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Cross-species protection: *Schistosoma mansoni* Sm-p80 vaccine confers protection against *Schistosoma haematobium* in hamsters and baboons

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Abstract

The ability of the *Schistosoma mansoni* antigen, Sm-p80, to provide cross-species protection against *Schistosoma haematobium* challenge was evaluated in hamster and baboon models. Pronounced reduction in worm burden (48%) and in tissue load (64%) was observed in hamsters vaccinated with recombinant Sm-p80 admixed with Glucopyranosyl Lipid Adjuvant-Stable Emulsion (GLA-SE). Similarly, in baboons, the Sm-p80 / GLA-SE vaccine produced a 25% reduction in *S. haematobium* adult worms and decreased the egg load in the urinary bladder by 64%. A 40% and 53% reduction in fecal and urine egg output, respectively, was observed in vaccinated baboons. A balanced pro-inflammatory (Th17 and Th1) and Th2 type of response was generated after vaccination and appears indicative of augmented prophylactic efficacy. These data

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on cross-species protection coupled with the prophylactic, therapeutic and antifecundity efficacy against the homologous parasite, *S. mansoni*, reinforces Sm-p80 as a promising vaccine candidate. It is currently being prepared for GMP-compliant manufacture and for further pre-clinical development leading to human clinical trials. These results solidify the expectation that the Sm-p80 vaccine will provide relief for both the intestinal and the urinary schistosomiasis and thus will be greatly beneficial in reducing the overall burden of schistosomiasis.

Keywords

Schistosome Vaccine; Intestinal Schistosomiasis; Urinary Schistosomiasis; *Schistosoma mansoni*; *Schistosoma haematobium*; Sm-p80; Calpain; Nonhuman primate; Baboons

1. Introduction

Over a billion people are at risk of being impacted by the negative health-related manifestations of schistosomiasis in 74 countries; at present an estimated 200 to 400 million people are infected and an additional 779 million individuals are at a potential risk of contracting the parasite [1–4]. In addition, recent calculations based on health-related quality of life (HrQoL) point to a disturbing upward trend in the disease burden of schistosomiasis in endemic areas [5]. The chronic infection due to schistosome parasites can linger for decades causing disease of intestinal or urogenital system. Intestinal schistosomiasis is caused by *Schistosoma mansoni* and *S. japonicum*, and the etiological agent of urinary schistosomiasis is *S. haematobium*. Over 90% of schistosome infections worldwide are caused by *S. mansoni* and *S. haematobium* [1]. Individuals infected with *S. mansoni* and *S. japonicum* suffer from severe hepatic periportal fibrosis, which can lead to portal hypertension and hepatosplenomegaly [6;7]. In contrast, the clinical manifestations of *S. haematobium* infection include cystitis and urethritis with hematuria that can progress to bladder cancer [2;6–8]. In addition, *S. haematobium* induced genital schistosomiasis in women, predominantly in Africa, is becoming an emerging problem: these women are highly susceptible to the HIV-1 infection, a more rapid onset of AIDS due to vigorous viral replication and immunosuppression, and due to the high viral loads they also readily transmit HIV to others [9–11].

Schistosomiasis is still not fully contained and is spreading to new geographical areas despite considerable efforts to exterminate the intermediate snail host; improved sanitation infrastructure; and implementation of initiatives based on Mass Drug Administration (MDA) using praziquantel. At present, no approved vaccine exists for human use to prevent or treat schistosomiasis. A vaccine that protects from infectious cercariae; reduces egg-induced pathology; gradually tapers the transmission rates; and helps in purging adult worms would be an important breakthrough in control and ultimately eradication of this major neglected tropical disease.

The identification of *S. mansoni* calpain as an important antigenic protein in the surface membrane renewal/recycling, a mechanism by which schistosomes elude the detrimental host immune response, has provided an excellent target for a schistosome vaccine [12–14]. Calpain is an amphitropic protein that remains soluble until activated by Ca^{2+} at which time the protein becomes membrane bound [15]. Schistosome calpain has been localized at the host parasite interface of larval and adult worms [12;16;17]. Furthermore, calpain inactivation inhibits the C3b component of complement and 5-hydroxytryptamine signaling pathways that induce acceleration of surface membrane synthesis [12;14;18]. With regards to the vaccine development, the large subunit of calpain, Sm-p80, is now a leading vaccine candidate for prophylaxis of *S. mansoni* [19–21] and *S. japonicum* [22;23]. In the present

study, we have explored the potential of Sm-p80 to protect against *S. haematobium* in hamsters and in a pilot study using baboons. A schistosome vaccine that protects against the three major species of schistosomes and thus provides relief from the clinical manifestations associated with both the intestinal and the urinary schistosomiasis will be greatly beneficial in reducing the overall burden of the disease.

2. Materials and Methods

2.1. Animals and parasites

Female, Golden Syrian hamsters (*Mesocricetus auratus*) 4–5 weeks old were obtained from Charles River Laboratories International Inc., (Wilmington, MA). Baboons (*Papio anubis*), FA06 (♂, 6.14 year), CL81 (♀, 8.28 years) PI03 (♂, 9.69 years) and JU06 (♀, 6.63 years) were obtained from the University of Oklahoma Health Sciences Center (OUHSC) baboon breeding colony and housed in AAALAC accredited facilities at OUHSC. Baboons were pre-screened for intestinal and blood parasites and for antibodies that were cross-reactive to Sm-p80 and were found negative for both. *Schistosoma haematobium* (Egyptian strain)-infected snails (*Bulinus truncatus*) were procured from the Schistosomiasis Resource Center, Biomedical Research Institute, Rockville, MD under NIH-NIAID Contract No. HHSN272201000051. The use of baboons and hamsters in this study was approved by the Institutional Animal Care and Use Committee.

2.2. Preparation of vaccine formulation

Recombinant Sm-p80 (rSm-p80) was generated as described earlier [19;21;24]. In brief, the full length coding sequence of Sm-p80 was cloned into pCold II vector (GenScript Corp., Piscataway, NJ) and the protein was expressed in BL21 (DE3) *E. coli* strain. The expressed proteins were purified using Ni-nitrilotriacetic acid-agarose followed by Sephadex G-150 columns. Endotoxin levels in protein samples were analyzed with a Limulus amoebocyte lysate assay (Charles River Laboratories International, Inc., Wilmington, MA). The recombinant protein used in immunizations contained minimal endotoxin levels of approximately 0.06 EU/ml and are low enough to be used in humans and not to interfere with immunological readouts. Just prior to injection, recombinant Sm-p80 was admixed with Glucopyranosyl Lipid Adjuvant-Stable Emulsion (GLA-SE), a synthetic Toll-like receptor (TLR) 4 agonist, to make the final vaccine formulation. We have utilized GLA-SE because it is used in many vaccine compositions that are commercially licensed in the US and Europe, and more importantly GLA-SE is a potent stimulator of antigen presenting cells [25].

2.3. Vaccination schedule and determination of parasitological parameters

The schedule of vaccinations and their administration frequencies; challenge with cercariae; and necropsy timeline of hamsters (7 control, 10 experimental) and baboons (2 control, 2 experimental) is outlined in Table 1. Necropsies of hamsters and baboons; perfusion of worms through the portal system and removal from the mesenteric veins and determination of percent protection was carried out as described previously [19–21;26;27]. The liver, intestines and urinary bladder of individual animals (hamsters and baboons) were excised and digested overnight at 37°C in 4% KOH. The number of eggs was counted from each animal and egg burden in each tissue/organ was determined as described previously [19–21;26;27]. Additionally, for baboons only, the fecal and urine samples were collected at twice weekly intervals and processed for egg counting. The number of eggs present in each time point was determined by the mean of corresponding group and the percent reduction in egg excretion in urine/feces was calculated.

2.4. Serum antibody response to vaccination

For hamsters, serum samples obtained from 3 time points (pre-immunization, pre-challenge and pre-sacrifice) were used to test the total IgG response to Sm-p80. Briefly, after serial dilution, pooled sera from each time point were added to rSm-p80 coated 96 well plates. One hundred microliters (1:32000 dilution) horseradish peroxidase labeled anti-hamster secondary IgG (H+R) (Abcam, Cambridge, MA) and TMB substrate (Pierce, Rockford, IL) were used in the ELISA. Signal intensity was measured at 450 nm using a plate reader (Bioteck, Winooski, VT). All of the samples were assayed in triplicate and results were expressed as the mean $\pm$ S.D.

Serum samples from each individual baboon were collected every 4 weeks and used to determine the Sm-p80 specific antibody titers for the total IgG, IgG subtypes (IgG1, IgG2, IgG3, and IgG4), IgM and IgA as described previously [20;21;24]. All of the samples were assayed in triplicate. Antibody titer curves were plotted for each collection. The highest titer per time point was selected and results were expressed as the mean $\pm$ S.D.

2.5. Single cell analysis of Th1/Th2/Th17 cytokine secreting cells via ELISPOT

Peripheral blood mononuclear cells (PBMCs) from blood were isolated using HISTOPAQUE®-1077 (Sigma-Aldrich, St. Louis, MO). Briefly, PBMCs from each baboon were seeded (2×10^5 cells/100 μ l/well) into anti-IFN- γ , anti-IL-4 or anti-IL-17 coated 96-well plates (U-cyTech, The Netherlands). The cells were stimulated with 0.5 μ g ConA, 1.2 μ g ovalbumin or 1.2 μ g rSm-p80 and incubated at 37°C with 5% CO₂ for 48 h, allowing for production and capture of released cytokines. One hundred microliters of biotinylated detection antibody were loaded to each well and incubated overnight at 4°C. The next day, 100 μ l Streptavidin-HRP (1:100 dilution) was added and after 1 h incubation at 37°C, freshly prepared AEC (3-amino-9-ethylcarbazole) substrate solution (100 μ l, 1:33 dilution) was added and incubated for 20–30 min at room temperature to develop the spots. The reaction was terminated by washing with distilled water. All assays were run in triplicate. The spot-forming units (SFU) representing single cells were counted using an ELISPOT Bioreader 5000 (ImmunoBioSystem, The Colony, TX, USA). Antigen-specific SFUs were calculated in one million cells for each group.

2.6. mRNA expressions of cytokines detected by qRT-PCR

For baboons, PBMC, spleen and lymph node cells and for hamsters, PBMCs and spleen cells were used to detect the gene expression of different cytokines. The cells from different sources were cultured in the presence or absence of rSm-p80. The cultures were maintained at 37°C in 5% CO₂ for 24 hours. Total RNA was extracted from cells via TRIzol method (Invitrogen Corp., Carlsbad, CA). Reverse transcription reactions for the first strand cDNA synthesis were carried out as described previously [20;21;24]. Specific primers were designed via Primer 3 Plus or from previous hamster studies [28]. Expression levels of different cytokines [for hamsters: IL-4, IL-10, IL-12 β , IL-13, IL-21, IFN- γ , TNF- α and TGF- β 1 and for baboons: IL-1 α , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 α , IL-12 β , IL-13, IL-17, IL-18, IL-21, IL-22, IL-23, TNF- α , IFN- α , TGF- β 1 and TGF- β 2] and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were determined using a Stepone™ plus Real time PCR system. The relative cytokine mRNA expression was calculated by comparing the differences in the message levels of the control group with the respective experimental group after standardization with GAPDH through the DataAssist™ software package V3.0.

2.7. Statistical analysis

Significance between the two groups was calculated by Sigma Plot (version 11.0, Systat Software, Inc., Chicago, IL) using Student's t-test for two independent variables or the

Mann-Whitney-Wilcoxon rank-sum test. Bonferroni's adjustments were included for multiple comparisons to reduce the risk of reaching false conclusions based on chance and the number of groups. $P < 0.05$ is considered significant.

3. Results

3.1. Prophylactic efficacy of the Sm-p80-based vaccine against *S. haematobium* in hamsters: reduction in worm burden and eggs retention in tissues

The cross-species protective potential of Sm-p80 was tested using *S. haematobium* cercariae in a hamster challenge model. Several steps (e.g., vaccine and challenge dosage) were performed to optimize the *S. haematobium*-hamster model before these efficacy experiments were initiated. The Sm-p80-based vaccine showed a pronounced prophylactic effect resulting in the reduction of worms and eggs in tissues (Table 2). Specifically, a 48% reduction in worm burden was achieved in hamsters following vaccination with Sm-p80 in GLA-SE (Table 2). Similarly, 66% and 63% decreases in egg retention in liver and intestine, respectively, were observed (Table 2). No eggs were detected in the urinary bladder of hamster either from control or experimental groups.

3.2. Prophylactic efficacy of the Sm-p80-based vaccine against *S. haematobium* in baboons: reduction in worm burden, eggs retention in tissues and egg expulsion in feces/urine

Even though statistically significant data were obtained from the hamster model on prophylactic efficacy, the absence of eggs in the urinary bladder indicated a better model was needed and led us to develop and optimize a *S. haematobium* model in baboons. In pilot experiments using baboons, Sm-p80 in GLA-SE exhibited a 25% reduction of *S. haematobium* adult worms (Table 3). Surprisingly, no reduction in liver and intestine tissue egg load was observed in vaccinated animals (Table 3). However, a 64% reduction in egg load was detected in the urinary bladder (Table 3). Additionally, 40% and 53% decreases in fecal and urine egg output, respectively, were observed (Table 3). The trend of egg expulsion in feces and urine was found to be consistently lower in control experiments as compared to the Sm-p80-GLA-SE vaccinated baboons (Supplemental Material).

3.3. Protective antibody responses to Sm-p80 in hamsters and baboons

Due to the paucity of appropriate hamster reagents, only total IgG titers were determined in the sera obtained at week 0 (pre-immunization), week 12 (pre-challenge) and week 28 (Pre-sacrifice). Appreciable total IgG antibody titers were determined at pre-challenge (end point titer 51,200) and pre-sacrifice (end point titer 6,400) (Figure 1A). Similarly, in baboons, significant titers for IgA (end point titer 2,000) and IgM (end point titer 14,400) were recorded (Figure 1B). Robust antibody responses were observed for total IgG (end point titer 32,000) and IgG1 (end point titer 4,800) in baboons vaccinated with Sm-p80/GLA-SE (Figure 1B). In baboons, no signals were detected for certain IgG subtypes including IgG2, IgG3 and IgG4 (Figure 1B).

3.4. Impact of the Sm-p80 vaccine on cytokine secreting cells in baboons

Following 48 hours of incubation, Sm-p80 specific IFN- γ positive cell numbers were determined. In the experimental group, Sm-p80/GLA-SE, 128 Spot Forming Unit (SFU) per million PBMCs were detected in baboon ID # PI03 and 388 SFU/million PBMCs formed in baboon JU06; the mean of the group was 258 SFUs. In the control group, GLA-SE alone, only 34 IFN- γ secreting SFU/million PBMCs were detected (17 SFU/million PBMCs in baboon FA06, 51SFU/million PBMCs in baboon CL81) (Figure 2). This reflects a 7.59-fold increase in IFN- γ secreting SFUs in animals vaccinated with Sm-p80 compared to their

respective control group (adjuvant alone). With regards to IL-4 positive cells, no significant response to Sm-p80 stimulation in either control or experimental group was observed (Figure 2). However, in response to Sm-p80 stimulation, IL-17 secreting SFUs in the experimental group were 225 SFU/million PBMCs (group mean) compared to 24 SFU/million PBMCs (group mean) in the control group; this reflects a 9-fold increase in SFUs in animals vaccinated with Sm-p80 compared to adjuvant controls (Figure 2).

3.5. Assessment of cytokine mRNA profiles in hamsters and baboons post-vaccination

For hamster studies, after 24 h incubation in the presence of rSm-p80, the mRNA expression of eight cytokines in PBMCs and splenocytes was analyzed from cells isolated from control (GLA-SE) and experimental (Sm-p80/GLA-SE) groups of hamsters. When compared with the control group, the experimental group showed enhanced message synthesis of IFN- γ in both splenocytes and PBMCs (Figure 3A). In addition, distinct up-regulation of Th2 biasing cytokines (IL-4, IL-13) in PBMCs was recorded (Figure 3A). No statistically significant differences were observed in the mRNA levels of other cytokines studied (Figure 3A).

For studies in baboons, a total of 20 cytokines were studied and differences in their relative expression levels were recorded for PBMCs, splenocytes and lymph node cells following stimulation *in vitro* with rSm-p80 (Figure 3B). Briefly, in the experimental group (Sm-p80/GLA-SE), distinct down-regulation of the Th2, Th17 and other pro-inflammatory cytokines was recorded in PBMCs. Specifically the levels of IL-4, IL-6, IL-10, IL-21, IL-22, IL-1 α and IL-8 were decreased (Figure 3B). For the Th1 cytokines, only IL-18 was down-regulated in PBMCs (Figure 3B). However, in lymph node cells, pronounced up-regulation of Th1 and pro-inflammatory cytokines (IFN- γ , IL-12 β , IL-1 α and IL-8) was observed coupled with distinct down-regulation in IL-4 mRNA levels (Figure 3B). In splenocytes, up-regulation in message levels was observed for IFN- γ , IL-1 α , IL-1 β and TGF- β 1 only (Figure 3B).

4. Discussion

Sm-p80 was originally discovered from *S. mansoni* [12;29–31] and has been developed for over twenty years as a vaccine candidate for intestinal schistosomiasis caused by *S. mansoni* [19–21;24] and *S. japonicum* [22;23]. A previous exploratory study indicated that Sm-p80 may also be useful in protecting against *S. haematobium*, the causative agent of urinary schistosomiasis [6]. To further explore the cross-species protective potential of Sm-p80, vaccine efficacy studies were performed in hamsters and baboons. The data presented here on reduction in adult worm burden, decrease in tissue egg load and tapering in expulsion of eggs in feces/urine reinforces the premise that a Sm-p80-based vaccine could be useful in the control of both intestinal and urinary schistosomiasis.

The lack of an experimentally-tractable small animal model has significantly limited our knowledge of vaccine efficacy and mechanistic aspects related to the urogenital schistosomiasis pathogen, *S. haematobium* [32]. In our study, using the hamster model we were able to obtain statistically significant prophylactic efficacy data. However, deposition of eggs in tissues/organs was limited to liver and intestine in hamsters. Contrary to the previous findings [33;34], in this study no eggs were detected in the urinary bladder of hamsters either in the control or the experimental group. This drawback limits the suitability of hamsters as a model to study *S. haematobium*-associated pathogenesis to the fullest. On the other hand, baboons exhibited a wide spectrum of urogenital-associated pathological manifestations associated with *S. haematobium* and thus important pilot data on worm burden, egg retention in tissues and expulsion in feces/urine, for Sm-p80 vaccine, were obtained. This reiterates previous studies in which *S. haematobium* irradiated cercarial preparations were used in baboons and provided excellent efficacy [35–37]. In our study here in the baboon model significant reductions in worm burden, trapped eggs in the urinary

bladder, and egg expulsion in urine/feces were observed following vaccination with the Sm-p80 based vaccine. However, surprisingly, a negative vaccine-mediated effect was observed in eggs found in the liver and intestine, which is in disagreement with our previous studies using Sm-p80 vaccine in *S. mansoni*-baboon system [20;21;24]. We expect to further investigate the validity of this phenomenon by performing a larger study with more baboons in the study groups.

Immune correlate studies in both hamsters and baboons followed a similar pattern as has been observed previously with *S. mansoni*-mice/baboon system using Sm-p80-vaccine with the exception of enhanced IL-4 message levels in the hamster studies [19–21;24;26;27]. Data presented here indicate that an appropriately balanced pro-inflammatory (Th17 and Th1) and to some extent, Th2 biased immune responses were generated following vaccination with the Sm-p80 vaccine and these appeared to be a requirement for protection against *S. haematobium* challenge. The principal antibody in hamsters in which Sm-p80-mediated protection was conferred was IgG. Similarly in baboons IgG and its subtype IgG1 was correlated with protection, surprisingly though no IgG2 was detected in the protected baboons. In humans, typically IgG4 is associated with Th2 type responses but IgG1 has also been implicated in many cases as a Th2 type of antibody [38–40], and it has also been shown to be IL-12 and IFN- γ driven in a few systems [41;42].

To date, exhaustive efficacy studies using Sm-p80 in different vaccine formulations and immunization strategies have been carried out in three different experimental animal models of infection and disease [mouse (n=1,795), hamsters (n=94) and baboon (n=99)] [6;19–21;24;26;27;43–46]. Cumulatively, the Sm-p80 vaccine has shown pronounced (i) prophylactic efficacy, (ii) egg-induced tissue pathology resolving efficacy, (iii) antifecundity effect, (iv) therapeutic efficacy, (v) resolution of acute Schistosomiasis, and (vi) cross-species protective efficacy. Based on these data, it is logical to assume that the Sm-p80-vaccine has a high likelihood of success against both intestinal and urinary schistosomiasis. With the completion of proof of concept studies in animals models and completion of initial process development, recombinant Sm-p80 vaccine is now entering cGMP compliant manufacturing activities leading to an IND filing in the next 2–3 years with the final goal of Phase I/II human clinical trials as soon as 2017. We believe that a logical, rational, and practical approach to the control of schistosomiasis should encompass Mass Drug / Vaccine Administration or MDVA: a vaccine implementation scheme that would entail treating infected individuals with praziquantel and then vaccinating them with a schistosome vaccine; especially one that is efficacious against the three major species of schistosomes that cause over 90% the disease, i.e., with a vaccine like the Sm-p80 formulation presented here.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Sm-p80 vaccine originally developed for *Schistosoma mansoni* confers protection against *S. haematobium*
- Sm-p80-vaccine has a high likelihood of success against both intestinal and urinary schistosomiasis
- Schistosomiasis control should encompass Mass Drug / Vaccine Administration or MDVA: a vaccine implementation scheme that would entail treating infected individuals with praziquantel and then vaccinating them with a schistosome vaccine

Figure 1A

Figure 1B

Figure 1.

Figure 1A. Serum total IgG titers in immunized hamsters. Sera were pooled for each time points. Three time points, pre-immunization (Week 0), pre-challenge (Week 12) and pre-sacrifice (Week 28) were used to determine total antibody titers. No signals for Sm-p80-specific antibodies were detected in sera obtained prior to immunization in either GLA-SE control or rSm-p80+GLA-SE experimental groups. The values represent the mean of three experiments $\pm$ standard deviation.

Figure 1B. Titers of anti-Sm-p80 antibodies in immunized baboons. ELISAs were performed with sera obtained from each baboon (every four weeks) in their respective control and experimental groups. The peak antibody titers for total IgG, IgG1, IgM and IgA in control and vaccinated groups are shown. No signals were detected for IgG2, IgG3 and

IgG4 groups for either GLA-SE control or rSm-p80+GLA-SE experimental group. The values represent the mean of three experiments $\pm$ standard deviation.

Figure 2.

Detection of IFN- γ , IL-4 and IL-17 secretory cells in the peripheral blood mononuclear cells (PBMC) of baboons. IFN- γ , IL-4 and IL-17 secreting cells were assayed by ELISPOT. Spot forming units (SFUs) were calculated in one million cells. The SFUs for cells obtained from the control group (GLA-SE) are shown as blue bars, and green bars represent rSm-p80 stimulated cells from the experimental group (Sm-p80+GLA-SE).

Figure 3A

Figure 3B

Figure 3.

Figure 3A. Relative fold changes of cytokine mRNA expression in vaccinated hamsters. After 24 h incubation with rSm-p80, RNA was extracted from stimulated pooled PBMCs and splenocytes. After standardization using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) through DataAssist™ software V3.0, the relative ratio of cytokine mRNA expression was compared between control group and experimental group.

Figure 3B. Relative fold changes of cytokine mRNA expression by qRT-PCR. After 24 h incubation with rSm-p80, RNA was extracted from stimulated pooled PBMCs, lymph node cells and splenocytes. The relative ratio of cytokine mRNA expression was compared between the control group (GLA-SE) and the experimental group (Sm-p80+GLA-SE) after

24 h stimulation by rSm-p80. The relative cytokine mRNA expression was calculated after standardization using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) through DataAssist™ software V3.0.

Table 1

The schedule of vaccinations and their administration frequencies; cercarial challenge and necropsy timeline of hamsters and baboons

Groups	1 st Immunization (Week 0)	2 nd Immunization (Week 4)	3 rd Immunization (Week 8)	Infection (Week 12)	Sacrifice
<i>Hamster model</i>					
GLA-SE Control	GLA-SE (10 µg)	GLA-SE (10 µg)	GLA-SE (10 µg)	200 <i>S. haematobium</i> cercariae	Necropsy (Week 28)
rSm-p80+GLA-SE	rSm-p80 (100 µg) + GLA-SE (10 µg)	rSm-p80 (100 µg) + GLA-SE (10 µg)	rSm-p80 (100 µg) + GLA-SE (10 µg)	200 <i>S. haematobium</i> cercariae	Necropsy (Week 28)
<i>Baboon model</i>					
GLA-SE Control	GLA-SE (50 µg)	GLA-SE (50 µg)	GLA-SE (50 µg)	1000 <i>S. haematobium</i> cercariae	Necropsy (Week 31)
rSm-p80+GLA-SE	rSm-p80 (250 µg) + GLA-SE (50 µg)	rSm-p80 (250 µg) + GLA-SE (50 µg)	rSm-p80 (250 µg) + GLA-SE (50 µg)	1000 <i>S. haematobium</i> cercariae	Necropsy (Week 31)

Table 2

Reduction in adult worm numbers and in tissues (liver and intestine) of hamsters following vaccination with Sm-p80 vaccine

Group	Animal Number	Worm burden	Tissue egg burden per gram		Mean total tissue egg burden per gram	Worm reduction	Tissue egg reduction		Gross tissue egg reduction
			Liver	Intestine			Liver	Intestine	
GLA-SE	C1	92	7288.89±1048.87	19994.92±721.97	13641.90±2897.66				
	C2	75	15738.89±713.91	18566.88±909.78	17152.88±816.91				
	C3	53	5466.67±143.69	17764.64±800.50	11615.65±2773.86				
	C4	0	0.00±0.00	0.00±0.00	0.00±0.00				
	C5	4	7627.78±369.73	4316.67±154.86	5972.22±761.78				
	C6	4	194.44±56.38	93.82±19.90	144.1317±34.95				
	C7	2	0.00±0.00	0.00±0.00	0.00±0.00				
Group Mean GLA-SE (±SE)			5188.10±2185.08	8676.70±3624.50	6932.40±1182.37				
rSm-p80 +GLA-SE	E1	16	2150.00±67.36	1778.12±191.23	1964.06±123.03				
	E2	54	4172.22±102.89	16923.08±222.06	10547.65±2853.28				
	E3	18	7073.96±362.48	2690.632±142.86	4882.293±995.51				
	E4	0	0.00±0.00	0.00±0.00	0.00±0.00	47.66%	66.32%	62.65%	64.03%
	E5	0	0.00±0.00	0.00±0.00	0.00±0.00				
	E6	0	0.00±0.00	0.00±0.00	0.00±0.00				
	E7	23	674.34±50.25	2350.00±134.72	1512.17±380.17				
	E8	16	1916.67±327.59	8244.44±339.03	5080.56±1430.56				
	E9	19	487.94±61.05	124.46±23.59	306.20±86.39				
	E10	26	997.81±117.20	293.69±26.47	645.75±166.36				
Group Mean rSm-p80+GLA-SE (±SE)			1747.29±721.90	3240.44±1717.18	2493.87±524.37				

Note: No eggs were detected in the urinary bladder of hamsters from control and experimental groups

Reduction in adult worm numbers, in tissue egg load and in excretion of eggs in feces and urine in baboons following vaccination with Sm-p80 vaccine

Table 3

Group	Baboon	Worm burden	Tissue egg burden per gram		Mean total tissue egg burden per gram	Egg burden (feces /g) ^a (urine / ml) ^b	Worm reduction		Tissue egg reduction		Gross tissue egg reduction	Egg reduction feces ^a urine ^b
			Liver	Intestine					Liver	Intestine		
GLA-SE	FA06	224	52.78±14.54	1114.79±86.31	445.29±336.40	31.22±8.54 ^a 3.34±2.23 ^b						
	CL81	239	33.12±13.52	13.71±7.85	1231.08±1207.68	40.10±9.43 ^a 85.00±67.19 ^b						
Group Mean GLA-SE (±SE)		231.50 ±7.50	42.95± 9.92	564.25± 171.06	838.18± 587.54	35.66±6.27 ^a 44.17±33.52 ^b			No Protection	No Protection	28.99%	39.93% ^a 52.91% ^b
rSm-p80 +GLA-SE	PI03	160	5.41±3.42	1501.65±178.59	520.45±490.80	30.78±5.68 ^a 9.97±8.28 ^b						
	JU06	186	430.29±68.64	248.88±35.83	669.97±334.51	12.06±2.74 ^a 31.63±18.83 ^b						
Group Mean rSm-p80 + GLA-SE (±SE)		173.00±13.00	217.85±71.94	875.27±207.87	692.52±184.59	21.42±3.74 ^a 20.80±9.48 ^b						