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Schistosoma egg-induced liver pathology resolution by Sm-p80-based schistosomiasis vaccine in baboons

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Summary

Schistosomiasis remains a serious chronic debilitating hepato-intestinal disease. Current control measures based on mass drug administration are inadequate due to sustained re-infection rates, low treatment coverage and emergence of drug resistance. Hence, there is an urgent need for a schistosomiasis vaccine for disease control. In this study, we assessed the anti-pathology efficacy of *Schistosoma mansoni* large subunit of calpain (Sm-p80)-based vaccine against schistosomiasis caused by infections with *Schistosoma mansoni* in baboons. We also evaluated the disease transmission-blocking potential of Sm-p80 vaccine. Immunisations with Sm-p80-based vaccine resulted in significant reduction of hepatic egg load in vaccinated baboons (67.7% reduction, $p = 0.0032$) when compared to the control animals, indicative of reduction in pathology. There was also a significant reduction in sizes of egg-induced granulomas in baboons immunised with Sm-p80 vaccine compared to their control counterparts. Egg hatching rate analysis revealed an overall 85.6% reduction ($p = 0.0018$) in vaccinated animals compared to the controls, highlighting the potential role of Sm-p80 vaccine in disease transmission. The findings on anti-pathology efficacy and transmission-blocking potential presented in this study have formed the basis for a large-scale double-blinded baboon experiment that is currently underway.

Keywords: Schistosomiasis; Sm-p80 vaccine; Anti-pathology; *Schistosoma mansoni*.

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INTRODUCTION

Schistosomiasis remains a major global health problem that currently affects over 230 million people in 78 countries.[1–3] The disease is caused by infections with a helminth parasite *Schistosoma*. It is the second most socioeconomically important parasitic disease with over 3 million disability-adjusted life-years (DALYs) attributed to the disease.[4] Clinical manifestations of schistosomiasis include anaemia, diarrhoea, weight loss, liver fibrosis, and other organ damage, which could prove fatal if left untreated.[1,5–8] The current schistosomiasis control program predicated on mass drug administration of praziquantel (PZQ) is inadequate due to low coverage, unabated re-infection rates and reports of the emergence of PZQ-resistant strains of schistosomes. In addition, schistosomiasis is now being reported in regions previously known to be schistosomiasis-free.[9,10] Schistosomiasis elimination could only be attainable through an integrated approach with an effective vaccine acting as the fulcrum.[3,11]

Infection occurs when humans come into contact with fresh water contaminated with *Schistosoma mansoni* cercariae. The cercariae penetrate the host skin, transforming into migrating larvae, which in turn travel through the circulation and finally mature into adult male and female worms. The worms form pairs, mate, and the female starts laying eggs, some of which are released into the environment and the rest trapped in various tissues within the human host. Human cases of *S. mansoni* infections develop severe chronic intestinal/hepatic schistosomiasis.[12]

The pathology due to schistosomiasis occurs mainly from the parasite eggs trapped in the host tissues, particularly the liver and intestine.[7] The chronic disease occurs as a result of the host's immune reaction to the trapped eggs evoking granulomatous reaction in the liver.[13] The parasite eggs are destroyed and the secreted egg antigens neutralised by the granulomas. However, this process also leads to the development of fibrosis in the host tissues which results in hepatic vascular occlusion, and then portal hypertension, spleen enlargement, ascites and fatal bleeding from gastrointestinal varices.[12] The severity of the chronic clinical manifestations is dependent on the duration and intensity of infection.[13] Although the majority of egg-induced pathology is seen in the liver and intestine (for *S. mansoni* and *S. japonicum* infections) and the urogenital tract (for *S. haematobium* infections) of infected individuals,[6] egg-induced granulomas have also been reported from other tissues such as the adrenal glands, skeletal muscles, brain, lungs and skin.[14] Experimental schistosomiasis studies using *S. mansoni*/mouse models including gene knockout and/or transgenic animals have shown that formation of granulomas is a consequence of the induction of marked $CD4^+$ Th2 cellular responses by

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schistosome egg antigens, leading to the production of Th2-type cytokines such as IL-4 and IL-5, accompanied by eosinophilia and IgE-dependent hypersensitivity reaction.[12,15]

Transmission of schistosomiasis is dependent on the eggs released into the environment by infected hosts. Therefore, a vaccine that ameliorates egg-induced pathology as well as having a direct effect on the viability on the eggs released into the environment would be greatly beneficial in reducing the overall morbidity and transmission of the disease. *Schistosoma mansoni* antigen, Sm-p80, is a leading schistosomiasis vaccine that has consistently provided protection (worm burden reduction) against *S. mansoni* infections in both mouse and baboon models.[16–20] In this present study, we assessed the anti-pathology efficacy of Sm-p80-based vaccine against *S. mansoni* infections in immunised baboons and also evaluated the potential of Sm-p80-based vaccine as a transmission-blocking vaccine.

METHODS

Animals and parasites

Male and female non-human primates, olive baboons (*Papio anubis*) aged 3.5 to 7.5 years old were obtained from the University of Oklahoma Health Sciences Center, USA (OUHSC) baboon breeding colony and housed in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facilities at OUHSC. The Institutional Animal Care and Use Committees (IACUC) of Texas Tech University Health Sciences Center (TTUHSC) and OUHSC approved the use of baboons in this study (protocol no. 11-160-I).

Schistosoma mansoni (NMRI strain)-infected *Biomphalaria glabrata* snails were obtained from the NIAID Schistosomiasis Resource Center (Biomedical Research Institute, USA).

Preparation of Sm-p80 vaccine

The recombinant Sm-p80 (rSm-p80) used for vaccination was produced as already described.[21] In brief, the full length coding sequence of Sm-p80 (GenBank accession number M74233) cloned into a pCold II vector (GenScript, USA) was expressed in *Escherichia coli* BL21 (DE3) strain via isopropyl β-D-1-thiogalactopyranoside induction. Expressed protein was purified by using Profinity Immobilized Metal Affinity Chromatography (Bio-Rad, USA) followed by gel filtration using Sephadex G-150 columns. Western blot analysis of purified rSm-p80 (His-tagged) has been described elsewhere.[21] Endotoxin levels in protein samples were analysed with a Limulus amebocyte lysate assay (Charles River Laboratories International, USA). The recombinant protein used in immunisations contained acceptable endotoxin levels (approximately 0.06 EU/mL) approved for human use by the US Food and Drug Administration.

Immunisation strategy and parasite challenge

Six baboons were randomly divided into control (n = 3) and experimental (n = 3) groups. The control group received 50 μg Toll-like receptor 4 agonist glucopyranosyl lipid adjuvant formulated in a stable emulsion (GLA-SE) while the experimental group received 250 μg rSm-p80 with 50 μg GLA-SE at weeks 0, 4 and 8. Four weeks after the last immunisation, all of the baboons were exposed to 1000 *S. mansoni* cercariae at the deposit site of the axillary cavity. All baboons were euthanised 8 weeks post cercarial challenge. Details of necropsy and efficacy determination procedures have been published previously.[17,22]

Sm-p80-specific antibody response following vaccination

Blood samples were obtained from each baboon prior to each immunisation, prior to cercarial challenge and at necropsy. Antibody response following vaccination was determined by enzyme-linked immunosorbent assay (ELISA) as already described.[21] Briefly, 96-well plates were coated with rSm-p80 (1.2 μg/well). Sm-p80 specific antibody titres for total IgG were determined using HRP labelled anti-monkey secondary antibodies (Alpha Diagnostics, USA). The data obtained are presented endpoint titres as described elsewhere.[23]

Assessment of liver pathology and histology

At necropsy, whole baboon livers were assessed for gross anatomy. In order to fully assess the extent of the liver pathology, resections were obtained from the right posterior medial segment, right anterior medial segment, left medial segment and left anterior lateral segment (Fig. 1A) for histological analysis. For histology, liver sections were fixed in 10% formalin. Sections were embedded in paraffin (5 μm thick), dewaxed and subsequently stained with haematoxylin and eosin (H&E) in order to analyse egg-induced granulomas. The number of granulomas was quantified in a 1 × 1 cm^2 square area from each segment (1a, 1b, 2a, 2b) (Fig. 1A and B). The diameter of each granuloma was measured via a straight line bisecting the central egg (Fig. 1C)[24–26] and the cross section area of each granuloma in square micrometres (μm^2) was calculated assuming as an area of circle. All liver section images were captured using a light microscope at 100× and 400× magnification.

Preparation of *S. mansoni* eggs and egg hatching determination

Schistosoma mansoni eggs were isolated from infected baboon livers following published methods with slight modifications.[27] Briefly, 6–7 g of infected liver tissue was homogenised in 1.2% sodium chloride (NaCl) solution and then passed through a series of sieves (425, 180, 106 and 45 μm). The flow through collected was centrifuged at 1500 rpm for 5 min. The supernatant was discarded and the pellet washed twice with cold 1.2% NaCl by centrifuging. After the final wash, the purified eggs were re-suspended in

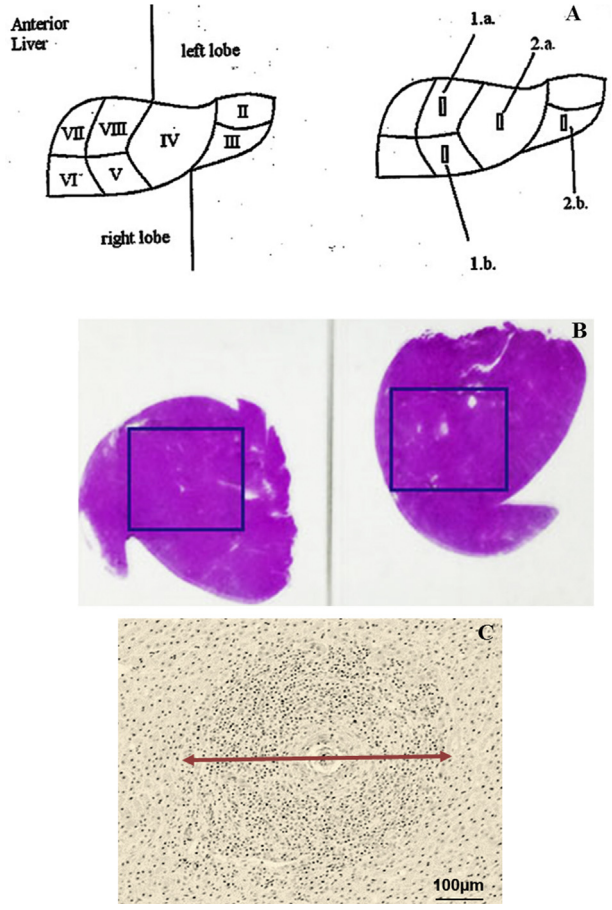

Fig. 1 Representative liver resections and granuloma measurement. (A) Pictorial representation of locations where liver sections were obtained: 1.a., right posterior medial segment; 1.b, right anterior medial segment; 2.a., left medial segment; and 2.b., left anterior lateral segment. (B) Representative liver sections stained with H&E. Blue squares are 1 × 1 cm^2. (C) A representative H&E stained granuloma surrounding a schistosome egg observed at 200× magnification. Red arrow represents the diameter of the granuloma.

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