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Saposin-like proteins are expressed in the gastrodermis of *Schistosoma mansoni* and are immunogenic in natural infections

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Summary
Background: Schistosomes are parasitic blood flukes that inhabit the portal blood system of humans. Ingested red cells are lysed in the gastrodermis to enable the parasites to digest hemoglobin. Saposin-like proteins (SAPLIPs) have been reported from the gastrodermis of related flukes, and at least one is hemolytic and a promising vaccine antigen. We now provide the first report of SAPLIPs from schistosomes and explore their role in host—parasite interactions.
Methods: We identified expressed sequence tags encoding a family of SAPLIPs from *Schistosoma mansoni* and produced one (termed *Sm*-SLP-1) in recombinant form using baculovirus. The anatomic site of SLP-1 expression within the worm was assessed and its recognition by sera from chronically infected humans and mice was determined. The vaccine efficacy of *Sm*-SLP-1 was tested in a mouse model.
Results: Full-length sequences were obtained for two cDNAs, *Sm-slp-1* and *Sm-slp-2*. The *Sm-slp-1* open reading frame contained a single SAPLIP domain while *Sm-slp-2* had a double domain. *Sm*-SLP-1 was immunolocalized to the gastrodermis of adult worms, but did not confer protection in a murine vaccination model of schistosomiasis. Mice infected with *S. mansoni* generated a specific antibody response to *Sm*-SLP-1. Individuals who were infected with *S. mansoni* had IgG that recognized *Sm*-SLP-1. IgG levels were statistically higher in individuals with heavy infection.
Conclusions: *Sm*-SLP-1 is expressed in the gastrodermis of *S. mansoni*. It is immunogenic in humans and mice, but is not protective as a vaccine in its current form. Schistosome SAPLIPs warrant further attention to elucidate their roles in host—parasite interactions and to further explore their potential as vaccine and diagnostic antigens.

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Introduction

Human schistosomes infect more than 200 million people worldwide with at least 600 million more at risk of infection,[1] making them a leading cause of parasite-induced morbidity. The World Health Organization (WHO) estimate of 0.5% disability weight assigned to schistosomiasis was recently revised by King et al. to 2–15%.[2] There are three major species of human medical significance, *Schistosoma haematobium*, *Schistosoma japonicum*, and *Schistosoma mansoni*.

Schistosomes are exclusive blood feeders, and it is estimated that female *S. mansoni* parasites can ingest approximately 330 000 erythrocytes per hour.[3] The hemoglobin (Hb) degradation pathway in schistosomes has been partially elucidated,[4,5] however the step immediately preceding this, hemolysis, has received far less attention. Erythrocytes ingested by schistosomes are thought to be lysed by the action of a hemolysin(s) within the esophagus and intestine,[6,7] which is proposed to form pores in the erythrocyte membrane.[8] Hemolysins may play additional roles in the parasite – egg extracts of *S. japonicum* contain lytic molecules that are thought to aid their movement through the tissues and into the gut lumen for excretion.[9] Despite these earlier studies, molecule(s) involved in hemolysis and general pore formation have yet to be characterized in any detail from schistosomes.

Recently, lytic proteins were identified from two liver fluke species: clonorin from *Clonorchis sinensis*[10] and FhSAP1 and FhSAP2 from *Fasciola hepatica*.[11] The proteins share sequence homology to amoebapores, pore-forming peptides from *Entamoeba histolytica*,[12] and belong to the saposin-like protein (SAPLIP) family of distantly related polypeptides that have six conserved cysteine residues forming three disulfide bridges.[13–16] SAPLIPs are found in all animals ranging from protozoa to mammals, and where known, their function seems to involve interactions with lipids. Clonorin, from *C. sinensis*, is expressed exclusively in the gut of adult flukes[10] and was proposed to lyse ingested host cells for nutritional purposes. FhSAP2 of *F. hepatica* is found in the excretory/secretory (ES) products, which act externally of the parasite,[11] and although it has not been localized to a defined tissue within the parasite, this protein might also be involved in feeding. Recombinant FhSAP2 is partially protective in vaccine trials using rabbits,[11] indicating that helminth pore-forming proteins might be potential vaccine candidates.

Here we describe the identification and characterization of two cDNAs encoding SAPLIPs from *S. mansoni*, *Sm-slp-1* and *Sm-slp-2*. The SLP-1 protein is expressed in the gastrodermis of the parasite and is recognized by antibodies from infected mice and human subjects, but does not appear to be an efficacious vaccine antigen in a murine model of *S. mansoni* in its current form.

Materials and methods

Sequence identification, cDNA library PCR, and sequence analysis

Clonorin, the saposin-like protein from the liver fluke *C. sinensis*, was used to search GenBank (nr) and dbEST datasets by BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/).[17] An *S. japonicum* expressed sequence tag (EST) was identified and subsequently used to identify homologous sequences from *S. mansoni*. cDNA and predicted protein sequences were analyzed using MacVector version 7.2., and predicted signal peptides were assessed using SignalP (http://www.cbs.dtu.dk/services/SignalP/).[18] Where ESTs did not encode full-length cDNA sequences (*Sm-slp-2*), the 5′ and 3′ termini were obtained by PCR using a combination of gene-specific oligonucleotide primers designed from the EST sequences and vector-derived primers that flanked the cloning site of an *S. mansoni* adult worm cDNA library constructed in λZAP-CMV (Stratagene). Primers used were as follows: Sm2F (5′ TCGTCCACTCGAAACTCCGGA) to find the 3′ end and Sm2GCR (5′ CTCCGGAGTTTCGAGTGGACG) to find the 5′ end. Multiple sequence alignments were assembled with ClustalW using the amino acid sequences between the first and sixth cysteine residues of each individual SAPLIP domain.

Expression and purification of recombinant proteins in baculovirus

Recombinant proteins were expressed in a baculovirus shuttle plasmid (pMelBac, Invitrogen) fused to an N-terminal melittin signal peptide. Primers incorporating a six-residue GC-rich clamp followed by restriction sites for *Sac* I (5′) and *Nco* I (3′) were designed to span the entire open reading frames (ORFs) of *Sm*-SLP-1 and *Sm*-SLP-2 without their predicted signal peptides. 3′ Primers contained sequence encoding a 6×His tag and stop codon for downstream purification using metal ion affinity chromatography. The following primer sequences were used for SLP-1: Sm1pmbF (5′ GCGCGCGAGCTCTACTCTGTCAAGAATGTGGAT); Sm1pmbR (5′ GCGCGCCCATGGTTAATGGTGATGGTGATGATGACATAAAGGAGTCAATTTGCA); and for SLP-2: Sm2pmbF (5′ GCGCGCGAGCTCAACAAAATTAATTTACTTACTAAG); Sm2pmbR (5′ GCGCGCCCATGGTTAATGGTGATGGTGATGATGGTAGAGGATAAGTTTGAAAAG). Expression and purification of recombinant proteins in both pMelBac and in a modified pMelBac plasmid, termed pHotWax, were as previously published.[19] Removal of C-terminal purification tags (V5 and His epitopes) from recombinant proteins expressed in pHotWax was performed using AcTEV Protease (Invitrogen) according to the manufacturer's instructions. To assess the ability of recombinant proteins to dimerize, Western blots of both native (samples were not boiled or reduced) and reduced/denatured *Sm*-SLP-1, as well as adult schistosome extracts (SmTX)[20] were probed with *Sm*-SLP-1-specific antiserum. Hemolysis assays using recombinant SLPs were conducted as described previously.[21]

Antibody production and immunolocalization

Antisera against recombinant *Sm*-SLP-1 (expressed in pMelBac) formulated with Freund's complete (first immunization) and incomplete (second and third immunizations) adjuvants were raised in female CBA/CaH mice as previously described.[22] Immunolocalization using fluorescence microscopy was performed on paraformaldehyde-fixed adult worm sections of *S. mansoni* as published elsewhere.[22] All animal research was approved by the Animal Ethics Committee of the Queensland Institute of Medical Research.