Immunization against Egyptian *Schistosoma mansoni* infection by multivalent DNA vaccine

Mahmoud H Romeih\(^1\)#&, Hanem M Hassan\(^1\)#&, Tarek S Abou Shousha\(^2\), and Mohamed A Saber\(^1\)

\(^1\)Department of Biochemistry and Molecular Biology and \(^2\)Department of Pathology, Theodor Bilharz Research Institute, Giza, Egypt

The development of multivalent vaccines consisting of several antigens is a novel approach to creating broad-range protection against different parasite strains and parasite life cycle stages. We have previously confirmed that the schistosome *Sm*21.7 and *Sm*Fimbrin (*Sm*Fim) proteins could induce protection in mice. Therefore, this study aimed to construct the multivalent DNA vaccine *Sm*21.7-*Sm*Fim/pBudCE4.1 and evaluate its immune efficacy. The open reading frames of two *Schistosoma mansoni* genes, *Sm*21.7 and *Sm*Fim, were inserted into the eukaryotic expression plasmid pBudCE4.1 designed for the independent expression of two genes in mammalian cells. To evaluate the *in vitro* expression of the multivalent *Sm*21.7-*Sm*Fim/pBudCE4.1 DNA vaccine and its immunological effect in mice, the recombinant plasmid *Sm*21.7-*Sm*Fim/pBudCE4.1 was used to transfect 293T cells, and the expression of mRNA and proteins was examined using reverse transcription-polymerase chain reaction and Western blot analysis. Then the ability of *Sm*21.7-*Sm*Fim/pBudCE4.1 to protect against *S. mansoni* challenge infections was analyzed according to worm burden and egg reduction rates after vaccination of mice. Vaccinated mice showed a significant level of protection (56%), and a decrease in the number and size, and change in the cellular profile, of granulomas. Egg reduction in liver and intestine was 41.53% and 55.63%, respectively, as determined relative to mice that received the empty vector only. In addition to reductions in worm viability, worm fecundity and egg hatching ability were observed following challenge infection in the immunized group. Results showed that *Sm*21.7-*Sm*Fim/pBudCE4.1 could express *Sm*21.7 and *Sm*Fim mRNA and proteins. Enzyme-linked immunosorbent assay and Western blot analysis indicated that immunized mice generated specific immunoglobulin G against *Sm*21.7-*Sm*Fim/pBudCE4.1. These results suggest that vaccination with multivalent *S. mansoni* DNA vaccine (*Sm*Fim-*Sm*21.7/pBudCE4.1) not only induces a significant reduction in worm and egg burdens, but also significantly reduces the size of egg granulomas. In summary, the multivalent vaccine stimulated specific immunity with a significant level of protection and has anti-pathological effect.

**Keywords** *Schistosoma mansoni*; *Sm*21.7; *Sm*Fimbrin; multivalent DNA vaccine; immune response

Schistosomiasis is one of the most important parasitic diseases in tropical areas. Approximately 200 million people worldwide currently suffer from the infection, which causes more than 500,000 deaths each year [1–4], and genital schistosomiasis might increase the risk of HIV infection [5]. In Egypt, *Schistosoma mansoni* is still a serious public health problem despite efforts over many years to control this parasitic infection [6]. Although praziquantel is an effective drug for the treatment of schistosomiasis, reinfection and the drug resistance of the parasite have become a problem [7]. Therefore, the development of an effective vaccine against schistosomiasis is important [8,9]. The major challenge in the development of antischistosomal vaccines is to use defined antigens to stimulate an appropriate immune response that leads to resistance [10,11].

Several promising candidate vaccine antigens have been characterized and their primary sequences were derived for *S. mansoni*. These antigens include the glycolytic enzyme triose-phosphate isomerase, a 28 kDa glutathione-
S-transferase (GST), the myofibrillar protein paramyosin, an integral membrane protein (Sm23), calpain, and S. mansoni 21.7 kDa protein (Sm21.7) [12–17]. As the efficacy of these vaccines against schistosomiasis remains uncertain, the identification and characterization of new antischistosomal vaccine molecules remains a priority. The development of a vaccine is an important long-term and challenging goal in the control of schistosomiasis [18].

DNA vaccination was introduced in 1990 by a study that showed the induction of protein expression on direct intramuscular injection of plasmid DNA in myocytes [19]. Protective immunity conferred by DNA vaccines has been shown in many animal models of various diseases including HIV, tuberculosis, and cancer [20–22]. Nucleic acid vaccination against schistosomiasis has recently been investigated using a panel of plasmids encoding schistosome antigenic proteins such as Sm21.7 [23], Sj26GST, Sj79, and Sj22.7 [4,24,25], S. japonicum paramyosin [26,27], and S. mansoni 23 kDa [28,29] and 28 kDa GST [30]. Progress has been made towards development of DNA vaccines against viral and bacterial pathogens, showing protection and lasting immunity [31]. Application of this new vaccination technology with regard to parasitic infection provides new hope for significant advances in antiparasitic vaccine research. The development of multivalent vaccines consisting of several antigens is a novel approach to creating broad-range protection against different parasite strains and parasite life cycle stages [32].

In our previous study, immunoscreening of S. mansoni cDNA expression libraries resulted in isolation of two immunogenic clones. The first clone was designated SmFimbrin (SmFim; GenBank accession No. L33405), containing a 2.4 kb cDNA insert with an open reading frame of 2.0 kb codes for a deduced 651 amino acid protein that shows a striking homology with chicken fimbrin and human plastin. Immunofluorescence revealed that SmFim was localized in the tegumental region of adult S. mansoni worms. The role of SmFim protein as a protective antigen was assessed by immunization of mice, and the level of protection was approximately 40% [33]. The complete sequence of the second clone, designated Sm21.7 (GenBank accession No. U30663), has an open reading frame that encodes a protein of 184 amino acids. The translation product of this cDNA has a deduced molecular mass of 21.7 kDa [17]. The role of Sm21.7 as a protective antigen was fully supported by the active immunization of the permissive host (Swiss albino mice and C57 Bl/6 mice). These experiments led to a significant level of protection (41%) against challenge infection [23].

In this study, we constructed a multiple DNA vaccine using the full length of Sm21.7 and SmFim genes that have been previously shown to be immunogenic in mice [23]. The full lengths of these genes were inserted into the eukaryotic expression plasmid pBudCE4.1. The plasmid was designed for simultaneous expression of two genes, driven by a human cytomegalovirus (CMV) and a human elongation factor-1α (EF-1α) promoter. The construct was tested for immunogenicity and protective efficacy as a multivalent DNA vaccine in Swiss albino mice.

Materials and Methods

Parasites, animals, and crude antigen

An Egyptian strain of S. mansoni cercariae and naïve female Swiss albino mice (6–8 weeks of age) were obtained from the Schistosome Biological Supply Program, Theodor Bilarz Har Research Institute (Giza, Egypt). The animals were divided randomly into two groups, each consisting of 10 mice, and were kept under standard laboratory conditions. In addition, two more groups of animals were used, infected and non-infected, each comprising five mice. Soluble worm antigen protein (SWAP) was prepared in our laboratory according to a standard protocol [34].

Construction of Sm21.7-SmFim/pBudCE4.1 multivalent DNA vaccine

The cDNAs containing the entire coding region of two S. mansoni genes, Sm21.7 and SmFim, were subcloned into the eukaryotic expression vector pBudCE4.1 (Invitrogen, Carlsbad, USA). The two genes were isolated from schistosomes and adult worm cDNA libraries, respectively. The pBudCE4.1 vector was designed for the independent expression of two genes from a single plasmid in CMV and EF-1α promoter for high-level expression. It also contains the Sh ble (ZeoR) gene for efficient selection in both mammalian cells and Escherichia coli with the selection agent Zeocin. Briefly, a pair of primers was synthesized according to the DNA sequence of each gene. For the Sm21.7 gene, the forward primer was P1, 5'-ATAA-GAATGGCGGGCGAGCATGGAAAAATTTATCTAAACT-TATTTAAC-3', and the reverse primer was P2, 5'-CGCCGGCTCGAGGCTTACGCTTTGATGACGCAGCAAG CAGTATGAT-3', containing NotI and XhoI restriction sites, respectively (underlined). For the SmFim gene the forward primer was P3, 5'-CGGCGCGATCTCTCGGCTC CATTAGTTTTTGTACATCATTTAAA-3', and the reverse primer was P4, 5'-ACGGTTCGACGACATGGCAA GTGCTATCAATCAATTAAAC-3', containing BamHI and SalI restriction sites, respectively (underlined). In addition, the Kozak sequence was added to the position...
of initiator. The Sm21.7 and SmFim genes were amplified by PCR and subjected to DNA agarose gel electrophoresis, then the fragments [NotI/XhoI (approximately 500 bp) and BamHI/SalI (approximately 2400 bp)] were purified using QIAquick gel extraction kit (Qiagen, Valencia, USA) and digested with the appropriate restriction enzymes. Thereafter, the purified fragment BamHI/SalI was ligated to the CMV vector promoter, and the purified fragment NotI/XhoI was ligated to the EF-1α promoter. The recombinant plasmid was transformed into E. coli DH5α competent cells and identified by restriction enzyme digestion, PCR, and sequencing to ensure that the insertions were cloned correctly after subcloning of each fragment. The resulting recombinant expression plasmid encoding the full length of the two genes (designated Sm21.7-SmFim/pBudCE4.1) was called the “multivalent DNA vaccine”. The large-scale preparation of the DNA plasmid was carried out using the Qiagen EndoFree Plasmid Maxi kit (Qiagen, Hilden, Germany). The multivalent DNA vaccine plasmid encoding the full length of the two genes was used throughout these experiments.

**DNA vaccination**

In three independent protection experiments, mice were divided into two groups. In group I, mice were injected intramuscularly in right and left tibialis anterior muscles with 100 µg purified DNA multivalent vaccine (50 µg/mouse). In group II, mice were injected intramuscularly in right and left tibialis anterior muscles with 100 µg purified non-recombinant pBudCE4.1. The mice of group II (control pBudCE4.1 plasmid) provided an indication for the naturally induced immune response due to the effect of injecting the pBudCE4.1 vector under the same conditions. Three weeks after the first injection, the groups of mice were subsequently boosted with 100 µg/mouse DNA, and after another 3 weeks, the mice were boosted a second time with 50 µg/mouse DNA [35]. The mice were challenged with 100 S. mansoni cercariae/mouse by the tail immersion method and perfused 7 weeks later [36].

**Blood sample collection**

Blood samples were collected from tail veins of all mice prior to immunization and thereafter at 2 weeks and 4 weeks, and finally at 7 weeks post-challenge. Sera were separated and stored at –20 °C.

**In vitro expression of Sm21.7-SmFim/pBudCE4.1**

To observe the expression of the two genes (Sm21.7 and SmFim) encoded by the multivalent Sm21.7-SmFim/pBudCE4.1, the recombinant plasmid was transfected into 293T cells using the calcium phosphate method. The transcription of Sm21.7 and SmFim mRNA and synthesis of Sm21.7 and SmFim proteins were examined by reverse transcription-polymerase chain reaction (RT-PCR) and Western blot analysis, respectively. Briefly, the 293T cells (1.2×10⁶ cells/plate), a gift from Dr. Steven J Triezenberg (Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, USA), were plated onto 10 cm diameter plates (Nunc, Rochester, USA) and maintained for 24 h in 7 ml Dulbecco’s modified Eagle’s medium (Gibco, Gaithersburg, USA) containing 10% fetal bovine serum (PA Biological Co.), 100 U/ml penicillin, and 100 µg/ml streptomycin. DNA mixtures were introduced into 293T cells using calcium phosphate transfection. One microgram of DNA was diluted in 100 µl deionized water, and 2 M calcium chloride (14 µl) was added to the DNA. An equal amount (114 µl) of 2xHEPES-buffered saline was added slowly in a drop-wise manner. After 30 min of incubation at room temperature, the medium was changed then cultured for 1–2 d. Supernatants were collected post-transfection and centrifuged for 30 min at 3000 rpm at 4 °C (Beckman Coulter) to remove cellular debris. The clarified supernatants were either frozen at –70 °C for further analysis or used immediately for RNA isolation. The 293T cells were washed twice with phosphate-buffered saline (PBS), followed by protein extraction using lysis buffer containing 1×TBS, NonidetP-40 at 10 µl/ml, 20 mM phenylmethylsulfonyl fluoride, 1 µM pepstatin, and 1 µM leupeptin. The mRNA and protein levels of Sm21.7 and SmFim encoded by Sm21.7-SmFim/pBudCE4.1 plasmid were determined by RT-PCR and Western blot, respectively.

**Western blot analysis**

The SWAP or 293T cell protein samples were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12% gel) [36], and transferred to a nitrocellulose membrane (Hybond C; Amersham Pharmacia Biotech). The membrane was blocked (4% non-fat dry milk) for 2 h at room temperature, then incubated for 1 h at room temperature with the appropriate primary antibody sera at a dilution of 1:5000. Then it was washed in 10 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.05% Tween-20 for 30 min with shaking. It was then incubated for 1 h at room temperature with horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G (IgG; 1:3000 final dilution; Pierce Biotechnology, Rockford, USA) followed by washing in 10 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.05% Tween-20 for 30 min with shaking. The signal was detected with a chemiluminescence detection kit.
(SuperSignal West Femto Maximum Sensitivity Chemiluminescent Substrate; Pierce Biotechnology).

**Enzyme-linked immunosorbent assay (ELISA)**
To determine the presence of Sm21.7 and SmFim antibodies in vaccinated and control mice sera, the pre-immune and post-vaccination sera were tested for specific IgG by ELISA. Briefly, ELISA was carried out in micrometer plates coated with purified SWAP antigen (3 µg/well) prepared in PBS. The plates were incubated overnight at 4 ºC then washed three times with PBS and 0.05% Tween-20 (PBST). Blocking solution was added at 100 µl/well. The plates were incubated at 37 ºC for 1 h then washed three times with PBST and dried on tissue paper. Sera were diluted 1:1000 in PBST and tested in triplicate; normal sera were used as the negative control. Diluted sera were applied at 100 µl/well and the plates were covered and incubated in a water bath at 37 ºC for 90 min. The plates were washed three times with shaking using PBST. The alkaline phosphatase-conjugated goat anti-mouse IgG was added (100 µl/well) then the plates were covered and incubated at 37 ºC for 45 min, and washed three times with PBST. Substrate solution was prepared immediately before use and applied at 100 µl/well, then incubated in the dark at room temperature for 15–30 min. NaOH (0.4 M at 100 µl/well) was added to stop the reaction. Absorbance was measured at wavelength 405 nm using an ELISA reader [37].

**Infection and determination of worm burden**
The vaccinated and control mice groups were challenged with 100 S. mansoni cercariae by tail immersion 3 weeks after the last boosting [38,39]. The percentage of the resistance was calculated by perfusion of the adult worms from the portal veins at 7 weeks after challenge infection. The worm reduction rate (% protection) was calculated and the livers and intestines were collected and the eggs were counted according to the method described previously [40].

**Enumeration of eggs in liver and intestine**
Total egg counts were expressed for each group of mice as the mean number of eggs/mg of mouse liver or intestine. The whole livers and intestines of vaccinated and control mice were weighed and a known portion (0.5 g) was removed to a screw cap glass tube and frozen until digestion. For digestion, 5 ml of 5% potassium hydroxide was added to each tube and incubated at 37 ºC until the tissue was completely digested (10–12 h). Then 50 µl digest was placed on a glass slide and eggs were counted under a microscope [41]. 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 groups of animal’s scarifications, a part of the liver tissue was immediately fixed in 10% buffered formalin solution and processed in paraffin blocks. Sections (5 µm thick) were cut on albuminized glass slides and stained by hematoxylin-eosin for routine histopathological examination, granuloma count, measurement of granuloma diameter, cellular profile, and Masson trichrome staining for assessment of tissue fibrosis.

The cellular profiles of liver egg granulomas were studied, with calculation of the percentage of different types of cells forming the granuloma in the different groups. The type of the granuloma, whether cellular, fibrocellular, or fibrous, was defined according to the cellular to fibrous ratio. The percentage of egg granulomas containing intact or degenerated miracidia was also calculated.

Liver egg granulomas were counted in five successive low-power fields (10×), and their diameters were measured using graduated eyepiece lenses, considering only lobular granulomas containing central ova. Two perpendicular maximal diameters were measured to get the mean diameter for each granuloma, then the mean granuloma diameters were calculated for the group.

**Statistical analysis**
Data were expressed as the mean±SD. P<0.05, determined by Student’s t-test, was considered significant.

**Results**

**Construction and identification of Sm21.7-SmFim/pBudCE4.1 recombinant plasmid**
The entire coding sequences of the Sm21.7 and SmFim genes were amplified by PCR using primers P1/P2 and P3/P4, respectively. Approximately 500 bp and 2400 bp for Sm21.7 and SmFim, respectively, were identified. The fragments were subcloned into the expression vector pBudCE4.1 digested with the same restriction enzymes, NotI and XhoI for Sm21.7 and BamHI and SalI for SmFim, to construct the recombinant plasmid Sm21.7-SmFim/pBudCE4.1. The identification was confirmed by PCR (Fig. 1) and sequencing.

**In vitro expression of Sm21.7-SmFim/pBudCE4.1 recombinant plasmid**
Multivalent DNA vaccine against Egyptian *Schistosoma mansoni* infection

To estimate the efficacy of recombinant plasmid *Sm*21.7-*SmFim*/pBudCE4.1 to produce the *Sm*21.7 (21.7 kDa) and *SmFim* (68 kDa) proteins in mammalian cells, the recombinant plasmid was transfected into 293T cells in parallel to pBudCE4.1 alone as a control using the calcium phosphate method. The expressions of *Sm*21.7 and *SmFim* mRNA and proteins were examined by RT-PCR and Western blot analysis using sera from immunized mice with *Sm*21.7-*SmFim*/pBudCE4.1, respectively. PCR products of the expected sizes (500 bp for *Sm*21.7 and 2400 bp for *SmFim*) were obtained using cDNA derived from transfected cells as templates. No product was amplified without RT 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. 2(A)]. The presence of *Sm*21.7 and *SmFim* proteins of transfected cells was confirmed by Western blot analysis. The two *S. mansoni* antigens of 21.7 kDa and 68 kDa were strongly recognized in transfected cells with multivalent DNA vaccine plasmid with respect to the untreated cells or cells transfected with pBudCE4.1 alone [Fig. 2(B)].

**Antibody responses in vaccinated mice**

The presence of antigen-specific IgG antibodies was ex-
Multivalent DNA vaccine against Egyptian Schistosoma mansoni infection

amined in the sera from immunized mice with using ELISA and the SWAP protein. Sera obtained from mice prior to immunization and thereafter at 2 weeks and 4 weeks, as well as finally at 7 weeks post-challenge, were analyzed quantitatively by ELISA for the levels of total IgG. The high levels of anti-multivaccine total IgG were detected in week 7 serum from mice vaccinated with multivalent DNA vaccine [Fig. 3(A)]. The two *S. mansoni* antigens of 21.7 kDa and 68 kDa were strongly recognized by sera from vaccinated mice, showing a specific humoral response against the two proteins [Fig. 3(B)]. Thus, the vaccination with multivalent DNA vaccine successfully induced the production of specific anti-*Sm*21.7 and anti-*Sm*Fim antibodies in mice. In contrast, no schistosome antigen was recognized by the sera from mice immunized with pBudCE4.1 vector.

**Protection induced by multivalent DNA vaccine**

Vaccinated and control mice were challenged with 100 cercariae each and the number of worms recovered after 7 weeks was assessed. At the time of perfusion, the body weight of vaccinated and challenged mice was significantly heavier than that of non-vaccinated infected control animals, suggesting overall better health status. The egg counts showed a significant difference either in terms of reduced worm burden or number of eggs present, isolated from the liver or mesentery. The animals showed a significant protection in mice following challenge infection (56%; *P*<0.01), as shown in Fig. 4. There was a substantial reduction in the mean number of eggs/mg of tissue in liver (41.53%; *P*<0.05) and intestine (55.63%; *P*<0.05) of the immuno-challenge group mice, when compared with the pBudCE4.1 blank vector group (Fig. 5). A significant reduction in the fecundity of the parasites (eg, number of eggs per female worm, −29.90%; *P*<0.05) was observed in the liver and intestine after vaccination with multivalent vaccine. Taken together, the multivalent DNA vaccine permitted a better growth of mice and reduced worm burden, egg number, and worm fecundity.

![Fig. 3 Antibody responses measured by enzyme-linked immunosorbent assay and Western blot analysis](image)

(A) Serum immunoglobulin G (IgG) antibody responses against crude soluble worm antigen protein (SWAP) in mice vaccinated with multivalent DNA vaccine against *Schistosoma mansoni*. Responses were measured prior to immunization then at 2, 4, and 7 weeks post-challenge. Antibody levels of mice were determined by enzyme-linked immunosorbent assay in each group. High levels of anti-*Sm*21.7/*Sm*Fim total IgG were detected at week 7 in mice vaccinated with *Sm*21.7-*Sm*Fim/pBudCE4.1. (B) Antibody responses against SWAP in mice vaccinated with multivalent DNA vaccine at 7 weeks post-challenge, by Western blot analysis. 1, protein marker with low molecular weight; 2, sera from mice immunized with pBudCE4.1 vector alone; 3, sera from mice immunized with multivalent DNA vaccine *Sm*21.7-*Sm*Fim/pBudCE4.1.

![Fig. 4 Changes in mean worm burden in mice immunized against Schistosoma mansoni infection with multivalent DNA vaccine Sm21.7-SmFim/pBudCE4.1 and pBudCE4.1 alone](image)

The percentage of protection was calculated by perfusions of adult worms from the portal vein at 7 weeks post-challenge infection. The percentage of protection was approximately 56% (*P*<0.01) in vaccinated Swiss albino mice.
Fig. 5 Changes in egg count in mice immunized against Schistosoma mansoni infection with multivalent DNA vaccine Sm21.7-SmFim/pBudCE4.1 in liver and intestine vessels. There was a substantial reduction in the mean number of eggs/mg tissue of livers (41.53%; P<0.05) and intestines (55.63%; P<0.05) of the immunochallenge group mice when compared to controls (pBudCE4.1 vector alone).

Histopathological changes in granuloma
Liver sections of both immunized and control groups at 7 weeks post-challenge were studied for granuloma count, size, and cellular profile. The histopathological examination showed a significantly (P<0.01) greater number of egg granulomas in the control group (12.0±4.7) than in the immunized group (7.0±3.2) [Fig. 6(A)]. The diameter of granulomas was significantly (P<0.05) larger in the control group (270.0±85.3) µm compared with the immunized group (160.0±77.3) µm [Fig. 6(B)]. In addition, the percentages of degenerated ova were significantly (P<0.01) higher in the immunized group (46.0%±16.6%) compared with the control group (32.5%±14.0%) [Fig. 6(C)].

The most striking feature of the cellular profile of egg granulomas was that the percentages of eosinophils and neutrophils in the granulomas were much higher (P<0.05) in the control group, 46.7%±7.5% and 63.3%±12.4%, respectively, than those in the immunized group, 31.6%±9.5% and 20.0%±21.1%, respectively, whereas the percentages of lymphocytes were greater (P<0.05) in the immunized group (65.0%±8.9%) than in the control groups (33.0%±7.9%) (Fig. 7). In addition, the percentage of ova containing degenerated miracidia was greater in the immunized group than in the control group.

Sections of groups of mice immunized with multivalent DNA vaccine showed fewer and smaller egg granuloma, usually formed by the central egg surrounded by some mononuclear inflammatory cells and few eosinophils. In contrast, sections of the control groups showed a greater number of portal egg granulomas formed by an ovum surrounded by large numbers of eosinophils, neutrophils, and histiocytes as well as some macrophages and focal areas of eosinophilic necrosis. Masson trichrome staining showed more fibrocellular granuloma formed of central ova surrounded by more macrophages, lymphocytes, fibroblasts, and collagen fibers in the vaccinated group.

Fig. 6 Changes in granuloma count, diameter, and degenerated ova in liver sections from mice immunized against Schistosoma mansoni infection. (A) Changes in granuloma count (mean) in mice immunized with multivalent DNA vaccine Sm21.7-SmFim/pBudCE4.1 and pBudCE4.1 as a control in liver histopathology sections. There is a significant decrease in granuloma count (P<0.01) in the multivalent vaccine group (7.0±3.2) compared to the pBudCE4.1 group (2.0±4.7). (B) Changes in granuloma diameter in mice immunized with multivalent DNA vaccine Sm21.7-SmFim/pBudCE4.1 and pBudCE4.1 as a control in liver histopathology sections. There is a significant decrease in granuloma diameter (P<0.05) in the multivalent vaccine group (160.0±85.3) µm compared to the control group (270.0±85.3) µm. (C) Changes in degenerated ova in mice immunized with multivalent DNA vaccine Sm21.7-SmFim/pBudCE4.1 and pBudCE4.1 as a control in liver histopathology sections. There is an increase in degenerated ova (P<0.01) in the multivalent vaccine group (46.0%±16.6%) compared to the pBudCE4.1 group (32.5%±14.1%).
Multivalent DNA vaccine against Egyptian Schistosoma mansoni infection

Discussion

The current studies have used the "naked DNA" vaccine formulation. DNA vaccines offer a promising approach for vaccination. *In vivo* expression of antigens by DNA plasmids has been shown to be long-lasting [42], providing protracted stimulation to the host’s immune system. The optimized approach provided by DNA vaccine technology will produce vaccines ready for clinical and practical applications, as well as providing a greater understanding of the underlying complexity of immunity in parasitic infections. DNA vaccines induce strong humoral and cellular immunity and have the potential to increase immunogenicity through modifications of the vector or incorporation of adjuvant-like cytokine genes.

The development of multivalent vaccines consisting of several antigens is a novel approach to creating broad-range protection against different parasite strains and para-
site life cycle stages. Multivalent vaccines have a greater amount of protective epitopes and could be effective in a greater proportion of the population. However, in multivalent vaccines, the optimal association or combination of antigens must be assessed to obtain synergistic effects [32]. Vaccination studies against leishmaniasis in mice have identified various parasite antigens with varying degrees of protection as protein vaccines. When combined into multivalent DNA vaccines, these antigens have the ability to confer complete or enhanced protection [43]. In vivo expression of antigens by DNA plasmids has been shown to be long-lasting, perhaps providing protracted stimulation to the host’s immune system [44].

Complex parasites present a plurality of antigenic epitopes and the antigen presentation capability varies widely among different individuals. Immunization with a vaccine that stimulates immunity to a broad array of antigens is likely to be more efficacious than immunization with a vaccine for a single antigen to induce protective immune response against parasites [45]. In a previous work in our laboratory, a gene encoding fimbrin-like protein and a gene encoding 21.7 kDa tegumental protein were isolated and identified using immunoscreening of expressed cDNA libraries. Vaccination of mice with either single recombinant antigens or DNA encoding forms of these antigens induced partial protection against S. mansoni cercarial challenge infection as judged by worm reduction, although specific antibodies were generated [17,34]. The observation of SmFim and Sm21.7 proteins in the tegument and subtegumental layer would likely confirm the importance of these structures as targets of the host’s protective response to S. mansoni infection [23,34]. The tegument, the outer covering of the parasite, serves as an interface between the host’s immune system and the parasite. Thus antigens associated with the tegument are major foci for either vaccine development or immunodiagnostic reagents for schistosomiasis [45].

In this study, to extend this approach, we have constructed a multivalent DNA vaccine using the full length of Sm21.7 and SmFim genes inserted into the eukaryotic expression plasmid pBudCE4.1. The plasmid was designed for simultaneous expression of two genes, where the two genes were driven by a human CMV promoter and an EF1α promoter. The construct was tested for immunogenicity and protective efficacy as a multivalent DNA vaccine in Swiss albino mice. To test whether multivalent DNA vaccine (Sm21.7-SmFim/pBudCE4.1) was able to express the schistosome antigens in mammalian cells, in vitro transfection experiments were carried out using 293T cells as recipient cells. The two S. mansoni genes (Sm21.7 and SmFim) were successfully transcribed in the transfected 293T cells (Fig. 2). The results of Western blot analysis showed that the Sm21.7 (21.7 kDa) and SmFim (68 kDa) proteins were expressed in 293T cells transfected with the multivalent DNA vaccine compared with 293T cells transfected with pBudCE4.1 alone as the control.

In the case of schistosomiasis, vaccination with DNA has been shown to induce immune responses in rats, and partial protection against challenge in mice [14,23,46], underlining the potential of this method of vaccine delivery for this disease [32]. In this study, to determine whether the multivalent vaccine conferred protection against S. mansoni, all groups of mice were challenged with 100 cercariae 7 weeks after immunization and worm/egg burdens were observed. In all cases, on challenge infection with normal S. mansoni cercariae, worm reductions of 56% (P<0.01) were obtained as an average of three experiments, compared with non-vaccinated controls. The number of eggs in the liver and intestine was reduced by 41.53% (P<0.05) and 55.63% (P<0.05), respectively, compared to controls. A reduction in worm numbers is the “gold standard” for antischistosome vaccine development but, as schistosome eggs are responsible for both pathology and transmission, a vaccine targeting parasite fecundity and egg viability also seems to be entirely relevant [46]. The effective vaccine would prevent the initial infection and reduce egg granuloma-associated pathology [32,47]. In agreement with that, in this study, the reduction in worm burden in animals immunized with multivalent DNA vaccine was significantly higher than that in the control animals.

In the current study, multivalent DNA vaccine given by intramuscular inoculation resulted in expression in vivo, which induced specific antibodies in mice as detected by Western blot analysis and ELISA. Our results indicated that the multivalent DNA vaccine could induce significant cellular and humoral immune response. High levels of anti-Sm21.7 and anti-SmFim total IgG were detected in sera from mice vaccinated with multivalent vaccine after 2 and 4 weeks, with the maximum reached at 7 weeks after challenge. And the two S. mansoni antigens of 21.7 kDa and 68 kDa were strongly recognized by sera from vaccinated mice, showing a specific humoral response against the two proteins. Thus, the vaccination with multivalent DNA vaccine successfully induced the production of specific anti-Sm21.7 and anti-SmFim antibodies in mice. The specific antibodies were able to mediate a significant killing of schistosomula using peritoneal macrophages as effector cells. In contrast, the pre-immune sera and control serum had no specific reactivity towards these proteins.

To what extent the vaccine-induced humoral or cellular immune responses are involved in these protective effects needs to be investigated. Both might be required [48,49]. It is believed that S. mansoni infections induce an immune response dominated by Th2 cells, whereas a Th1 predominant response strongly correlates with resistance to infection [48]. Th1 responses are typically characterized by the secretion of γ-interferon (IFN-γ) and interleukin-2 (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 antischistosome infection by producing cytokines, such as IFN-γ and IL-2 [31,49]. 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 individuals with a high level of IFN-γ were significantly correlated with resistance to schistosome infection [50,51]. In animal models of 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 [52,53]. However, the data suggest a role of Th2-type cytokines for hepatic fibrosis in human S. mansoni [54].

In the current study, histopathological examination showed a significant decrease in granuloma count (P<0.01) in the vaccinated group (7.0±3.2) compared with the control group (12.0±4.7). There was a significant reduction in granuloma diameter (P<0.05) in the vaccinated group (160.0±77.3) μm compared with the control group (270.0±85.3) μm. The percentage of degenerated ova was increased significantly (P<0.01) in the DNA multiple vaccinated group (46.0%±16.6%) compared with the control group (32.5%±14.1%). There was also a predominance of neutrophils (63.3%±12.4%) and eosinophils (46.7%±7.5%) in egg granulomas of the control group compared with the vaccinated group (31.6%±9.5% and 20.0%±2.1%, respectively). Furthermore, there was a marked increase in the number of lymphocytes in egg granulomas of the vaccinated group (65.0%±8.9%) compared with the control group (33.0%±7.9%). The importance of eosinophils as effector cells has been reported previously [55], confirming that eosinophils dominate the cellular infiltrate in the acute granuloma, and they have been shown to participate in the killing of adult worms and ova due to highly reactive O₂ radicals and/or release of cationic or major basic proteins from granules. Inhibition of Th2-related cytokines IL-4 and IL-5 inhibits hepatic pathology and eosinophils, respectively [56]. In addition, the percentage of degenerated ova was increased significantly in the multivalent DNA vaccinated group compared to the control group.

Several powerful candidates for a cocktail vaccine, including paramyosin, triose-phosphate isomerase, and Sm23, have been reported [26]. However, further work remains to be accomplished, notably on the effects of vaccination and on the possibility of vaccinating animals after chemotherapy to eliminate ongoing infection. Indeed, a combination of chemotherapy and multivalent DNA vaccine strategy could well be the main hope for control of schistosomiasis.

In conclusion, we have successively constructed a multivalent DNA vaccine (Sm21.7-SmFim-pBudCE4.1) in pBudCE4.1 vector. The construct is promising in that it can elicit a protective immune efficacy against S. mansoni infection in mice. Intramuscular immunization with the multivalent DNA vaccine was able to induce humoral and cellular immune responses in mice. This work provides new insights into methods for developing highly protective antischistosome vaccines through preferential induction of specific Th polarization. The construct showed potential as a DNA vaccine and antipathological vaccine.

Acknowledgements

The authors would like to thank Professor David Arnosti at the Department of Biochemistry and Molecular Biology, Michigan State University (East Lansing, USA) for his valuable advice and the permission to use his laboratory. We would also like to thank Professor Steven J Triezenberg, Department of Biochemistry and Molecular Biology, Michigan State University for his kind supply of 293T cells and for permission to use his tissue culture laboratory.

References

Multivalent DNA vaccine against Egyptian Schistosoma mansoni infection


10 McManus DP. The search for a vaccine against schistosomiasis—a difficult path but an achievable goal. Immunol Rev 1999, 171: 149–161


13 Boulanger D, Reid GD, Sturrock RF, Wolowczuk I, Balloul JM, Grezel D, Pierce RJ et al. Immunization of mice and baboons with the recombinant Sm28GST affects both worm viability and fecundity after experimental infection with Schistosoma mansoni. Parasite Immunol 1991, 13: 473–490


33 Saber MA, Hamid AH. Molecular cloning, purification and characterization of Schistosoma mansoni fimbrin. Arab J Biotechnol 1998, 1: 1–16

34 Pearce EJ, James SL, Hiency S, Lanar DE, Sher A. Induction of protective immunity against Schistosoma mansoni by vaccination with schistosoma paramyosin (Sm97), a nonsurface parasite antigen. Proc Natl Acad Sci USA 1988, 85: 5678–5682


Multivalent DNA vaccine against Egyptian Schistosoma mansoni infection


