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***Schistosoma mansoni* antigen Sm-p80: Prophylactic efficacy of a vaccine formulated in human approved plasmid vector and adjuvant (VR 1020 and alum)**

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Abstract

Schistosomiasis is an important parasitic disease. Consensus is developing now that ideal control methods of the disease should be based on an integrated approach incorporating drug treatment, sanitation improvement, education, and an effective vaccine. With regards to the vaccine development, Sm-p80 has been shown to be a promising and strong immunogenic vaccine candidate. In the present study, Sm-p80-based vaccine formulated in alum was tested for its prophylactic efficacy in a mouse model. It was observed that vaccination using heterologous prime boost (DNA prime followed by boost with protein formulated in alum) and homologous prime boost (both prime and boost with protein formulated in alum) approaches, resulted in 61% and 55% reduction in worm burden, respectively. The protection was directly correlated with the induction of high titers of antibody responses that mainly included IgG, its isotypes, and IgM. In addition, both of the immunization approaches triggered a mixed Th1 and Th2 type response. Some involvement of Th17 specific immune response was also detected as indicated by the up-regulation of relevant cytokines. These results reinforce the potential of Sm-p80 as a viable vaccine candidate.

Keywords

Schistosoma; Sm-p80; Calpain; Vaccine; Prime-boost

1. Introduction

As one of the oldest parasitic diseases, the history of schistosomiasis can be traced back to the 20th Dynasty of ancient Egypt (Coon, 2005; Tan et al., 2007). The widespread

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distribution and transmission of this disease has enormous implications on the human health. Currently this disease is endemic in more than 76 countries and approximately 210 million people are infected and an additional 779 million people at risk of acquiring the infection (Steinmann et al., 2006; Hotez et al., 2007; Berriman et al., 2009). To reduce the threat of infection with parasites, a global approach based on mass drug administration has been employed in many countries (Fenwick et al., 2009; Webster et al., 2009). The treatment of schistosomiasis principally relies on a single drug, praziquantel, developed over 30 years ago. However, *Schistosoma mansoni* can develop tolerance or resistance to praziquantel, and up to now these mechanisms have been poorly understood (James et al., 2009). Emergence of drug resistance makes the long-term planning solely based on praziquantel uncertain. In addition, praziquantel cannot provide protection from re-infection (Berriman et al., 2009; James et al., 2009). Therefore, coordination of chemotherapy coupled with vaccination and other conventional approaches has the potential of achieving sustained control that may ultimately lead to the eradication of the disease.

To develop an effective vaccine, identification of a specific antigen as a vaccine candidate is a crucial task. To this effect we have targeted the large subunit of calpain (=Sm-p80) and have shown its protective and antifecundity potential in both the mouse and the nonhuman primate models (Siddiqui et al., 2003a; Siddiqui et al., 2003b; Siddiqui et al., 2005a; Siddiqui et al., 2005b; Ahmad et al., 2009a; Ahmad et al., 2009b; Ahmad et al., 2009c; Ahmad et al., 2010; Zhang et al., 2010a; Zhang et al., 2010b;). In our continual efforts to improve and refine the efficacy of a Sm-p80-based vaccine, in the present study, we have investigated the feasibility of using alum as an adjuvant for presenting Sm-p80 to the host immune system.

2. Materials and methods

2.1 Hosts and parasites

All of the animals (female C57BL/6 mice) were purchased from Charles River Laboratories International Inc. (Wilmington, MA, USA). The National Institutes of Health (NIH) supported Schistosomiasis Resource Center (Biomedical Research Institute, Rockville, MD, USA) provided the *S. mansoni* infected *Biomphalaria glabrata* snails.

2.2. Construction of plasmid, purification of DNA and recombinant protein

For DNA vaccine preparation, the large subunit of *S. mansoni* calpain, (Sm-p80) was inserted between *Bam*HI and *Bgl*II sites of VR1020 (Vical Incorporated, San Diego, CA, USA). The expression of Sm-p80 was ascertained by western blot after transfection into CHO and COS-7 cells as described earlier (Ahmad et al., 2009c; Zhang et al., 2010a; Zhang et al., 2010b). The plasmid DNA was obtained using the conventional alkaline lysis method was further purified on Sepharose CL4B columns. The purified DNA was then ethanol precipitated and resuspended in sterile, endotoxin-free saline. For recombinant protein expression, Sm-p80 was subcloned into pCold II vector (GenScript Corp., Piscataway, NJ) and expressed in *Escherichia coli* strain BL21 (DE3) (Invitrogen Corp., Carlsbad, CA). The details of expression and purification have been described previously (Ahmad et al., 2009a; Ahmad et al., 2009b; Ahmad et al., 2009c; Zhang et al., 2010a; Zhang et al., 2010b). Endotoxin levels were determined via *Limulus* amoebocyte lysate assay (Charles River Laboratories International Inc., Wilmington MA) respectively. The plasmid DNA as well as recombinant protein used in immunizations contained minimal endotoxin levels (approximately 0.06 EU/ml) which are acceptable levels, approved for human use by the United States Food and Drug Administration.

2.3. Immunization strategy

Two different immunization regimens were carried out simultaneously. Each of the two immunization regimens consisted of 30 animals, 15 animals each allocated for control and experimental groups, respectively. Fifteen mice were further divided into 3 sub-groups of 5 for the three sets of experiments. Each of the three experiments was performed independently. The details of experimental protocol are summarized in Table 1.

2.4. Challenge infection, necropsy of animals, worm and egg burden determinations

All of the animals were challenged with 150 cercariae of *S. mansoni* via tail exposure method four weeks after the second boost. The animals were sacrificed 6 weeks post-challenge and the adult worms were recovered. The number of worms recovered from each mouse (worm burden) was recorded and percentage reduction in worm burdens in vaccinated versus control animals was calculated. After sacrifice, liver and intestine samples were collected from each animal and digested in 4% KOH. The number of eggs present in the tissue was determined and percent reduction in egg production was calculated.

2.5. Antibody response assay

Blood samples were collected from all of the animals at 2 week intervals for the duration of the immunizations and the isolated sera were used to determine the various antibody titers via an enzyme linked immunosorbent assay (ELISA) (Ahmad et al., 2009a; Ahmad et al., 2009b; Ahmad et al., 2009c; Zhang et al., 2010a; Zhang et al., 2010b). Briefly, after coating each well of the microtiter plate with 1.2 µg recombinant Sm-p80, the plates were washed 3 times, and incubated with the serially diluted test sera and subsequently with the optimally diluted horseradish peroxidase-labeled secondary antibody. All of the samples were assayed in triplicate. Results are expressed as end point titers calculated from a curve of optical density verses serum dilution to a cut-off of two standard deviations above background control values.

2.6. Cell proliferation and Cytokine production Assay

For splenocyte proliferation assays and determinations of the Th1/Th2 cytokines (IL-2, IL-4, IL-10 and IFN-γ), single cell suspensions were prepared from the spleens of the control and experimental groups of animals as described elsewhere (Siddiqui et al., 2003a). The splenocytes (5×10^5 cells per well) were stimulated with either 0.5 µg concanavalin A (ConA) or 1.2 µg recombinant Sm-p80 in a final volume of 200 µl medium. After 48 h incubation with 5% CO₂ at 37°C, 100 µl supernatant was removed for cytokine production assay. Th1/Th2 cytokines (IL-2, IL-4, IL-10, and IFN-γ) were measured using a murine cytokine Th1/Th2 ELISA panel kit (eBiosciences Inc., San Diego, CA, USA), following the procedure provided by the manufacturer. The remainders of the cell cultures were continued for cell proliferation assays as described previously (Ahmad et al., 2009a; Ahmad et al., 2009b; Ahmad et al., 2009c; Zhang et al., 2010a; Zhang et al., 2010b).

2.7. Flow cytometry

The single cell suspension of splenocytes were adjusted to 10^7 /ml cells and maintained in RPMI-1640 supplemented with 20% (v/v) fetal calf serum in 24-well plates in the presence of 100 ng/ml phorbol-12-myristate-13-acetate (PMA; Sigma), 1 µg/ml ionomycin or 12 µg/ml rSm-p80 overnight at 37°C in a humidified atmosphere containing 5% CO₂. Four hours before the end of incubation period, Brefeldin A was added in a final dilution of 1:1000. Direct staining of the cultured cells was carried out as per the procedures provided by the manufacturers (eBiosciences Inc., San Diego, CA, USA). Briefly, FITC-labeled anti-mouse CD3 antibody and Peridinin-chlorophyll proteins-Cy5.5 (PerCP-Cy5.5) labeled anti-mouse CD8 antibody were reacted with the cells for 30 min at 4°C in the dark (Jia et al., 2006). The

cells were washed with 10 mM phosphate buffered saline-Tween (PBS-T, pH 7.4). The fixation and permeabilization of the cells were carried out as per the method provided with the kit (eBiosciences Inc., San Diego, CA, USA). Phycoerythrin (PE)-labeled anti-mouse gamma interferon antibody and anti-mouse interleukin 4 (IL-4) antibodies were added separately to the cells and incubated for 20 min at room temperature in the dark. After washing, these cells were then re-suspended in 500 μ l PBS. Intracellular expression of IFN- γ and IL-4 by CD3⁺CD8⁻ and CD3⁺CD8⁺ cells was estimated by flow cytometry (Glück et al., 2007). Data were analyzed using BD CellQuestTM Pro software.

2.8. RT-PCR for determinations of cytokines profiles

Total RNA was extracted from splenocytes of C57BL/6 mice before and after stimulation of the cells with rSm-p80. TRIzol reagent was used for the extractions of RNA according to the protocols provided by the manufacturers (Invitrogen, Carlsbad, CA, USA). The details of reverse transcription reactions for first strand cDNA synthesis, the expression of various cytokines by semi quantitative PCR and visualization by electrophoresis in 2% agarose gel have been described elsewhere (Ahmad et al., 2009a; Ahmad et al., 2009b; Ahmad et al., 2009c; Zhang et al., 2010a; Zhang et al., 2010b). After acquiring the images of the RT-PCR ethidium bromide-stained agarose gels, quantification of the bands were performed by Quantity One Computer Program (Bio-Rad, Hercules, CA, USA).

2.9. Statistical analysis

Significance between different immunization groups was calculated by one-way ANOVA and in two group significance by paired t-test using SPSS computer program (version 13.0; SPSS Inc). Bonferroni adjustments were included to reduce the risk of reaching false conclusions. Results were considered statistically significant when the analysis returned a $P < 0.05$.

3. Results

3.1. Reduction of worm burden and egg load

The protective and egg reduction effect of the Sm-p80-based vaccine using two immunization regimens was determined. Mice inoculated with the first regimen (heterologous immunization, combination of Sm-p80 DNA and recombinant protein with alum) demonstrated a 60.99% reduction in worm burden, compared with control group that received only the empty vector VR1020 and alum ($P \leq 0.013$). A distinct egg reduction was also observed in this group of animals (23.32% reduction in egg count) as compared with the control group ($P \leq 0.05$). The worm burden reduction in the second vaccine regimen group (homologous immunization, rSm-p80 with Alum) was found to be 54.98%. The egg load in this vaccine regimen group showed a reduction of 21.23% (Table 2).

3.2. Humoral immune response during the immunization period

High Sm-p80-specific total IgG titers were observed in sera samples collected from mice immunized in accordance with the two [heterologous prime-boost (DNA prime-protein formulated with alum boost) and homologous prime-boost (protein formulated with alum prime and boost)] experimental protocols. The IgG titer started rising 2 weeks after prime injection and kept increasing to reach a peak at 12 week or 10 week (homologous immunization) thereafter with every booster. The end point IgG titer for the two experimental regimens was 1:204, 800 (Figure 1A). IgG1 started rising at 2 week after prime vaccination and continued to increase to a peak of 1:12, 800 at 10 week or 8 week in the heterologous and homologous immunization regimens, respectively (Figure 1B). Similarly, IgG2a titer begun rising at 2 week post initial vaccination and kept going up and

peaked at 12 week or 10 week (homologous) (Figure 1C). The titer of IgG2b started rising 4 weeks after the initial immunization in heterologous groups of animals and reached a peak of 1:25, 600 at 12 week. While in homologous immunization regimen the IgG2b started increasing right from the second week of inoculation and reached a titer of 1: 51, 200 at 10 week (Figure 1D). IgG3 antibody titer in both the immunization protocols began rising only 4 weeks post inoculation and reached the highest level of 1:25, 600 or 1:12, 800 at 12 or 10 week for heterologous and homologous groups of animals (Figure 1E). As expected, the IgM titer showed a moderate increasing trend after the initial inoculation and reached its peak of 1:6, 400 or 1:12, 800 (homologous) at 8 week, which gradually declined in the later weeks of vaccination irrespective of the immunization regimen (Figure 1F). However, IgM antibody was undetectable in the sera of animals just prior to sacrifice. We have also examined the titer of various immunoglobulins in the sera of animals collected just before sacrificing them. The IgG titers for both the homologous and heterologous group of animals were 1:102, 400 while IgG1 titer for the two immunization regimens were 1:3, 200 and 1:6, 400 (homologous) respectively. The titers of IgG2A stood at 1:25, 600 for both the immunization protocols. IgG2b titer was 1:6, 400 and 1:12800 for the heterologous and homologous groups of animals while IgG3 titer stood at 1:12, 800 and 1:6, 400 for the two vaccinations regimens respectively.

3.3. T cell proliferation responses and cytokine productions

As ascertained by splenocyte proliferation, the rate of proliferations in both of the immunization protocols were 26.27% and 21.24% higher than corresponding control groups after stimulation by recombinant Sm-p80. Nevertheless, rSm-p80-driven proliferation of splenocytes was 29.64% and 3.97% lower than the stimulation induced by ConA, in heterologous and homologous immunization protocols, respectively. As shown in Figure 2, there has been an appreciable amount of the secretions of IL-2 and IFN- γ cytokines in both of the immunization regimens as compared with their respective controls. The concentration of IL-2 was 10-fold higher in the heterologous and 37-fold higher in homologous immunization protocols. A 5- and 6- fold increase in the secretion of IL-4 has also been observed in both the vaccination regimen as compared to their respective control groups. In addition, in both of the immunization protocols, a 9- and 17-fold increase in the secretion of IFN- γ was observed (Figure 2). However no significant difference in the production of IL-10 was noticed in either of the vaccination protocols as compared to their respective control groups. The data presented for both, the cell proliferation assay as well as the cytokine assays, are the mean of three independent experiments.

3.4. Assessment of cytokine mRNA profiles

An attempt was made to assess the mRNA expression of 29 different cytokines [IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, MIP-2 (IL-8 in humans), IL-9, IL-10, IL-11, IL-12 α , IL-12 β , IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, TNF- α , IFN- γ , TGF β 1, TGF- β 2] using semi-quantitative RT-PCR. It was observed that besides conventional Th1 (IL-2 and IFN- γ) and Th2 type cytokines, (IL-4 and IL-10), the expression of a number of other cytokines including IL-3, IL-5, IL-6, MIP-2, IL-9 and IL-12 were up-regulated when compared with their respective control groups (Figures 3).

3.5. Th1/Th2 assay using flow cytometry

CD3 gate was set to crop the cell group. All of the cells shown in Figure 4 were CD3 positive after gating. In the presence of rSm-p80, CD8- IFN- γ producing cells increased by 8% in the case of heterologous (Sm-p80 DNA prime, protein-alum boost) immunization regimen; and 18% increase was observed in the case of homologous (protein-alum vaccinated group) groups of animals. The production of IL-4 increased 10% and 5% in both of the immunization regimens, respectively.

4. Discussion

Schistosomiasis is one of the neglected tropical diseases and is considered to be the second-most socioeconomically devastating parasitic infection after malaria (Fenwick, 2006). Enthusiasm for the Millennium Development Goals has provoked a global mobilization in support of mass drug treatment implementation. As a result of this, millions of people in Africa alone have been treated with praziquantel to control schistosomiasis (Bergquist et al., 2005; Hotez et al., 2009). However, the continual use of this approach has been questioned by some, for example, Bergquist et al., maintain that, “simplistic approach of exclusive drug treatment might not be sufficient and, in the worst-case scenario, might even exacerbate pathology” (Bergquist et al., 2005). Additionally it has gradually been realized that an effective control of schistosomiasis may not be achieved without improved sanitation and an effective vaccine. Therefore robust efforts to develop a protective vaccine to be used in combination with chemotherapy and improved sanitation in order to curb the menace of schistosomiasis are required. Our continuing research and investigations by others have repeatedly shown that the large subunit of calpain (Sm-p80) has great potential as a vaccine candidate (Jankovic et al., 1996; Ohta et al., 2004; Siddiqui et al., 2003b; Siddiqui et al., 2003a; Osada et al., 2005; Siddiqui et al., 2005a; Siddiqui et al., 2005b; Ahmad et al., 2009a; Ahmad et al., 2009b; Ahmad et al., 2009c; El Ridi et al., 2009). There are also reports that calpains was found to be excreted/secreted by the ex vivo lung stage schistosomula. These antigens when prepared in a recombinant or multiple antigen peptide form, elicited highly significant reduction of 47–55% in challenge worm burden and egg load (El Ridi et al., 2001; El Ridi et al., 2009; Tallima et al., 2009; El Ridi et al., 2010). Based on these studies, El Ridi and co-workers indicated that larval ES antigens principally elicit Th1 and Th17 type of effectors responses in murine study.

In the present study an attempt has been made to investigate the combination of FDA approved, human-use plasmid, VR1020, as a vector in combination with alum as an adjuvant in heterologous (DNA prime-protein boost) and homologous (protein–alum prime and boost) approaches. It was observed that Sm-p80 protected mice at 61 and 55 % using the two approaches. Both of the immunization regimens with Sm-p80 induced nearly 2 fold higher levels of IgG, IgG2a, IgG2b and IgG3 than a recombinant Sm-p80 protein vaccine using oligodinucleotide (ODN) as an adjuvant as previously reported (Ahmad et al., 2009c). While in comparison with naked DNA vaccine, the total IgG and its isotypes titer increased 4 to 6 folds, especially for IgG3 (Ahmad et al., 2010).

It has also been reported by many researchers that the levels of pathogen specific cellular immunity can substantially be enhanced by administering vaccines in a heterologous prime/boost regimen (Beveridge et al., 2007; Gilbert et al., 2006; Harari et al., 2008; McShane et al., 2004). Since the heterologous prime/boost vaccination strategies can improve the magnitude and quality of T-cell responses and can amplify the epitope coverage that could be the reasons why we obtained a significantly higher protection levels in animals vaccinated using this approach as compared to homologous protocols. On the other hand, homologous prime boost vaccination strategies in other system have shown to induce moderate but highly persistent protective immune responses when examined for a period of 16 months after the limited vaccination regimens (Kolibab et al., 2010).

Recombinant Sm-p80 stimulation of splenocytes from the vaccinated mice evoked elevated expression of cytokine mRNA skewed toward Th1 response, as observed in the present study. Since the splenocytes used in the present study were collected at the time of perfusion, so the cytokine profiles discussed in this investigation could be not only the results of immunization alone but also the challenge infection might have had its impact. However, the differences between the control and the experimental group should provide

important information on the Sm-p80 induced production of cytokines. The classic paradigms of IFN- γ , IL-2 and IL-12 showed an elevated expression in both of the employed immunization strategies. IL-12 is a T cell stimulator, and closely related with the fluctuation of IFN- γ and IL-2 and stimulates the production of IFN- γ and reduces IL-4 mediated suppression of IFN- γ (Boehm et al., 1997). IFN- γ and Th1 cellular immune responses appear to play a key role against the infection. Conversely, IL-2 stimulation seems to link the maintenance of IL-12 signal transduction in natural killer cells by modulating the expression of IL-12 receptor (Brombacher et al., 2003). Elevated expression of IL-6 is interesting as IL-6 is a double-edge sword, pro-inflammatory and anti-inflammatory cytokine (Diehl et al., 2002). As a result of this dual activity, IL-6 may exert its anti-inflammatory role through inhibitory effects on TNF- α and IL-1, and activation of IL-10. But most importantly IL-6 entangles into another key immune response against parasite that is in combination with other cytokine to result in the differentiation of Th17 cells (Bettelli et al., 2007). Interestingly, previous studies have shown that schistosome larvae activate endothelial cells to produce IL-6 to escape the inflammatory reaction that develops in the lungs of infected hosts (Angeli et al., 2001). Furthermore, one of the effects of IL-6 has also been linked to the cytotoxicity of normal human platelets towards the young larvae of *S. mansoni* *in vitro* (Pancre et al., 1990). Elevated up-regulation of MIP-2 in the present study in both of the immunization protocols is very significant as IL-8 (in humans) is involved in antibody-dependent cell-mediated cytotoxicity involving neutrophils (Mitchell et al., 2003; Reali et al., 1995). Increased concentrations of IL-8 also enhance the phagocytic ability of neutrophils during the immune and inflammatory responses to pathogens (Gougerot-Podcalá et al., 1996). The increased expression of IL-9 is important since IL-9, a pleiotropic cytokine and has recently been demonstrated as a key mediator that affects Th17 and Treg function as a result it differentiate naïve T cell into Th17 synergizing with TGF- β and suppressed Treg cell function (Elyaman et al., 2009). The pleiotropic activities of IL-9 and its potential role in Th2 cytokine-mediated pathologies in schistosome infections has previously been postulated (Fallon et al., 2000). The up-regulation of mRNA expression of IL-21 in the presence of rSm-p80 can be deduced as a cascade loop after Th17 induction (Bettelli et al., 2008). The probable relationships between up-regulation of IL-6, MIP-2, IL-9, IL-21 and protection against the worms could be that the up-regulation of these cytokines might have in turn led to procurement of pro-inflammatory macrophages that might have promoted Th1 responses as reported previously (Brombacher et al., 2003).

The formulation and immunization protocol of vaccine needs to be tuned to match the immune response for acquiring the maximum protection. DNA vaccine has good capability to induce both humoral and cellular immune responses in rodents and nonhuman primates, however, in humans; this capability is limited due to low-levels of immunogenicity (Lu, 2009). Recent studies have suggested that heterologous prime-boost, i.e., the same antigen is sequentially delivered in different types is more immunogenic than the homologous prime-boost (Koopman et al., 2008). The findings in HIV vaccine research exhibited that the combination of DNA/protein vaccine regimen contributed to robust Th1 and Th2 response, and weak CD8 response with undetectable virus load if compared with the simple modality of DNA or protein vaccination (Lu, 2009). In the present study we have used both heterologous and homologous immunization protocols. The mixed modality in the case of heterologous vaccination regimen involving DNA prime protein boost generated both Th1 and Th2 immune responses as can be seen by the cytokine profiles and flow cytometry analyses. This concurs with the previous report that vaccination against *S. mansoni* requires the simultaneous induction of both humoral and cell mediated immunity (Jankovic et al., 1999).

Considering the humoral immune reaction in the two experimental groups, we did not find apparent differences as we expected that the mixed modality provoked higher levels of

antibodies than the homologous vaccination approach. Aluminum hydroxide adjuvant may be responsible for these results. Aluminum adjuvants sustain long-lasting strong antibody response and elicit primarily Th2 immunity (Lambrecht et al., 2009). The protection levels obtained in the present study using a heterologous prime boost are slightly higher (roughly 4%) compared to our previous report using CpG as adjuvant (Ahmad et al., 2009c); this difference could be attributed to the use of an efficient vector (VR-1020) in this study compared to a lesser efficient vector, pcDNA3 in the previous study. However, homologous immunization regimen using rSm-p80 plus CpG, the protection was recorded to be almost 70% (Ahmad et al., 2009c), compared to rSm-p80 plus Alum (55%) as reported in this study. Therefore our premise that induction of Th1 response plays an important role in conferring protection is a valid one but it is also obvious that an antibody response is also important to achieve maximum levels of reduction in worm burdens. One of the possible mechanism (s) of Sm-p80 mediated protection of schistosomiasis could be antibody dependent cellular cytotoxicity, where the production of toxic nitrogen oxide metabolites could have been implicated to be key mechanism of microbicidal activity of *S. mansoni* (Pearce et al., 1986;Gazinelli et al., 1992; Gazzinelli et al., 1994; Stockinger et al., 2007).

In summery the present results combined with our previous data clearly indicate that Sm-p80 is a promising vaccine candidate. The present results demonstrate that a heterologous prime/boost protocol using Sm-p80 DNA and recombinant protein with alum may be considered for designing future immunization strategies against schistosomiasis. Based on the present results it is also indicated that the selection of appropriate immunization formulation and protocol is vital to maximally enhance the immune efficacy of Sm-p80.

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p80 alone with alum, respectively) elicited strong humoral immune responses. The levels of IgG (A), IgG1 (B), IgG2a (C), IgG2b (D), IgG3 (E) and IgM (F) in animals vaccinated via these two immunization regimens. All of the values represent as mean of three experiments • standard error.

Figure 2.

The cytokine productions of splenocytes. After rSm-p80 re-stimulation for 48 h, the concentration of secreted cytokines from splenocytes was measured by ELISA. The values represent as mean $\pm$ SD. (*) indicates statistical significance as compared to the relevant control groups.

Figure 3.

Cytokine mRNA expression assay by RT-PCR. After 24h incubation with rSm-p80, RNA was extracted from stimulated splenocytes and semi quantitative RT-PCR was preformed. Panel one (1–4) represents the heterologous immunization protocol. Lanes 1, 2, VR1020 plus alum; lanes 3, 4, Sm-p80-VR1020 plus rSm-p80 formulated in Alum. Panel two (5–8) is the homologous immunized protocol. Lanes 5, 7, alum, 6, 8, rSm-p80+Alum. Lanes 1, 3, 5, 7, medium control and lanes 2, 4, 6, 8, rSm-p80 stimulation.

Figure 4.

Intracellular IFN- γ and IL-4 secretions analysis by flow cytometry after 24 h stimulation with rSm-p80. Gating is done using CD3 membrane marker. The production of IFN- γ and IL-4 from positive CD3 cells were clearly seen after FACS analysis.

Table 1

Immunization strategy using Sm-p80 with Alum as adjuvant in experimental heterologous (DNA prime protein boost) or experimental homologous (recombinant Sm-p80 prime and boost) regimen.

Vaccine Group	Primary Immunization (week -0)	First boost (Week-4)	Second boost (Week-8)
Heterologous Control	100µgVR1020 DNA	150 µg Alum	150 µg Alum
Heterologous Experimental	100µgSm-p80-VR1020 DNA	25 µg rSm-p80 in 150 µg alum.	25 µg rSm-p80 in 150µg alum.
Homologous Control	150 µg alum	150 µg alum	150 µg alum
Homologous Experimental	25 µg rSm-p80 in 150 µg alum	25 µg rSm-p80 in 150 µg alum	25 µg rSm-p80 in 150 µg alum

The anti-worm and anti-egg effects in C57BL/6 mice after immunization with heterologous and homologous vaccine protocols.

Table 2

Vaccine group	A. Worm burden; B. Egg count, no. per gram*		Average of 3 Exp.	% Reduction (A, in worm burden; B, in egg production)
	Exp. 1	Exp.2		
Heterologous Control				
(VR1020+Alum)	A. 47.60±1.67	A. 29.50±5.00	A. 33.75±2.17	A. 37.76±2.82
	B. 8823.71 (5 ^a)	B. 5122.12 (4 ^a)	B. 2718.93 (4 ^a)	B. 5554.92±1775.53
Heterologous				
Experimental (Sm-p80-VR102+rSm-p80 +Alum)	A. 16.71±3.17	A. 12.00±3.85	A. 15.60±5.55	A. 14.73±2.36 ^b
	B. 3642.63 (5 ^a)	B. 7159.66 (5 ^a)	B. 1975.16 (5 ^a)	B. 4259.15±1528.05
Homologous Control	A. 31.40±5.23	A. 38.60±5.56	A. 18.25±7.59	A. 30.21±3.21
	B. 6439.19 (5 ^a)	B. 13931.30 (5 ^a)	B. 5061.36 (5 ^a)	B. 8477.28±2755.86
Homologous				
Experimental (rSm-p80+Alum)	A. 12.60±2.15	A. 23.60±6.19	A. 4.60±1.28	A. 13.6±2.93 ^c
	B. 6161.34 (5 ^a)	B. 11384.48 (5 ^a)	B. 3382.05 (5 ^a)	B. 6975.95±2345.73

Note. Data are the mean no. ± Standard error, unless otherwise indicated.

^a = animal number

^b = compared to VR1020+Alum, significant difference ≤ 0.013

^c , compared to Alum group, significant difference ≤ 0.05