Efficacy of combined SMS01 DNA and protein as a cocktail vaccine against *Schistosoma mansoni* infection

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Abstract: Schistosomiasis control currently relies primarily on chemotherapy which is both expensive and temporary; therefore there is an urgent need for an effective vaccine. One of the main strategies for vaccine development is the identification of specific antigen(s) that elicit highly protective immune response in immunized hosts. Several defined vaccine candidate antigens of *Schistosoma mansoni* have shown promise in animal vaccination experiments. In a previous study, single-gene vaccination with SMS01 recombinant protein was shown to elicit partial protection against *Schistosoma mansoni* challenge infection. Here we show that the vaccination of mice with a SMS01 (Sm21.7) vaccine cocktail significantly enhance protective responses against *S. mansoni* infection. To evaluate the usefulness of combined SMS01 DNA and protein as a cocktail vaccine against infection, the vaccination models were applied using DNA vaccination and SMS01 fusion protein or both. Consequently, the gene coding for SMS01 immunogenic protein inserted into mammalian expression vector pcDNA1, used as DNA vaccine, in combination with recombinant protein produced in pET-3a system. Then the ability of these combinations to induce a protection against *S. mansoni* infection was analyzed according to worm reduction rate and egg reduction rate after vaccination of mice. In addition the level of IgG antibody response was determined by ELISA. Results showed a significant reduction in worm burden in animals immunized with protein, DNA or combined vaccine, has been observed as 63%, 55% and 46% respectively compared to the control group. ELISA results showed that all the vaccinated groups have produced a high IgG titre and the highest IgG titre was produced by fusion protein group when compared to the control. Antifecundity effects of the three treatments have been observed, and the oogram pattern indicated that the dead ova were very high and the highest level was obtained using fusion protein. In spite of this result, the advantages of DNA vaccination model cannot be denied as being easier and economic. Thus, vaccination against *S. mansoni* remains a long-term prospect because wide ranges of time and tremendous amount of work are needed for continuous development and optimization of vaccine cocktails. [The Journal Of American Science. 2007;3(4):113-126]. (ISSN: 1545-1003).

Key Words: Schistosoma *mansoni*; Liver worms; SM21.7; Vaccine; SMS01; vaccine cocktails

1.Introduction

Schistosomiasis is still a major helminthes infection at the beginning of the 21st century and an important public health problem in many countries. As the second major parasitic disease in the world after malaria, schistosomiasis affects 200 million people, 800 million being exposed to the risk of infection (WHO 2002). It is also estimated that 20 million individuals suffer from severe consequences of this chronic and debilitating disease responsible for at least 500.000 deaths per year (Capron *et al.* 2002a). In Egypt, there is extensive documentation that the government's efforts have been successful in reducing both the prevalence and morbidity of this disease (Engles et *al.* 2002). However, schistosomiasis is still endemic in rural areas of Egypt and in spite of the low endemicity level, transmission still occurs. Four species are of direct medical importance to man; *S. mansoni*, *S. haematobium*, *S. japonicum* and *S. mekongia.* Despite the development of active and relatively safe drugs, the development of human schistosomiasis vaccine is recognized as priority to

complement existing control measures (Bickle et *al.* 2001; Bergquist 1995). Although praziquantel is an effective drug for the treatment of schistosomiasis, reinfection and the drug resistance of the parasite have become a problem. Therefore, the development of an effective vaccine against schistosomiasis is important to control this disease (Bergquist *et al*.2002).

In the past few years, many vaccine strategies have focused on defense against invasion of cercariae, to reduce worm burden by inducing humoral immunity with schistosome vaccine candidates, but the high-level antigen induced-specific antibodies could not adequately protect the host from infection (Bergquist 1998; Chen *et al.* 2003). 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 (Capron *et al*. 2002b). Several promising candidate vaccine antigens have been characterized and their primary sequences derived for *S. mansoni*. These antigens include the glycolytic enzyme triose-phosphate isomerase (*Sm* TPI) (dos Reis MG *et al*. 1993 and Reynolds *et al*. 1994), a 28 kDa glutathione-S-transferase (*Sm*28) (Balloul *et al*.1987 and Boulanger *et al.* 1991), Sm20.8 (Mohamed *et al.*1998), the myofibrilar protein paramyosin Sm97 (Pearce *et al*.,1988), an integral membrane protein *Sm*23 (Da'dara *et a*l. 2002) *S. mansoni* calpain (Karcz *et al*. 1991) and *S.mansoni*(Ahmed *et al.* 2001).

Nucleic acid vaccination against schistosomiasis has lately been investigated using a panel of plasmids encoding schistosome antigenic proteins such as Sm21.7 (Ahmed *et al*. 2006), Sjc26GST (Zhou *et al.* 2005), Sj62 kDa (Zhang *et al.* 2007), *Schistosoma japonicum* paramyosin (Fonseca *et al*. 2005) and *Schistosoma mansoni* 23(Da'dara *et al.*2001), 28 GST (Dupre *et al.* 1997). In such a previous work, it was estimated that SMS01 (Sm21.7) has induced high level of protection against *S. mansoni* challenge infection (Ahmed *et al.* 2001). The goal of this research was to study the response of using recombinant DNA and recombinant protein as a vaccine in the protection of experimental animals challenged wit *S.mansoni* infection. To achieve that, gene coding for immunogenic protein inserted in mammalian expression vector, used as DNA vaccine, in combination with recombinant protein of this gene produced in pET-3a system.

2.Materials and Methods

2.1.Mice, parasites, and infection

An Egyptian strain of *S. mansoni* was maintained in golden hamsters, *Biomphalaria alexandrina* snails and animals were purchased from the Schistosome Biologic Supply Center, Theodor Bilharz Research Institute (Giza, Egypt). All animal presented here had been approved by the local government based on national regulations. We have used female Swiss albino mice (N = 70, age: ~4 weeks, weight: ~18 g) and New Zeeland female rabbits (3.5-4.5Kg). Animals were kept in groups under environmentally controlled conditions (temperature: ~25°C; humidity: ~70%; 12 hour light/dark cycle) and had free access to water and food.

2.2. Preparation and purification of recombinant SMS01 DNA

The DNA was prepared according to Ahmed *et al.* (2001). Briefly, a pair of primers was synthesized according to the DNA sequence of the SMS01, *Bam*H1 adaptors linked to forward and reverse primers and the Kozark sequence was added to the position of initiator. The forward primer was 5′CATCTGGATCCATGGATAGTCC and the reverse 5′TAACGGATCCCTAGTTACTTGG. The amplified sequence was ligated into the eukaryotic expression pcDNA1/Amp expression vector (Invitrogen, Corp, SanDiago, CA), which was previously digested with *Bam*H1 and treated with alkaline phosphates. The structure was verified by

restriction digestion and sequencing. Large-scale preparation of the plasmid was carried out by using the alkali lysis method, followed by double banding on CsCl-EtBr gradient (Sambrook *et al.,* 1989). Then DNA was resuspended in phosphate buffer saline (PBS) for vaccination. The SMS01-pcDNA1 plasmid encoding the full length SMS01 was used throughout these experiments.

2.3. Expression of recombinant protein in pET-3a system

The recombinant protein was expressed in pET-3a system using IPTG method according to (Studier *et al.* 1986; Rosenberg 1987). In brief, the bacterial colony, which contained the recombinant plasmid with SMS01 DNA sequence cloned into pET-3a, was cultured overnight in LB-Amp medium. The culture was then diluted 1:10 (V/V) in fresh LB medium and grown for *2-3* hrs at 37°C (till OD 600 was equal to 0.6). Then the IPTG was added to the final concentration 2mM and the incubation was continued for further 3hrs. The cells were centrifuged at 6,000 rpm (Sorvall, GSA rotor) at 4°C for 10 minutes and the pellet was resuspended in 1:50 (V/V) of the total volume of the bacterial culture in PBS, and then sonicated for 2-3 minutes on ice. The crude lysate was centrifuged at 12,000 rpm in Sorvall SS34 rotor at 4°C for 10 minutes and clear supernatant was further clarified by filtration through 0.45µm filter according to Smith and Johnson (1988).

2.4. Purification of recombinant protein using ion exchange chromatography

Recombinant protein was purified from the bacterial lysate by passing over a cation exchange column, SP-Sephadex C-50 (Pharmacia). The protein was eluted with a pH gradient (pH from 7.2 to 10.8) made with 0.1 M Tris-glycine buffer. The SMS01 recombinant protein was eluted at pH 9.4 (corresponding with the predicted PI of the protein). The eluted fractions were evaluated by SDS-PAGE to identify the fractions of the purified protein.

2.5. Detection of the purified fused protein using SDS-PAGE

Sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate the fused and purified SMSO1 protein (Laemmli 1970; Russel and Blair 1977). In brief, separated gels were composed of 10% acrylamide and stacking gel was formulated as *5%* acrylamide. Electrophoresis was carried out at a constant 150 volt in an electrode buffer pH 8.3. The gel was used for Western blot analysis or stained with coomassie brilliant blue for 30 minutes.

2.6. Western blot analysis

To detect the antigenic proteins immobilized on nitrocellulose membranes, the sensitive technique of Brunette (1981) was carried out. Recombinant proteins were separated on SD S-PAGE and transferred onto on PVDF membrane in a transfer unit (Mini Trans Blot Bio-Rad) using lx transfer buffer. The transfer was carried out at 80 volt for 2 hrs at 4°C. The membranes were blocked with 5% non-fatty dry milk in TBST for 2hrs then, incubated for 60 minutes with the primary antibody (rabbit serum) at room temperature with gentle agitation. Then the blots were washed with 3 changes of TBST and incubated at room temperature for 1 hr with alkaline phosphatase conjugated secondary antibody (goat anti rabbit IgG fraction). Furthermore the membranes were washed 3 times with TBST for color development solution (BCIP/NBT) until signals become clearly visible. Finally the membranes were rinsed with TBST then immersed in stopping solution for 1 minute air dried and stored protected from light.

2.7. Preparation of antibodies against SMS01 fusion protein

The purified fused protein SMS01 was used as an antigen to immunize New Zealand female rabbits (Green and Manson 1992). For primary immunization, the antigen in PBS was emulsified with complete Freund's adjuvant (100µg/animal) and used to inject the animals subcutaneously in multiple locations. Four weeks later, a booster dose of the antigen (50 µg/animal) emulsified with incomplete Freund's adjuvant (without the bacterial extract) was injected. After three weeks, an activating dose of the antigen emulsified with

incomplete Freund's adjuvant was injected into the animals. Blood samples were collected by ear vein puncture 2 weeks after each immunization and sera were used for western blot analysis.

2.8. Vaccination experiments

In order to assess the importance of SMS01 as a vaccine candidate, the groups of female Swiss albino mice were injected intramuscularly. The first group was injected with (50 µg /mouse) with purified SMS01-pcDNA1 DNA. The second group was injected with the SMS01 recombinant protein emulsified with incomplete Freund's adjuvant. The mixed group was injected with a cocktail vaccine (DNA and protein) as discussed before, but a space of time was left between the two injections to reduce the mortality rate. It has been recorded that death may be occurred at once if both injections were given at the same time. For that we can say that a chemical shock may take place in this case. Then the fourth group (control) of mice was not vaccinated. In each group, the zero time was detected briefly after which the first injection was performed. Three weeks later, after the first injection each mouse in each group was boosted with either 50 µg DNA or 50 µg proteins or with both as in case of the cocktail group. The animals were also boosted for a second time as to predict the best immunological response. Blood samples were collected and the sera were tested by ELISA for the production of antibodies against recombinant antigen

2.9. Challenge infection and evaluation of the worm burden

Vaccinated and control groups of mice, three weeks after the last immunization, were exposed to 100 *S. mansoni* cercariae for challenge by the tail immersion method (Oliver and Stirewalt 1952). Six weeks later, the animals of each group were necropsied and worms were recovered from the hepatic and portomesenteric vessels using the perfusion technique and the percent of protection was calculated using the formula (% Protection = (C-T/C) X 100). Where(C: the mean worm burden of the control animals, T: the mean worm burden of the tested animals) and dead animals were excluded.

2.10. Enzyme-Linked Immunosorbant Assay (ELISA)

The ELISA was done as described by **Hillyer *et al* (1979).** Briefly, microtitre plates were coated with 3-5µg/ml of recombinant SMS01 protein in 0.05M carbonate buffer pH9.6(l00 µl /well), and incubated at room temperature overnight. Plates were washed twice with PBS/Tween (2-3 minutes each)then dried on tissue paper. The plates were blocked for non specific binding with PBS containing 1% BSA (100 µl/well). Then plates were incubited at 37°C for one hour followed by 3 washes with PBS/Tween. Addition of 100µl of diluted serum/well was added followed by incubation for 1.15 hr at 37°C. Washing 3 times with PBS/Tween (l00 µl /well) was repeated then 100µl/well of diluted secondary antibody (1µl/ml in PBS-Tween) was added and plates were incubated at 37°C for 45 minutes. Subsequently, the plates were washed with PBS/Tween for 3 times with shaking. Substrate solution (NBT and BCIP) was added and the plates were left in dark for 30 minutes. The reaction is finally stopped using 0.4N NaOH(l00µl/well) and the absorhance was measured at 405 nm using Bio Tek ELISA reader (Roitt *et al*. 1998).

2.11. Oogram pattern and tissue egg load

Three fragments of the small intestine (from the middle part of the small intestine) were cut longitudinally, washed with saline, compressed between two microscope slides, and examined under a low-power microscope. A total of 100 eggs per animal were observed, and the stage of each egg and the mean number of the different stages were recorded. The number of eggs/gm liver or intestine was calculated according to (Pellegrino et al.,1962). Viable eggs were counted and classified according to their degree of development into the following stages: Stage I: Embryo, one third the diameter of the egg shell. Stage II: Embryo, one half the diameter of the egg shell. Stage III: Embryo, two thirds the length of the egg shell. Stage IV: Embryo, occupying the entire egg shell.

The mature egg contains a fully, developed miracidium. Dead egg, appearing as semi transparent, granular or black eggs, were also counted.

The tissue egg load was determined as described by (Kloetzel et al.,1967). Where, 0.3 g of liver and small intestine was taken from each mouse and digested overnight in 5 mL KOH (5%). Then, after complete digestion, the samples were vortexed, and three aliquots of 100 µL each were examined microscopically. Subsequently, all *S. mansoni* eggs were counted. The hepatic and intestinal tissue egg loads were determined by multiplying the number of eggs in each 100µL sample by the total volume of KOH and dividing this value by the weight of the sample in gram.

2.9.3 Statistical analysis

Statistical significance was determined by student's t-test and significance was determined using a P value<0.05 as being significant.

3.Results

3.1 Identification of SMS01-pcDNA1

The SMS01 gene was amplified by PCR as (approximately 500 bP)which is the right size of the gene and was confirmed by sequence and restriction enzymes digestion as shown in Figure(1).

3.2 Expression and purification of protein

The SMS01 expressed protein by pET-3a system was purified on a cation exchange column using pH gradient. SMS01 protein was eluted at pH9.4 is the PI of the protein. The eluted fractions were analyzed by SDS-PAGE and confirmed by western blot analysis as a single band using sera from rabbit immunized with SMS01 recombinant protein (Figure 2).

3.3 Worm burden and the capacity of protection

The number of worms burden in sacrified animals in different groups was shown in Fig (3). Results showed that the vaccinated mice with SMS01 fusion protein have produced the best level of protection (63%) against infection with *S. mansoni* and vaccinated mice with SMS01 DNA have brought such a considerable protection level (about 55.36%). On the other hand, a protection of about 46.81% can be deduced in mice vaccinated with both of SMS01-pcDNA and the SMS01 fusion protein. The statistical analysis of the data obtained from the worm counts in all animals in the study is shown in Table (1). There were significant differences in worm burden between all vaccinated groups and the control($P < 0.001$). In addition a significant difference was also found between the group of vaccinted with fusion and the mixed group ($P < 0.01$).

3.4 Ova count and antifecundity effects

Ova count was determined as it is an indication for the antifecundity effects. Ova count in different groups was briefly illustrated in Figure **(4).** The immunized mice using SMS01 fusion protein as a vaccine against *S. mansoni* infection, has proved to be such a successful antifecundity vaccine as it has produced a remarkable reduction of ova count in liver (mean = 2630 ± 1135.59) and in intestine (6000 ± 1536.91). The reduction of ova count in immunized mice with DNA was (mean = 3684.21 ± 712.79) in liver and (mean=7684.21 ± 1500.09) in intestine respectively. The mixed group was produced the lower reduction of ova count (mean = 5094.73 ± 2084.19 in case of liver and mean=9368.42 ± 2962.92 in intestine) when compared to control group.

Statistical analysis of the data obtained from the ova counts in case of liver and intestine from all animals in different groups is summarized in Tables (2) and Table(3). Ova count in both of liver and intestine was proved to be highly significant with respect to the control in all vaccinated groups ($P < 0.001$). Concerning ova count in liver, noticeable difference could be seen between the DNA and protein groups ($P < 0.05$), as well as between the mixed group and each of the later ones ($P < 0.01$, $P < 0.001$ respectively). While in case of

the intestine, there was a considerable difference between the DNA and protein groups ($P < 0.01$). A significant difference could also be deduced between the mixed group and each of the DNA and the protein groups. As in case of the liver, same difference could be deduced between the mixed group and each of the other two groups.

3.5 Oogram pattern

The oogram pattern was considered to be such a very useful test, as to make such an accurate differentiation between groups concerning different stages of ova found after vaccination . In this work , it was found that the total number of dead ova could assign at which level the vaccine used was successful (Figure %). Results showed that all vaccinated groups have produced a highly significant increase in the number of dead ova with respect to the control ($P < 0.001$). Where the number of dead ova (Figure 5) was remarkably high (mean = 69.9 ± 5.40) in mice vaccinated with SMS01 fusion protein when compared with both DNA group (mean = 55.76 ± 4.24) and the mixed group (mean = 41.2 ± 4.25). The protein group has also produced such a considerable reduction in the immature stage (mean = 21.03 ± 6.76). In addition a noticeable significant difference could be found between the mixed group and each of the other two groups in case of dead ova ($P < 0.001$)(Tables 3 and 4).

Results showed that all the vaccinated groups have produced a significant reduction in the mature stage with respect to the control group. The mean number of the mature stage was, (9.39 ± 3.64), (10298 ± 5.05) and (13.03 ± 5.64) in mice vaccinated with protein, DNA and mixed group respectively when compared to the control group (Fig 5). The mature stage was highly reduced in case of all groups ($P < 0.001$) with respect to the control).

The protein group has produced the best significant difference in case of the immature stage ($P < 0.01$). Also, a slightly significant difference could be denoted concerning the mixed group ($P < 0.05$). Upon comparing between groups: A very high significant difference could be seen between the DNA group and the protein one ($P < 0.002$ in case of immature stage, $P < 0.001$ in case of dead ova). In addition, results showed no significant difference between the mixed group and the protein one ($P < 0.1$) concerning the mature stage. There is a significant difference between the mix group and the DNA group ($P < 0.01$) as well as the protein one ($P < 0.001$) was observed in immature stage (Tables 3 and 4).

3.6 Detection of Anti SMS01 IgG in immunized mice

ELISA was considered to be such a successful test for the detection of antibody titers in vaccinated group of animals (Table 5). Results showed that the highest IgG titre (mean = 1.77 ± 0.144) was obtained in vaccinated animals with fusion protein. While the DNA group has produced a considerable antibody titer (mean = 1.57 ± 0.28), the mixed group, has produced the lower IgG titre (mean = 1.38 ± 0.39) as showed in Figure (5). Statistical data as showed in Table (5) revealed that, all groups have produced a significant difference with respect to the control ($P < 0.001$). The IgG antibody titre in the protein group exceeds both that found in both of the DNA group and the mixed group by such a remarkable significant difference ($P < 0.01$, $P < 0.001$ respectively). A slightly low significant difference could be observed between the DNA group and the mixed group one ($P < 0.1$)

4. Discusion

Human schistosomiasis, a chronic and debilitating parasitic disease of the tropics, is ranked second after Malaria in terms of public health importance. At present, there is no vaccine available and chemotherapy is the cornerstone of schistosomiasis control. Praziquantel is the drug of choice (Utzinger *et al.* 2001). Inspite of safe and efficacious drugs, schistosomiasis still ranks high on the list of endemic diseases of public health importance in the world, in part due to rapid reinfection rates which demand frequent

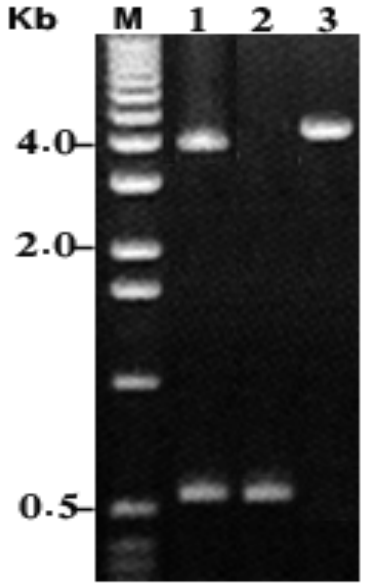

Fig (1): Agarose gel electrophoresis for the digestion product of recombinant pcDNA1-SMS01 clones DNA to determine the insert presence. M: 1Kb DNA marker. Lane (1) represents DNA of recombinant pcDNA1 clones digested with *BamHI*. Lane (3) PCR products of SMS01. Lane (3): SMS01 digested with *EcoRI*.

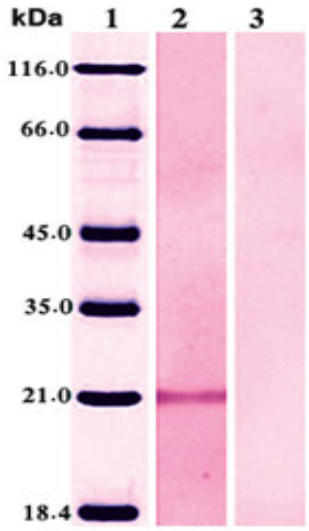

Fig (2): Western blot analysis showing the reactivity of vaccinated rabbit serum with SMS01 purified protein compared to the non recombinant pET-3a vector. Lane 1: High molecular weight protein, Lane (2): Specific band at molecular size of 21.7 kDa which is the molecular weight of the fused protein. Lane (3) Non recombinant bacterial lysate with no response.

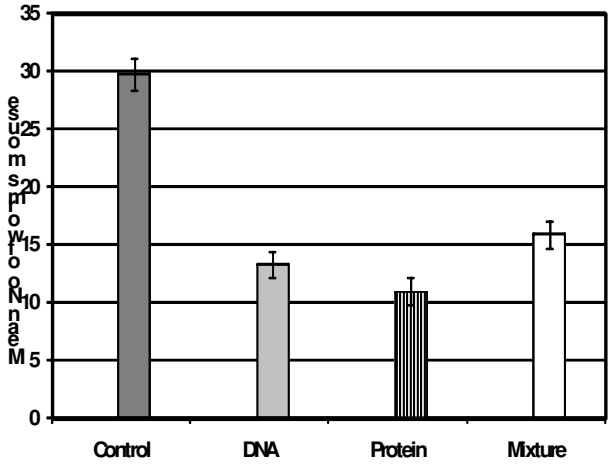

Figure (3): Changes of worm burden in immunized and control mice sacrificed six weeks after challenge infection. The percentage of protection was calculated by perfusions of adult worms at six week post-challenge infection. The vaccinated mice with SMS01 protein have reached the maximum level of protection (63%). While the DNA groups exhibit a considerable level of protection (55.36%), the lowest level (46.81%) was obtained by the mixed group compared to the control group.

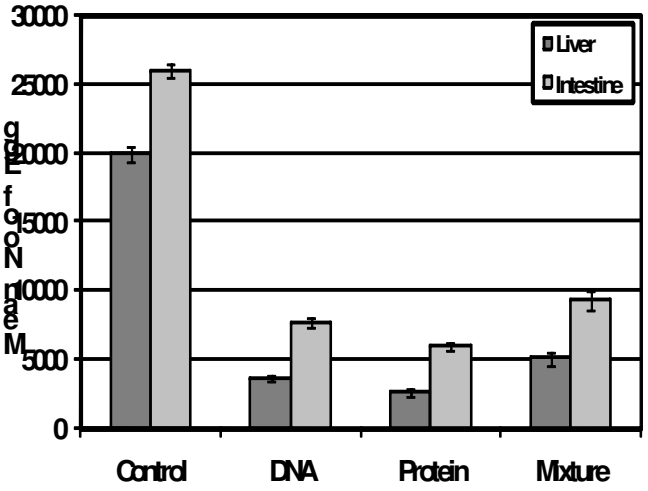

Figure (4): Changes in ova count in immunized and control mice (livers and intestines) sacrificed six weeks after challenge infection. Vaccinated mice with SMS01 has proved to be a successful antifecundity and produced a remarkable reduction in ova count in livers (mean=2630±113.90) and intestines (mean=600±1536.91). SMS01 DNA revealed a level of reduction about (mean =3684.21±721.79) in livers and (mean =7684.21±1500.09) in intestines, and finally the mixed group denoted a level of reduction in livers (5094.73±2084.19) and (9368.42±2962.92) in intestines when compared to the control group.

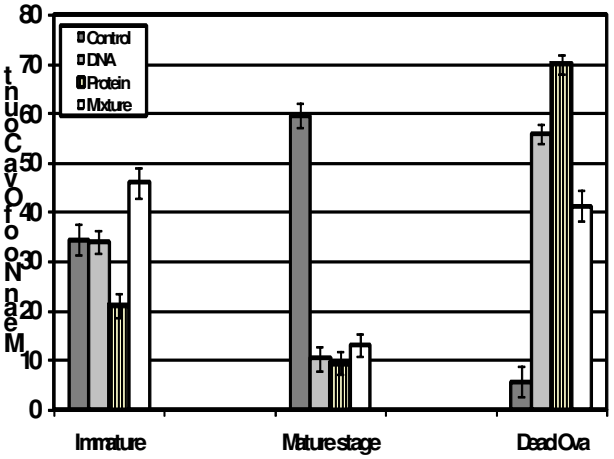

Figure (5): The oogram pattern in immunized and control of mice vaccinated with either the DNA or the protein or both and sacrificed six weeks after challenge infection. The number of dead ova was remarkably high in the protein group (mean=69.9± 5.40) when compared with DNA group (mean =55.67 ±4.24) or Mixed group (mean= 41.2±4.25). The protein group has also produced such a considerable reduction in the immature stage (mean = 21.03 ± 6.76). The mean number of the mature stage was, (mean=9.39 ± 3.64), (mean=10298 ± 5.05) and (mean=13.03 ± 5.64) in mice vaccinated with protein, DNA and mixed group respectively when compared to the control group.

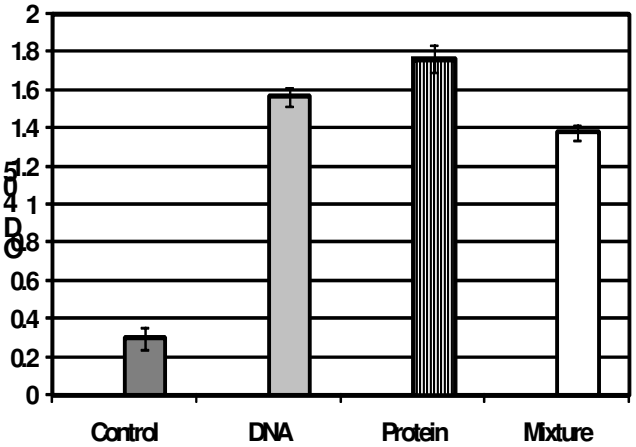

Figure (6): IgG antibody responses against crude adult worm antigens (SWAP) in mice immunized animals compared to the control groups at six weeks post-challenge and control. The protein group have produced the highest IgG titre (mean=1.77 ±0.14), considerable titre was produced by DNA group (mean=1.57 ±0.28). While mixed group was reached a lower tire (mean =1.38 ±0.39).

Table (1): Statistical analysis of Changes of worm burden in immunized and control mice sacrificed six weeks after challenge infection.

Group A	Group B	Mean difference	Standard error (±)	Significance
Control	DNA	16.45	2.23	< 0.001
	Protein	18.80	2.23	< 0.001
	Mix	13.90	2.23	< 0.001
DNA	Control	-16.45	2.23	< 0.001
	Protein	2.35	1.82	< 0.1
	Mix	-2.55	1.82	< 0.1
Protein	Control	-18.80	2.23	< 0.001
	DNA	-2.35	1.82	< 0.1
	Mix	-4.90	1.82	< 0.01
Mixed	Control	-13.90	2.23	< 0.001
	DNA	2.55	1.82	< 0.1
	Protein	4.90	1.82	< 0.01

Table (2): Statistical analysis in ova count in immunized and control livers of mice sacrificed six weeks after challenge infection

Group (A)	**Group (B)**	**Mean difference**	**Standard error (±)**	**Significance**
Control	**DNA**	**16315.78**	**580.4**	**< 0.001**
	Protein	**17370.00**	**575.4**	**< 0.001**
	Mix	**14905.26**	**580.4**	**< 0.001**
DNA	**Control**	**-16315.78**	**580.4**	**< 0.001**
	Protein	**1054.21**	**475.97**	**< 0.05**
	Mix	**-1410.52**	**482.04**	**< 0.01**
Protein	**Control**	**-17370.00**	**575.42**	**< 0.001**
	DNA	**-1054.21**	**475.97**	**< 0.05**
	Mix	**-2464.73**	**475.97**	**< 0.001**
Mixed	**Control**	**-14905.26**	**580.45**	**< 0.001**
	DNA	**1410.52**	**482.04**	**< 0.01**
	Protein	**2464.73**	**475.977**	**< 0.001**

retreatment. In addition the potential development of drug resistance emphasizes the need for a long-term approach such as the development of a protective vaccine (Da'dara *et al.* 2001).

One of the main strategies for vaccine development is based upon the identification of larval stage antigens that elicit highly protective immune response in vaccinated hosts (Hota-Mitchell *et al*. 1999). One of these important genes,

Table (3): Statistical analysis of Changes in ova count in immunized and control intestines of mice sacrificed six weeks after challenge infection

Group (A)	Group (B)	Mean difference	Standard error (±)	Significance
Control	DNA	18315.78	792.58	< 0.001
	Protein	2000.00	785.72	< 0.001
	Mix	16631.57	792.58	< 0.001
DNA	Control	-18315.78	792.58	< 0.001
	Protein	1684.21	649.92	< 0.01
	Mix	-1684.21	658.20	< 0.01
Protein	Control	-2000.00	785.72	< 0.001
	DNA	-1684.21	649.92	< 0.01
	Mix	-3368.42	649.92	< 0.001
Mixed	Control	-16631.57	792.58	< 0.001
	DNA	1684.21	658.20	< 0.01
	Protein	3368.42	649.92	< 0.001

Table (5): Statistical data of the ELISA readings in immunized and control mice sacrificed six weeks after challenge infection.

Group (A)	Group (B)	(t) test	Significance
Control	DNA	-19.94	< 0.001
	Protein	-40.14	< 0.001
	Mix	-11.62	< 0.001
DNA	Control	19.94	< 0.001
	Protein	-2.75	< 0.01
	Mix	1.70	< 0.1
Protein	Control	40.14	< 0.001
	DNA	2.756	< 0.01
	Mix	3.88	< 0.001
Mixed	Control	0.36	< 0.001
	DNA	-1.70	< 0.1
	Protein	-3.88	< 0.001

SMS01 (Sm2 1 .7) was identified as located in the tegumental region and dispersed among the parenchyma tissue of liver worms schistosome parasite (Ahmed *et al.* 2001). Since the tegument is the outer covering of the parasite and serves as an interface between the host immune system and the parasite the antigen associated with the tegument would be the major focus for deve1opnent of vaccine and/or immunodiagnostic reagents for schistosomiasis (Bergquist 1992). Therefore localization of the 21.7 kDa protein in tegument and subtegumental layers would likely confirm the importance of this protein as a target of all host's protective response to S. *mansoni* infection (Ahmed *et al. 2001)*

In this study to evaluate the efficacy of combined SMS01 DNA and protein as a cocktail vaccine against *Schistosoma mansoni* challenge infection, the vaccination models were applied using DNA vaccination and fusion protein or both. Thus SMS01 gene was cloned into the eukaryotic expression vector pcDNA1/Amp as to estimate its protective capacity. This vector, with its CMV promoter, has high transcription and expression levels in different mammalian cells (Wang *et al.* 1998). The expression of SMS01 recombinant protein was carried out in the pET-3a vector, and recombinant SMS01 protein was purified according to Ahmed et al. (1997).

In this work DNA vaccine was injected intramuscularly as it appeared to generate the best immune response (Fynan *et al.* 1993) while protein was injected intraperitoneally. All groups in the immunization experiments were challenged with 100 cercariae 4 weeks after the last boost and six weeks later worm burdens were analyzed, to detect their ability for protection against *S. mansoni* infection. Results showed that in all vaccinated groups induced statistically significant levels of protection to challenge infection. The groups of mice vaccinated with SMS01 DNA have significantly reduced the worm burden by (55.36%, $P < 0.001$) and by (46.81%, $P < 0.001$) in mixed group (Figure 3). The highest significant reduction levels were obtained by mice vaccinated with recombinant protein (63.08%, $P < 0.001$). No significant difference between the vaccinated groups could be detected, except in case of the recombinant protein and the mixed group ($P < 0.01$). A remarkable production of specific anti-SMS01 antibodies in female Swiss albino mice was found in all vaccinated groups, which was confirmed by ELISA ($P < 0.001$) in all groups, with respect to the control group (Table 1).

A significant difference in IgG titre could be detected between groups of mice vaccinated with DNA and recombinant protein ($P < 0.01$). Such a

noticeable difference could be detected between the mixed vaccinated groups with both of the DNA and the recombinant protein vaccinated group ($P < 0.1$ and $P < 0.001$ respectively). These data are in agreement with Anderson's hypothesis, which supported the idea of antibody involvement in the augmented protection of multiply vaccinated C57Bl/6 mice. Multiple vaccinations with irradiated cercariae led to an increase in the level of protection from 59 to 82%. Since antibody titre was elevated, it was concluded that the additional protection was the result of antibody mediated mechanisms (Anderson *et al.* 1999).

Our results showed that all vaccination groups of mice have induced an antifecundity effect. The total ova count in livers and intestines

Table (4): Statistical data of oogram pattern in immunized and control in mice sacrificed six weeks after challenge infection

Group (A)	Group (B)	Stage	Calculation of (t) test	Significance
Control	DNA Protein Mix	Immature Stage	0.10 2.91 -2.45	< 0.1 < 0.01 < 0.05
Control	DNA Protein Mix	Mature Stage	11.94 12.63 11.07	< 0.001 < 0.001 < 0.001
Control	DNA Protein Mixed	Dead Ova	-32.59 -34.41 -23.06	< 0.001 < 0.001 < 0.001
DNA	Control Protein Mixed	Immature Stage	-0.10 3.82 -3.46	< 0.1 < 0.002 < 0.01
DNA	Control Protein Mixed	Mature Stage	-11.94 0.45 -1.13	< 0.001 < 0.1 < 0.1
DNA	Control Protein Mixed	Dead Ova	32.59 -6.53 7.64	< 0.001 < 0.001 < 0.001
Protein	Control DNA Mixed	Immature Stage	-2.91 -3.82 -7.97	< 0.01 < 0.002 < 0.001
Protein	Control DNA Mixed	Mature Stage	-12.63 -0.45 -1.70	< 0.001 < 0.1 < 0. 1
Protein	Control DNA Mixed	Dead Ova	34.42 6.53 13.20	< 0.001 < 0.001 < 0.001
Mixed	Control DNA Protein	Immature Stage	2.45 3.46 7.97	< 0.05 < 0.01 < 0.001
Mixed	Control DNA Protein	Mature Stage	-11.07 1.13 1.70	< 0.001 < 0.1 < 0.1
Mixed	Control DNA Protein	Dead Ova	23.06 -14.53 -13.20	< 0.001 < 0.001 < 0.001

were significantly reduced in all groups compared to the control ($P < 0.001$). The mice groups vaccinated with recombinant protein showed the best antifecundity effect when compared to either the DNA or the mixed vaccinated group in liver. A noticeable difference in case of liver could be seen between the DNA and protein group ($p<0.05$), as well as between the mixed group, DNA and protein groups ($p<0.01$ and $p<0.001$) respectively. There was a considerable difference in case of intestine could be seen between the DNA vaccinated groups and protein group ($p<0.01$). Also, a significance difference could be deduced between the mixed group and each of DNA group and protein group. As in case of liver, same difference could be deduced between the mixed group and DNA or protein group. Furthermore concerning the viability of ova and oogram, it was concluded that the recombinant protein vaccinated groups showed the best protection model with respect to the control; it was the only group that showed a significant decrease in the immature stage ($P < 0.01$).

Our results showed that a successful decrease in the mature stage as well as a significant increase in the number of dead ova could be observed in all groups ($P < 0.001$) compared to the control. The mature stage was reduced in all groups ($p<0.001$) compared to the control group. The protein group has produced the best significant difference in the immature stage ($p<0.01$). Also, a slightly significance difference could be denoted concerning the mixed group ($p<0.05$). A very high significant difference could be seen between the DNA group and protein group ($p<0.001$ in case of dead ova. Noticeable significant difference could be found between the mixed group and the DNA group ($p<0.01$) as well as the protein group ($p<0.001$). There is no high difference could be seen between the mixed group and the protein group ($p<0.1$).

In genetic vaccination, the DNA vector carrying the genetic code for a pathogenic antigen is taken up into cells and transcribed in the nucleus. Messenger RNA is translated into protein in the cytoplasm. The gene product (protein antigen) is ultimately degraded by proteosomes into intracellular peptides. Being produced in the host cell, the antigen is processed through the MHC class I system and thus stimulates a cell mediated/cytotoxic T-cell response when presented on the cell surface (Gilsdorf 1994 ; McDonnell *et al.* 1996). In spite of the fact that this work has proved that vaccination with recombinant SMS01 created the best protection (vaccination) model against *S.mansoni* we can't deny the advantages of DNA vaccination criteria vaccination with DNA is probably more simple and cost-effective than with conventional protein preparations, thus raising hopes for use against infectious diseases in developing countries, where DNA vaccination may therefore, become the poor man's gene therapy(Chlichlia *el al.*2002).

5. Conclusion

The cocktail vaccination model was considered very successful against *Leishmania major* either by using plasmid DNA encoding TSA/LmSTI1 leishmanial fusion proteins (Ameen 2007) or by using DNA encoding cysteine proteinases (Rafati *et al.* 2001). In this work, the cocktail vaccination model has produced a successful protection results with respect to the control. On the other hand, it could be denoted that each of the DNA or the protein vaccination models was still more protective against *S. mansoni* than the cocktail one. Still, there is a long way from an ideal vaccine as we not deny that experimental animals vaccination models can not adequately represent the human situation.

Actually studies on experimental models have been highly productive and are still much needed but may not adequately represent the human situation. SMS01 may be such a crucial antigen, and several strategies remained to be tried such as; changing vaccination protocols (amount of vectors. route of infection, number of boosters and intervals), vectors (choosing stronger promoter) and adjuvant molecules (JL-12 for example), check protection after chemotherapy. We may still have a long way from an ideal vaccine that gives complete protection against schistosome infection but hopefully progressing in the right direction.

6. Acknowledgments

This work was supported by a grant from Theodor Bilharz Research Institute (Grant No 74 M, to MA Saber) and by The Academy of Scientific Research and Technology (grant to M M Sadek).

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