22.7 had been previously described.

Herein, we produced constructed Sj22.7 DNA vaccine pcDNA3-Sj22.7 and tested its vaccination strategies in mice.

Materials and Methods

Parasites and animals

The snails used for schistosome infection were obtained from Hunan Provincial Institute of Schistosomiasis Research (Yueyang, China). Cercariae were collected from exposing infected snails. Female Kunming mice (18–20 g, 6–8 weeks old) were purchased from the Department of Zoology, Xiangya School of Medicine, Central South University (Changsha, China). All animals were maintained in our animal facilities for the duration of the experiments. Animal experiments were performed according to the protocols approved by Central of Animal Health of Xiangya Animal Care and Use Committee.

Construction and preparation of DNA vaccines pcDNA3-Sj22.7

The cDNA containing the entire coding region of Sj22.7 gene (GenBank accession No. AY815219) was isolated from schistosomula cDNA library. Briefly, a pair of primers was synthesized according to the DNA sequence of Sj22.7. The forward primer was P1, 5'-GCGGTACCACTAAC-ATGGGAGAAGAGA-3' and the reverse primer P2, 5'-ACGGGCCCCCTAAATGTCCTGATTACCTC-3' containing *Kpn*I and *Apa*I restriction sites (italicized), respectively.

The target gene was subcloned into the eukaryotic expression vector pcDNA3 (Invitrogen, Carlsbad, USA). This vector containing a strong human cytomegalovirus promoter, an ampicillin-resistance gene and a neomycin-resistance gene was used as a DNA vaccine vector. The Sj22.7 was amplified using a Hema 480 thermal cycler in a total volume of 100 μ l mixture with 2 μ M of each primer (P1 and P2), 0.2 μ g of cDNA library of *S. japonicum* adult worm as template, 0.2 mM of deoxyribonucleotide triphosphate (TaKaRa, Tokyo, Japan) and 1 U of *Taq* polymerase (TaKaRa). The PCR was performed first by 95 °C for 3 min; then 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min; and finally 72 °C for 8 min. The *Kpn*I/*Apa*I fragment (622 bp) was purified using Agar gel DNA purification kit (TaKaRa) and then ligated to the pcDNA3 vector to obtain recombinant plasmid pcDNA3-Sj22.7. The recombinant plasmid was transformed into *Escherichia coli* DH5 α competent cells and identified by restriction enzymes digestion, PCR and sequencing.

In vitro transient transfection of HeLa cells

To estimate the efficacy of recombinant plasmid pcDNA-Sj22.7 to produce the 22.7 kDa-protein in mammalian cells, the recombinant plasmid was transfected into HeLa cells. Briefly, HeLa cells were seeded into 24-well tissue culture plates at 1.5×10^5 cells per well and grown to 90% confluence in DMEM (Gibco, Rockville, USA) supplemented with 100 U/ml penicillin, 100 μ g/ml streptomycin (Amresco, Solon, USA) and 10% (V/V) heat inactivated fetal calf serum (FCS) (Hyclone, Logan, USA) at 37 °C in 5% CO₂. Then, the cells were transfected with the plasmid DNA with Lipofectamine 2000 (Invitrogen) at the ratio of 1 μ g DNA:2 μ l lipid per well in serum-free DMEM at 37 °C for 12 h. Cells were then grown for 48 h in DMEM containing FCS. The mRNA and protein levels of Sj22.7 were determined by RT-PCR and indirect immunofluorescence microscopy, respectively. Briefly, the slides with transfected HeLa cells were incubated with

rabbit anti rSj22.7 serum (1:100, prepared in our lab) and then with goat anti-rabbit IgG conjugated to fluorescein isothiocyanate (FITC) (1:10, Boster, Beijing, China). After washing, slides were examined under a fluorescent microscope (Olympus, Tokyo, Japan). When yellow green fluorescence appeared in the transfected cells, the reaction was defined as indirect fluorescent antibody test (IFAT) positive. HeLa cells transfected with the empty plasmid pcDNA3 and sera from normal mice were used as negative controls, respectively.

DNA vaccination

The mice were divided randomly into three groups, and each group consisted of 25 mice. Mice were immunized by intramuscular injection with one kind of the following regimens in 100 μ l of sterilized normal saline: (1) 100 μ g of pcDNA3-Sj22.7, (2) 100 μ g of pcDNA3, or (3) control with no plasmid DNA. The mice in the third group served as a challenge infection control. Twenty-four hours after being injected with 50 μ l of 50 mg/ml bupivacaine hydrochloride in the quadriceps femoris muscles (hind legs), mice were inoculated intramuscularly with 100 μ g of empty plasmid pcDNA3 or recombinant plasmid pcDNA3-Sj22.7 in the same area. Mice were immunized 3 times (2 weeks interval).

Expression of the DNA vaccine in musculature of immunized mice

Two weeks after the last immunization, two mice from each group were killed and parts of the liver tissues were immediately fixed in 10% buffered formalin solution and processed in paraffin blocks. The sections of 5 μ m were cut on albuminized glass slides. Expression of Sj22.7 *in vivo* was examined by indirect immunofluorescence. Sections were stained by immunofluorescence method using rabbit anti-rSj22.7 serum (1:100 dilution in PBS-T) and FITC conjugates of goat anti-rabbit IgG (1:10). Fluorescence was observed with an immunofluorescence microscope.

Blood sample collection for antibody assay

Blood samples were collected from tail veins of all mice before immunization and thereafter at 2, 4 and 6 weeks. Pooled serum samples were prepared from each group by mixing an equal volume of serum from each group, then used for an enzyme-linked immunosorbent assay (ELISA) and Western blot analysis.

Enzyme-linked immunosorbent assay

To determine the anti-Sj22.7 antibody titer in collected

sera from mice vaccinated with pcDNA3-Sj22.7 and control. The pre-immune and post vaccination sera were tested for specific IgG antibody level by ELISA. The antigen used in ELISA was Sj22.7 purified protein [18]. The secondary antibody used in the ELISA was alkaline phosphatase-conjugated goat anti-mouse IgG (Promega, Madison, USA). Color reaction was developed by the addition of *o*-phenylenediamine (OPD) (Sigma, St. Louis, USA) in citrate phosphate buffer and stopped with 50 μ l of 5% sulfuric acid per well. The absorbance was read at 490 nm using an ELISA reader (BioRad, Hercules, USA).

Western blot analysis

S. japonicum adult worm antigen (AWA) was prepared in our lab to confirm the specificity of antibodies to pcDNA3-Sj22.7 [19]. AWA was separated by 12% sodium dodecylsulfate-polyacrylamide gel electrophoresis. After electrophoresis, the gels were either Coomassie brilliant blue R-250-stained to visualize the protein bands or transferred onto nitrocellulose membrane to analyze using Western blot. For Western blot, the membrane was blocked with 5% skimmed milk in PBS containing 0.5% Tween-20 (blocking solution). Vaccinated serum samples (1:200) were used as the primary antibody and peroxidase-conjugated goat anti-mouse IgG (1:5000) (Amresco) was used as the secondary antibody. Specific binding was detected with H₂O₂ and diaminobenzidine (DAB) (Sigma) as a chromogenic substrate.

Preparation and cultivation of spleen cells

Two weeks after the last immunization, three mice from each group were killed and their spleens were removed under aseptic conditions. The suspension of single spleen cells was prepared after removing erythrocytes by hypotonic lysis and resuspended in RPMI 1640 (Gibco) by vigorous pipetting. The cell suspension was added into the 96-well flat-bottomed tissue culture plates (Sigma) at 200 μ l/well, then cultured at 37 °C in a humidified atmosphere with 5% CO₂.

T-lymphocyte proliferation assay

The T-lymphocyte proliferation [20] was detected with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Spleen cell suspensions from immunized and control mice were prepared in RPMI 1640 supplemented with 10% FCS. Splenocytes were cultured at 37 °C with 5% CO₂ in a 96-well tissue culture plate at a concentration of 5 \times 10⁵ cells/well in the presence of medium, 10 μ g/ml recombinant antigen or 5 μ g/ml concanavalin A (ConA) (Sigma). The cells were cultured

for 3 d followed by incubation with 10 μ l of MTT per well for 4–6 h. After incubation, 100 μ l of dimethyl sulfoxide (DMSO) was added into each well. The plates were shaken slowly for 10 min. The absorption at 570 nm of each well was measured using a microtiter plate reader (BioRad).

Challenge infection and parasite loads

Two weeks after the last immunization, mice were challenged with 40 ± 1 normal *S. japonicum* cercariae by abdominal skin penetration. All mice were killed and perfused at day 42 after infection, and the immunoprotection was assessed by worm reduction rate and egg reduction rate. The egg counts in livers and intestines of the mice were determined by microscopic examination after digestion with 4% potassium hydroxide for 16 h at 37 °C. Fecundity was expressed as the number of eggs per female worm. The ratio of the liver eggs versus intestinal eggs was also determined. Control mice were not immunized but treated identically. For each group of mice, total egg counts were expressed as the number of eggs per gram of mouse liver or intestine.

Histopathological examination

After killing animals in different groups, parts of the liver tissues were immediately fixed in 10% buffered formalin solution and processed in paraffin blocks. The sections of 5 μ m were cut on albuminized glass slides and stained by hematoxylin and eosine for routine histopathological examination for counting granuloma and size. Liver egg-granulomas were counted in five successive low power fields (10 \times), and their diameters were measured using graduated eyepiece lens, considering only lobular granulomas containing ova in the center. Two perpendicular maximal diameters were measured, getting the mean diameter for each granuloma, and then calculating the mean granuloma diameter for the group.

Cytokine detection

Six weeks post-infection, spleen cells were cultured in RPMI 1640 containing 10% FCS on stimulation with 5 μ g/ml Con A or 10 μ g/ml recombinant antigen (rSj22.7) at 37 °C with 5% CO₂. Supernatant fluids were collected at 72 h, and IFN- γ and IL-4 assay were carried out using ELISA kits according to the manufacturer's instructions (Boster). The cytokine concentrations were calculated according to the standard curve.

Statistical analysis

Data were expressed as mean \pm SD. $P < 0.05$ determined by ANOVA or Student's *t*-test was considered significant.

Results

Construction and identification of DNA vaccine

The entire coding sequence of the Sj22.7 amplified from cDNA adult worm library using primers P1/P2 was approximately 622 bp (**Fig. 1**). The fragment was cloned into the expression vector pcDNA3 digested with the same restriction enzymes *Kpn*I and *Apa*I to construct recombinant plasmid pcDNA3-Sj22.7. With the pcDNA3-Sj22.7 as a template and P1/P2 as primers, the product of PCR showed a similar size to the insert (**Fig. 2**). The identifica-

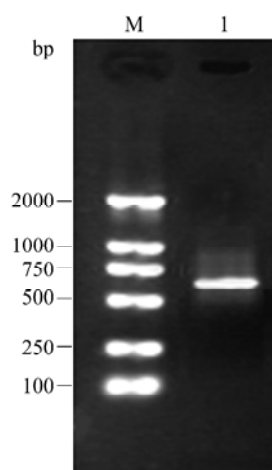


Fig. 1 The polymerase chain reaction (PCR) amplification of Sj22.7 gene

M, DL2000 ladder marker; 1, PCR product of Sj22.7.

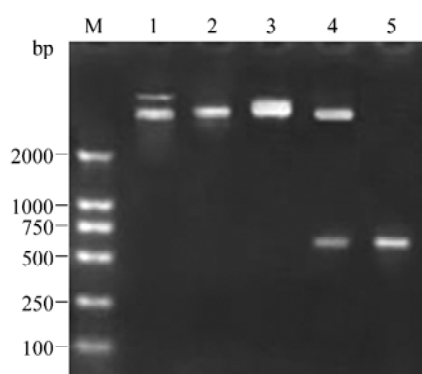


Fig. 2 Restriction enzymes and polymerase chain reaction (PCR) identification of the recombinant plasmid pcDNA3-Sj22.7

M, DL2000 ladder marker; 1, pcDNA3 plasmid; 2, pcDNA3 digested by *Kpn*I and *Apa*I; 3, pcDNA3-Sj22.7 plasmid; 4, pcDNA3-Sj22.7 digested by *Kpn*I and *Apa*I; 5, PCR product of pcDNA3-Sj22.7.

tion was confirmed by sequencing.

Transient expression of Sj22.7

The transient expression of Sj22.7 in transfected HeLa cells was determined using RT-PCR. PCR product of the expected size (622 bp) was obtained using cDNA derived from transfected cells as a template. To eliminate the possibility that the Sj22.7 PCR product was produced because of plasmid DNA contamination, RNA alone was used as a template for PCR. No product was amplified without reverse transcription of the RNA. Similarly, no product was obtained using cDNA derived from cells transfected with control plasmids or cells without undergoing transfection as a template (**Fig. 3**). The presence of Sj22.7 in the plasma and on the surface of transfected cells was confirmed by immunofluorescence microscopy (**Fig. 4**). These results showed that the pcDNA3-Sj22.7 construct could be expressed in mammalian cells.

Expression of DNA vaccine in muscle

To determine whether the Sj22.7 protein could be expressed in the muscle after the injection of pcDNA3-Sj22.7, indirect immunofluorescence assays were carried out. Results showed that Sj22.7 could express in muscle following intramuscular injection with pcDNA3-Sj22.7 (**Fig. 5**).

Antibody response to DNA vaccination

The presence of antigen-specific IgG antibodies was

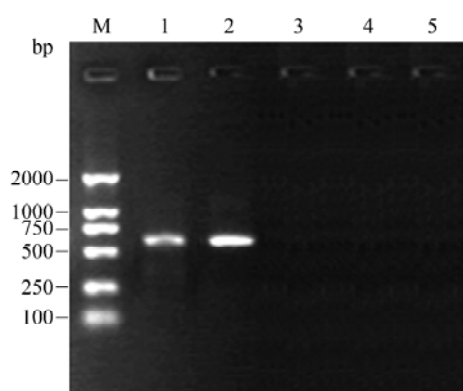


Fig. 3 Identification of HeLa cells transfected with pcDNA3-Sj22.7 by reverse transcription-polymerase chain reaction (RT-PCR)

M, DL2000 ladder mark; 1, PCR amplification of Sj22.7; 2, pcDNA3-Sj22.7 transfected HeLa cells; 3, pcDNA3 transfected HeLa cells; 4, untreated HeLa cells; 5, RNA used as template.

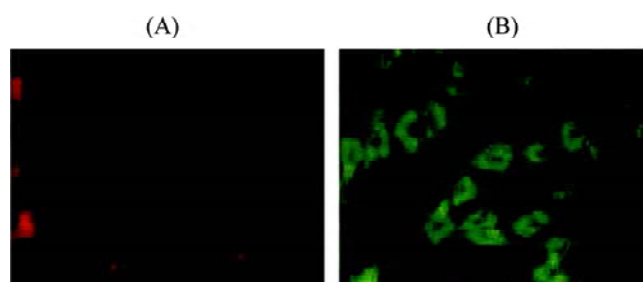


Fig. 4 *In vitro* expression of Sj22.7 in HeLa cells

Immunofluorescence microscopy indicates the presence of Sj22.7 protein in the plasma and on the surface of HeLa cells reacted with immune serum. (A) pcDNA3 transfected HeLa cells reacted with serum of mice from immunized with fusion protein. (B) pcDNA3-Sj22.7 transfected HeLa cells reacted with serum of mice from immunized with fusion protein. Magnification, 200 \times .

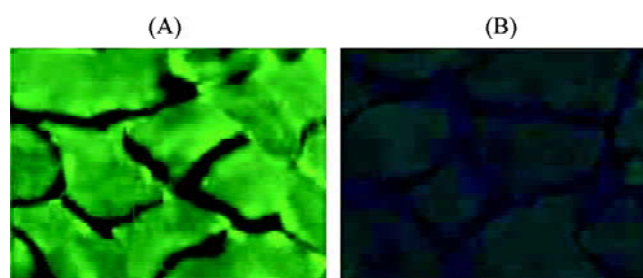


Fig. 5 Results of mouse musculature tested by indirect immunofluorescence method

(A) Recombinant plasmid group. (B) Blank plasmid group. Magnification, 400 \times .

examined in the serum from immunized mice using Western blot analysis. An *S. japonicum* antigen of 22.7 kDa from AWA was strongly recognized by serum from mice vaccinated with pcDNA3-Sj22.7 (**Fig. 6**, lane 1), showing that a specific humoral response against the Sj22.7 protein. No schistosome antigen was recognized by the serum from mice vaccinated with pcDNA3 (**Fig. 6**, lane 2). Serum obtained from mice at weeks 0, 2, 4 and 6 was analyzed quantitatively by ELISA for the levels of total IgG. In agreement with the results of Western blot analysis, high levels of anti-Sj22.7 total IgG were detected in the serum from mice vaccinated with pcDNA3-Sj22.7 after 6 weeks (**Fig. 7**).

T-lymphocyte proliferation response

The T-lymphocyte proliferation responses are shown in **Fig. 8**. T lymphocytes from mice immunized with pcDNA3-Sj22.7 showed a significant proliferation response

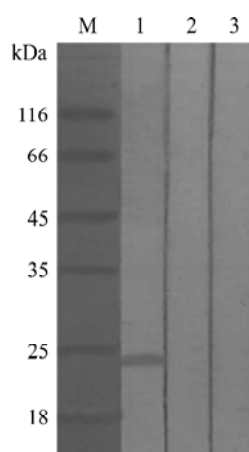


Fig. 6 Western blot analysis of sera from mice immunized with pcDNA3-Sj22.7

M, protein marker with low molecular weight; 1, sera from mice immunized with pcDNA3-Sj22.7; 2, sera from mice immunized with pcDNA3; 3, sera from mice immunized with NS.

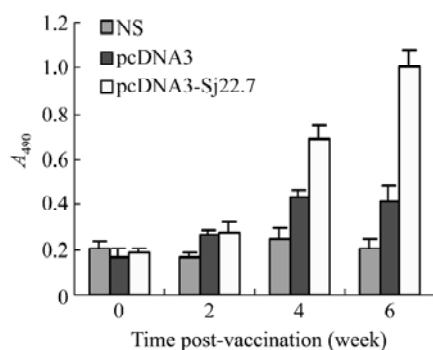


Fig. 7 Serum IgG antibody levels in mice immunized with DNA vaccine

Antibody levels of mice were determined by enzyme-linked immunosorbent assay (ELISA) in each group. High levels of anti-Sj22.7 total IgG were detected in week 6 serum from mice vaccinated with pcDNA3-Sj22.7.

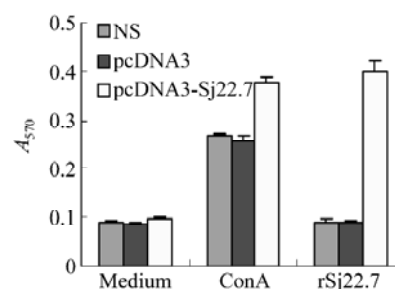


Fig. 8 Proliferation level of mice spleen T lymphocytes

This test was carried out at the end of the sixth week (day 42) after vaccination. T lymphocytes from mice immunized with pcDNA3-Sj22.7 showed a significant proliferation response to ConA or rSj22.7.

to ConA or rSj22.7 ($P < 0.01$, compared with medium or pcDNA3 group).

Protection induced by DNA vaccine

Vaccinated and control mice were challenged with 40 ± 1 cercariae each and the number of worms recovered 6 weeks later was assessed. The results related to the effect of the DNA vaccine on mouse pathology and parasite development are summarized in **Table 1**. At the time of perfusion, the bodyweight of vaccinated and challenged mice was significantly heavier than that of non-vaccinated infected control animals, suggesting an overall better health status. The egg counts showed a significant difference either in terms of reduced worm burden (-29.70% , $P < 0.01$) or number of present eggs, isolated from the liver or mesentery. The animals showed a significant protection in mice following challenge infection. There was a subsequent reduction in the number of eggs in the liver (-47.25% , $P < 0.05$) and intestine (-51.73% , $P < 0.05$) of the pcDNA3-Sj22.7 immunized group when compared with the pcDNA3 blank vector group. A significant reduction in the fecundity of the parasites (e.g., number of eggs

Table 1 Worm and egg loads in mice vaccinated with the DNA construct expressing Sj22.7

Group	Control	pcDNA3 ^a	pcDNA3-Sj22.7 ^b
Numbers of mice	10	10	10
Worm burden	26.6 \pm 3.0	25.7 \pm 2.2 (-3.38% , $P > 0.05$)	18.7 \pm 2.4 (-29.70% , $P < 0.05$)
Numbers of eggs per gram of liver	32836 \pm 1706	31451 \pm 1077 (-4.22% , $P > 0.05$)	17323 \pm 1646 (-47.25% , $P < 0.05$)
Numbers of eggs per gram of intestine	32145 \pm 1549	30552 \pm 1033 (-4.96% , $P > 0.05$)	15516 \pm 1605 (-51.73% , $P < 0.05$)
Numbers of eggs per worm in liver	5389.55 \pm 280.01	5063.94 \pm 173.41 (-6.04% , $P > 0.05$)	3993.60 \pm 379.46 (-25.90% , $P < 0.05$)

Mice were either not treated (control) or vaccinated three times with the empty vector pcDNA3 or with pcDNA3/Sj22.7, then challenged with 40 ± 1 cercariae of *S. japonicum* and perfused 42 d later. ^a compared with control ($P > 0.05$); ^b compared with the empty vector pcDNA3. The reduction was induced by the pcDNA3-Sj22.7 vaccination ($P < 0.05$).

Table 2 Mean diameter of egg granulomas in the liver of mice

	NS	pcDNA3 ^a	pcDNA3-Sj22.7 ^b
Number of granulomas	50	50	50
Diameter of egg granulomas (μm)	364.30 \pm 48.1750	349.68 \pm 51.69 (−4.01%, $P>0.05$)	239.78 \pm 48.99 (−34.18%, $P<0.05$)

^a compared with NS ($P>0.05$); ^b compared with the empty vector pcDNA3 ($P<0.05$).

per female worm; −25.90%, $P<0.05$) was observed in the liver and intestine after vaccination with pcDNA3-Sj22.7. Taken together, the Sj22.7 DNA vaccine permitted a better growth of mice and reduced worm burden, egg number and worm fecundity.

Histopathological changes in granuloma

Liver sections of both immunized and control groups at 6 weeks post-infection were studied for granuloma count and size. The histopathological examination showed a significantly greater number of egg granulomas in the control group than in the immunized group. The diameter of granuloma was significantly larger in the control group compared with the immunized group (**Fig. 9** and **Table 2**). In addition, the percentages of degenerated ova were higher in the immunized group compared with the control groups.

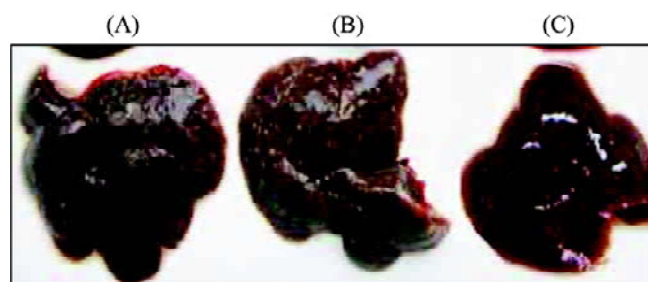


Fig. 9 Comparison in livers of different groups 42 d after cercariae infection

(A) NS control group. (B) Blank plasmid group. (C) Recombinant plasmid group.

Histopathological examination of vaccinated and control infected mice were carried out using hematoxylin and eosin stain. Sections of group immunized with pcDNA3-Sj22.7 showed less number and smaller egg granuloma usually formed by the central egg surrounded by some mononuclear inflammatory cells and few eosinophils. In contrast, the control groups showed greater number of

portal egg granulomas formed of an ovum surrounded by large number of eosinophils, neutrophils and histiocytes (**Fig. 10**).

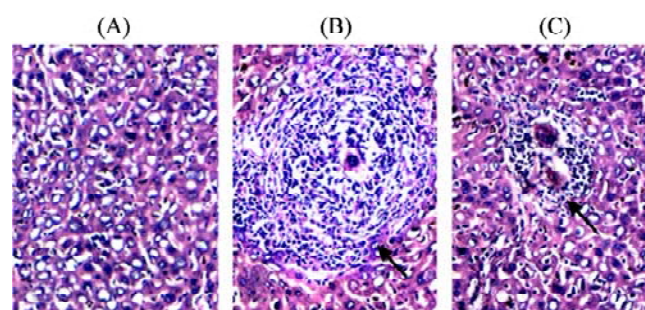


Fig. 10 Mouse liver slices 42 d after infection of cercariae (stained with HE, 20 \times 10)

(A) Normal mouse liver slice. (B) Blank plasmid group. (C) Recombinant plasmid group. The arrow shows egg granuloma.

Cytokine responses

The cytokine profile induced in pcDNA3-Sj22.7 vaccinated or control animals was measured. ELISA results obtained from culture supernatants harvested at 72 h showed that, in response to rSj22.7, spleen cells from the pcDNA3-Sj22.7 group produced higher level of T helper cell type 1 (Th1)-associated cytokine IFN- γ and lower level of the T helper cell type 2 (Th2)-associated cytokine IL-4 ($P<0.05$) as shown in **Fig. 11**. Splenocytes of immunized mice produced an average of 100.67 pg/ml of IFN- γ and 47.32 pg/ml of IL-4 following ConA stimulation (120.67 pg/ml and 68.29 pg/ml respectively).

Discussion

Nucleic acid immunization can be an effective vaccination technology that delivers DNA constructs encoding specific immunogens into host cells, inducing both antigen-

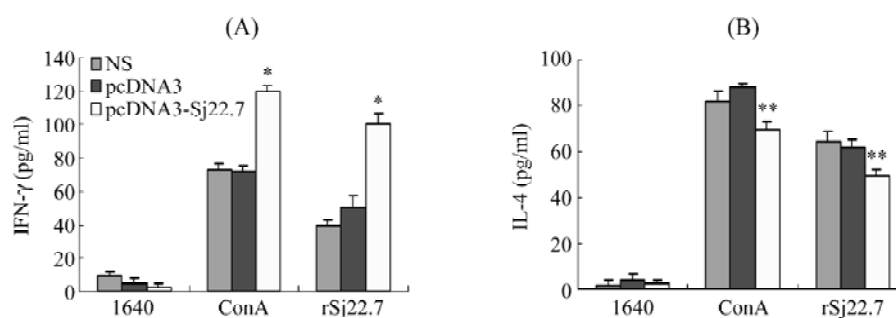


Fig. 11 Cytokine IFN- γ (A) and IL-4 (B) responses of spleen cells from mice

The results are presented as mean \pm SD for each group. Statistically significant difference of pcDNA3-Sj22.7 vaccinated mice compared to control groups is denoted by asterisk (* P <0.01; ** P <0.05).

specific humoral and cellular immune responses. Since the first demonstration of protective immunity against viral challenge induced by DNA vaccination using a plasmid DNA encoding influenza A nucleoprotein, various degrees of success has been achieved, and the main methods of plasmid-DNA application are intramuscular injection and intradermal delivery into skin [21]. In the case of schistosomiasis, vaccination with DNA has shown to induce immune responses in rats, and partial protection against challenge in mice [22], underlining the potential of this method of vaccine delivery for this disease. Previously, we identified a gene encoding a pairing-associated protein identical to the Sj22.7 AWA by two-dimensional gel electrophoresis and mass spectrometry [16]. Several investigators have suggested that the 22.7-kDa antigens of *S. japonicum* adult worms are important vaccine candidates [17]. In the present study, we produced the Sj22.7 DNA vaccine pcDNA3-Sj22.7, tested the pcDNA3-Sj22.7 vaccination strategies in mice and assessed the immunogenicity and protective efficacy of Sj22.7 as a DNA vaccine.

To test whether pcDNA3-Sj22.7 was able to express the schistosome antigen Sj22.7 in mammalian cells, transfection experiments were carried out with HeLa cells as recipient cells. The Sj22.7 gene was successfully transcribed in the transfected HeLa cells. The results of IFAT showed that the Sj22.7 gene was expressed in HeLa cells transfected with pcDNA3-Sj22.7. The presence of Sj22.7 in the plasma and on the surface of transfected cells was also confirmed by immunofluorescence microscopy. These results showed that the pcDNA3-Sj22.7 construct could be expressed and lead to the direct synthesis of the immunogen in eukaryotic cells. To determine whether the Sj22.7 protein was expressed in the muscle after the injection of pcDNA3-Sj22.7, indirect

immunofluorescence assays were carried out. Results showed that Sj22.7 could be expressed in muscle followed by intramuscular injection with plasmid DNA encoding Sj22.7. Therefore, the schistosome Sj22.7 was for the first time successfully expressed in mammalian cells both *in vitro* and *in vivo*.

To determine if the pcDNA3-Sj22.7 vaccine conferred protection against *S. japonicum*, all animals in each groups were challenged with 40 ± 1 cercariae 2 weeks after the last immunization, and 6 weeks later, worm/egg burden was analyzed. In all cases, the pcDNA3-Sj22.7 vaccine conferred a significant protection in mice against challenge infection, which leads to a worm reduction rate of 29.70% and an egg reduction rate of 47.25% (liver) and 51.73% (intestine). The reduction in worm burden in animals immunized with pcDNA3-Sj22.7 was also significantly higher than in animals immunized with the control. The administration of pcDNA3-Sj22.7 by intramuscular inoculation in the current study resulted in expression *in vivo*, which induced specific antibodies in mice as detected by Western blot analysis and ELISA. Vaccination can be targeted towards the prevention of infection or to the reduction of parasite fecundity. A reduction in worm numbers is the “gold standard” for anti-schistosome vaccine development but, as schistosome eggs are responsible for both pathology and transmission, a vaccine targeted on parasite fecundity and egg viability also seems to be entirely relevant [23]. The effective vaccine would prevent the initial infection and reduce egg granuloma associated pathology [24,25].

DNA vaccine pcDNA3-Sj22.7 could induce significant cellular and humoral immune response. High levels of anti-Sj22.7 total IgG were detected in the serum from mice vaccinated with pcDNA3-Sj22.7 after 4 weeks. Thus, pcDNA3-Sj22.7 vaccination successfully induced the

production of specific anti-Sj22.7 antibodies in mice. Simultaneously, T lymphocytes from mice immunized with pcDNA3-Sj22.7 showed a significant proliferation response to rSj22.7. To what extent the vaccine-induced humoral or cellular immune responses are involved in the protective effects needs to be investigated. Both might be required [26,27]. It is believed that *S. japonicum* infections induce an immune response dominated by Th2 cells, whereas a Th1 predominant response strongly correlates with resistance to infection [13]. A key distinction between the Th1 versus Th2 pathways lies in the source of different cytokines involved [28]. Th1 responses are typically characterized by the secretion of IFN- γ and IL-2. However, Th2 responses are characterized by the secretion of IL-4, IL-5, IL-6 and IL-10. The vaccination-induced Th1-type response plays an important role in anti-schistosome infection by producing cytokines, such as IFN- γ and IL-2 [29,30]. It has been shown that, at an early stage of infection, the host's response against the parasite is a Th1-type one. Epidemiological surveys of schistosomiasis showed that the individual with a high level of IFN- γ was significantly correlated with resistance to schistosome infection [31]. In animal models with schistosome infection, it has been observed that IFN- γ can suppress granuloma formation *in vivo*, and decrease the size of pulmonary granulomas and the extent of hepatic fibrosis [32,33]. However, the data suggest a role of Th2-type cytokines for hepatic fibrosis in human schistosomiasis *mansoni* [34]. In the present study, we found that spleen cells from the pcDNA3-Sj22.7 group produced abundant amounts of Th1-associated cytokine IFN- γ and significant reduction of the Th2-associated cytokine IL-4 ($P < 0.05$).

In conclusion, pcDNA3-Sj22.7 is a promising DNA vaccine that can elicit a protective immunity efficacy against *S. japonicum* infection in mice. In addition, the pcDNA3-Sj22.7 showed potential as a DNA vaccine and anti-fecundity vaccine. The intramuscular immunization with pcDNA3-Sj22.7 was able to induce the humoral and cellular immune responses in mice. We have confirmed that the DNA vaccine could induce strong Th1 responses that enhance protective immune responses against schistosomiasis. These data suggested a role for Sj22.7 as a vaccine candidate or as a novel target for anti-schistosome drugs.

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