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Schistosoma mansoni antigen Sm-p80: prophylactic efficacy using TLR4 agonist vaccine adjuvant glucopyranosyl lipid A-Alum in murine and non-human primate models

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ABSTRACT

Sm-p80, the large subunit of *Schistosoma mansoni* calpain, is a leading candidate for a schistosomiasis vaccine. The prophylactic and antifecundity efficacy of Sm-p80 has been tested in three animal models (mouse, hamster and baboon) using a multitude of vaccine formulations and approaches. In our continual effort to enhance the vaccine efficacy, in this study, we have utilized the adjuvant, synthetic hexa-acylated lipid A derivative, glucopyranosyl lipid A (GLA) formulated in aluminum (GLA-Alum) with recombinant Sm-p80. The rSm-p80+GLA-Alum immunization regimen provided 33.33%–53.13% reduction in worm burden in the mouse model and 38% worm burden reduction in vaccinated baboons. Robust Sm-p80-specific immunoglobulin (Ig)G, IgG1, IgG2a and IgM responses were observed in all immunized animals. The rSm-p80+GLA-Alum coadministration induced a mix of T-helper (Th) cells (Th1, Th2 and Th17) responses as determined via the release of interleukin (IL)-2, IL-4, IL-18, IL-21, IL-22 and interferon-γ.

Significance of this study

What is already known about this subject?

- Schistosomiasis is a neglected tropical parasitic disease affecting over 230 million people worldwide.
- Current control programs centered on mass drug administration of praziquantel are inadequate.
- Elimination of schistosomiasis is only attainable through integrated control programs with an effective vaccine serving as a fulcrum.
- The large subunit of *Schistosoma mansoni* calcium-activated neutral protease (calpain), Sm-p80, is a leading schistosomiasis vaccine.

What are the new findings?

- Sm-p80 vaccine formulated in synthetic hexa-acylated lipid A derivative, glucopyranosyl lipid A (GLA) formulated in aluminum (GLA-Alum) provided significant protection against *S. mansoni* infections in mice and baboons.
- GLA adjuvant formulated in Alum did not maximize the immunogenicity and efficacy of Sm-p80 vaccine.
- Immunizations with Sm-p80+GLA-Alum induced significant production of Sm-p80-specific antibodies (immunoglobulin (Ig)G, IgG1, IgG2a and IgM).
- Sm-p80-mediated balanced T-helper (Th) cells (Th1/Th2/Th17) immune responses are associated with immune protection in vaccinated animals.

INTRODUCTION

Schistosomiasis is a neglected tropical disease of significant public health importance that has the potential to impact up to an estimated one billion people worldwide. Approximately 237 million people are currently infected and an additional 800 million people are in danger of being exposed to this parasitic infection in 78 countries.[1–3] The estimated disability adjusted life years associated with schistosomiasis is 3.6 million,[4] and health-related quality of life indicate that schistosomiasis is potentially causing a much higher disease burden than was previously estimated.[5] Mass antiparasitic drug administration programs using praziquantel and other control strategies have made inroads in reducing the disease burden of schistosomiasis.[6] However, most experts agree that a sustainable and meaningful reduction in the disease burden and transmission would only be possible through the deployment of a vaccine in conjunction with current control programs.[4 7]

The large subunit of *Schistosoma mansoni* calpain, Sm-p80, is expressed in all *S. mansoni* life cycle stages.[8–10] Sm-p80 plays critical roles in schistosome tegument biogenesis/renewal, a mechanism employed to modulate and/or

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Significance of this study

How might these results change the focus of research or clinical practice?

- The results from this pilot study reinforce the continued development of Sm-p80 as a schistosomiasis vaccine. Due to its inefficiency at overriding the parasite-induced Th2 responses, GLA-Alum adjuvant may not be suitable for the next phase of schistosomiasis vaccine development.

evade their host immune attack.[8 11] With respect to schistosomiasis vaccine development, Sm-p80 is regarded as a leading candidate having demonstrated significant protection against *S. mansoni* challenge infections in addition to cross-species protection against *Schistosoma haematobium* and *Schistosoma japonicum* infections in rodent and baboon models.[10 12–16]

Adjuvants are utilized to improve or modulate the intrinsic immunogenicity of an antigen to selectively switch the onset of a specific cell-mediated response in addition to the antibody response.[17 18] Based on our previous efficacy studies using Sm-p80 antigen formulated in either glucopyranosyl lipid A in stable, oil-in-water emulsion (GLA-SE)[19] or alum hydroxide[16] which showed significant protection against *S. mansoni* infections, we hypothesized that Sm-p80 vaccine formulated with GLA adsorbed on alum hydroxide (GLA-Alum) would enhance its efficacy. GLA and Alum hydroxide adjuvants are currently used in many commercially available vaccine formulations and are potent stimulators of T-helper (Th)1 and Th2 cell immune responses, respectively.[20 21] In this present study, we assessed the protective efficacy of Sm-p80+GLA-Alum vaccine against *S. mansoni* infections in mice as well as in a pilot study using non-human primate model of infection and disease.

MATERIALS AND METHODS

Animals and parasites

Mice aged 3–4 week, female C57BL/6, were purchased from Charles River Laboratories International (Wilmington, Massachusetts, USA). Animal husbandry and all performed procedures were guided by the principles of the Institutional Animal Care and Use Committee. Olive baboons (*Papio anubis*) aged 11–16 years were bred in the Association for Assessment and Accreditation of Laboratory Animal Care and International-accredited facilities at the University of Oklahoma Health Sciences Center (OUHSC). Animals were prescreened for intestinal and blood parasites and for antibodies that are cross-reactive to Sm-p80. Infected *Biomphalaria glabrata* snails, the intermediate host of *S. mansoni*, were provided by Schistosomiasis Resource Center (Biomedical Research Institute, Rockville, Maryland, USA). On the day of challenge, infected snails were exposed under light to induce cercarial shedding. The number and viability of cercariae were counted under a light microscope.

Preparation of recombinant protein Sm-p80

The recombinant Sm-p80 protein (rSm-p80) was produced using prokaryotic expression system as previously described.[10] In brief, the full length *Sm-p80* gene cloned into pCold II (GenScript Corp., Piscataway, New Jersey, USA) and transformed into BL21 (DE3) *Escherichia coli* strain (Invitrogen Corp., Carlsbad, California, USA). The expression of recombinant protein was induced by 0.75 mM isopropyl β-D-1-thiogalactopyranoside. Expressed protein was purified by affinity chromatography followed by size exclusion chromatography. Endotoxin levels in purified protein samples were analyzed with a *Limulus* amebocyte lysate assay (Charles River Laboratories International) and the quality of rSm-p80 analyzed by sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) and western blotting.

Animal immunization and challenge

Formulated GLA-Alum was provided by PAI Life Sciences. For the mouse experiment, three independent vaccine trials were conducted with a total of 10 mice per trial, divided randomly into the control group (n=5) and the experimental group (n=5). Each mouse in the control groups was immunized with 5 µg GLA-Alum, while those in the experimental group were immunized with 25 µg rSm-p80 in combination with 5 µg GLA-Alum. Primary immunization followed by two boosters were administered intramuscularly at weeks 0, 4 and 8, respectively. Four weeks after the last immunization of each trial, the mice were challenged with 150 *S. mansoni* cercariae via tail exposure. For the pilot baboon experiment, six baboons were randomly divided into control (n=3) and experimental (n=3) groups. The control group received 50 µg GLA-Alum, while the experimental group received 250 µg rSm-p80 with 50 µg GLA-Alum at weeks 0, 4 and 8. Four weeks after last immunization, all the baboons were exposed to 1000 *S. mansoni* cercariae at the deposit site of the axillary cavity. Online Supplementary table 1 shows immunization protocol and the experimental schedule.

Animal necropsy, worm and egg burden

All animals were sacrificed at week 6 (mouse experiment) or week 8 (baboon experiment) post challenge. Adult worms were recovered by perfusion of the portal system and the mesenteric veins.[22 23] The liver and intestine of individual animals were excised for digestion overnight at 37°C in 4% potassium hydroxide.[23] Eggs were counted to determine the egg burden for each animal. The reduction (P) in worm and egg burdens were calculated by comparing control (C) and experimental (I) groups with a standard formula: $P\% = \frac{C-I}{C} \times 100\%$.[12]

Serum antibody response to vaccination

Blood samples were collected from individual animals at 2-week intervals (mouse experiment) or 4-week intervals (baboon experiment) and sera were isolated for antibody level determination using ELISA as already described.[12 15] Briefly, 96-well plates were coated with rSm-p80 (1.2 µg/well). Sm-p80-specific antibody titres for total IgG subtypes, IgA and IgM were determined using either horseradish peroxidase labeled antimouse or antimonkey secondary antibodies (Alpha Diagnostics International, San Antonio, Texas, USA). The results were expressed as mean of endpoint titres±SD as previously published.[24]