Amino Acid Transport in Schistosomes

CHARACTERIZATION OF THE PERMEASE HEAVY CHAIN SPRM1hc

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Schistosomes are human parasitic flatworms that constitute an important public health problem globally. Adult parasites live in the bloodstream where they import nutrients such as amino acids across their body surface (the tegument). One amino acid transporter, Schistosome Permease 1 light chain, SPRM1lc, a member of the glycoprotein-associated family of transporters (gpaAT), has been characterized in schistosomes. Only a single member of the SLC3 family of glycoproteins that associate with gpaATs is found following extensive searching of the genomes of Schistosoma mansoni and S. japonicum. In this report, we characterize this schistosome permease heavy chain (SPRM1hc) gene and protein. The 72-kDa gene product is predicted to possess a single transmembrane domain, a (βα)n (TIM barrel) conformation and a catalytic triad. Xenopus oocytes functionally expressing SPRM1hc with SPRM1lc import phenylalanine, arginine, lysine, alanine, glutamine, histidine, tryptophan, and leucine. Biochemical characterization demonstrates that in Xenopus extracts and in schistosome extracts SPRM1hc is associated into a high molecular weight complex with SPRM1lc that is disrupted by reducing agents. Quantitative real-time PCR and Western analysis demonstrate that SPRM1hc is expressed in each schistosome life stage examined (eggs, cercariae, schistosomula, adult males and females). SPRM1hc is widely distributed throughout adult male and female worms as determined by immunolocalization. Consistent with the hypothesis that SPRM1hc functions to facilitate nutrient uptake from host blood, immunogold electron microscopy confirms that the protein is distributed on the host-interactive tegumental membranes. We propose that surface-exposed, host-interactive, nutrient-transporting proteins like the SPRM1 heterodimer are promising vaccine candidates.

The disease is characterized by the presence of adult worms, or blood flukes, within the portal and mesenteric veins. These worms, living as male/female pairs, can survive for many years during which time the female produces hundreds of eggs per day. The primary pathological consequences of schistosome infection are the host immunological response to these eggs within host tissues (3).

Adult schistosomes, within the vertebrate blood stream, can feed in two ways. First, they can ingest blood through the mouth and into the gut where a battery of enzymes breaks down the material. Second, they can import nutrients directly across their body surface (or tegument) (4–7). The schistosome tegument is an unusual structure, being enclosed by two closely apposed lipid bilayers in the form of a normal plasma membrane overlain by a membrane-like secretion called the membranocalyx (8). Because the tegument lacks lateral membranes, its cytoplasm extends as a continuous unit, or syncytium, around the entire body of the worm (9, 10). The tegumental cytoplasm is connected by numerous, thin cytoplasmic processes to interconnected, cell bodies (or cytons) that lie beneath the peripheral muscle layers; these contain nuclei, endoplasmic reticula, golgi complexes, and mitochondria (9, 11). The import of nutrients across the tegument surface implies the presence of nutrient-transporting proteins (sometimes called permeases) in the tegumental plasma membrane. Such nutrient importing proteins must be exposed to the nutrients in the host blood and, as such, should be available for chemotherapeutic or immunological assault.

Several glucose transporter proteins have been identified in schistosomes (12) and one of these, Schistosome Glucose Transporter 4 (SGTP4), is detected in the host-interactive plasma membrane of intravascular Schistosoma mansoni (13, 14). Schistosomes synthesize a new tegument during invasion of their vertebrate host and SGTP4 is rapidly deposited into the surface of this new structure (14–16). A second glucose transporter (SGTP1) is found in the basal membrane of the tegument where it likely facilitates the distribution of glucose from within the tegument to the internal tissues (17, 18).

A single amino acid transporter (Schistosome Permease 1 light chain, SPRM1lc) has been characterized in schistosomes, which belongs to the glycoprotein-associated family of transporters (gpaAT)2 (19, 20). This ∼55-kDa protein is found in...
both larval and adult schistosomes and in a variety of tissues (20). When expressed within *Xenopus* oocytes, SPRM1lc promoted amino acid uptake but only when co-expressed with the human glycoprotein, h4F2hc (19). In this context, SPRM1lc facilitated the transport of the basic amino acids arginine, lysine, histidine, as well as leucine, phenylalanine, methionine, and glutamine (19, 20). The h4F2hc protein, acting as a chaperone, was necessary for SPRM1lc to reach the oocyte plasma membrane and function as an amino acid permease. A disulfide bond links h4F2hc and SPRM1lc (21). All characterized heterodimeric amino acid transporters function as obligatory amino acid exchangers (22, 23). Thus the role of SPRM1lc can be viewed as that of equilibrating concentrations of amino acids across the membrane rather than as that of an influx transporter.

Biochemical characterization demonstrates that, in schistosome extracts of all life cycle stages, SPRM1lc is associated into a high molecular weight complex that can be disrupted by reducing agents (20). This is consistent with the hypothesis that a significant fraction of the endogenous SPRM1lc is linked by a disulfide bond to the schistosome h4F2hc homolog. We call this protein the schistosome amino acid permease heavy chain or SPRM1hc. In this report, we clone cDNA encoding SPRM1hc and characterize the protein from this important human parasite.

**EXPERIMENTAL PROCEDURES**

Parasites—The Puerto Rican strain of *S. mansoni* was used. Cercariae were obtained from infected Biomphalaria glabrata snails and isolated parasite bodies were prepared as described (24). Parasites were cultured in RPMI medium supplemented with 10 mM HEPES, 2 mM glutamine, 5% fetal calf serum, and antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin) at 37 °C, in an atmosphere of 5% CO2. Adult parasites were recovered by perfusion and parasite eggs were isolated from infected mouse liver tissue as described (25).

Identifying SPRM1hc and SJSPRM1hc—SPRM1lc functionally associates with the human protein h4F2hc when both molecules are expressed in *Xenopus* oocytes. In this work we set out to identify and characterize the schistosome h4F2hc homolog. Analysis of the *S. mansoni* transcriptome revealed one EST (designated SmAE 607755.1) with sequence similarity to the amino acid permease heavy chain of *S. japonicum* (27). In this way a single close homolog was identified from *S. japonicum* that was designated SJPRM1hc (GenBank™ accession number AAW26021.1) but the clone appeared to lack the N-terminal coding region. To identify further potential coding DNA, the *S. japonicum* genome data base, housed at the Shanghai Center for Life Science and Biotechnology Information, was probed for sequence that extended upstream of the available coding DNA. Several hits were obtained and one (TISJA01484276) was used to identify the likely 5′-coding sequence for SJPRM1hc. The following oligonucleotides were used with *S. japonicum* cDNA to amplify by PCR two overlapping fragments comprising the entire potential SJPRM1hc coding sequence: SJPRMhcfor1, 5′-CTATCATTCTATTTGCAAAGCGG-3′ with SJPRMhcrev1, 5′-GCACTCACCCTCTAATAATTGGG-3′; and SJPRMhcfor2, 5′-GGTGTCGCGAAAATAAGAAGG-3′ with SJPRMhcrev2, 5′-CACACAGAAGCTAATTTCATCG-3′. Both PCR fragments were purified and sequenced. Comparisons between SPRM1hc and related permease heavy chain sequences were undertaken using the UPGMA best tree building method, with gaps distributed proportionally, using DS Gene software (Accelrys Inc.).

**Functional Expression of SPRM1hc in Xenopus laevis Oocytes**—To express SPRM1hc in *Xenopus* oocytes, the entire SPRM1hc coding DNA was first amplified by PCR using adult parasite cDNA and oligonucleotides, Hc-XO1 (5′-CCGCTCGAGATGAGTTGGACGGCTACCAATTG-3′) and Hc-XO2 (5′-CGCGGTATCCACATCCAATTGGAAACATATCTATACG-3′), using conditions as described (12). Underlined sequences denote restriction sites; XhoI for Hc-XO1 and BamHI for Hc-XO2. Next, the PCR product was gel-purified, digested with XhoI and BamHI and ligated into the similarly digested *Xenopus* expression plasmid, pSDeasy. TOP10 cells were transformed with the ligation mixture and recombinant transformants were selected on agar plates containing 100 μg/ml ampicillin. Plasmid was purified from several clones and the cloned inserts were sequenced. One plasmid, pNA0009, was linearized by digestion with PstI and cRNA was synthesized *in vitro*, as earlier described (19). Tritiated amino acids were used in uptake experiments involving *X. laevis* oocytes, as previously detailed (22). Background uptake was determined using oocytes expressing only the corresponding heavy chain (SPRM1hc or h4F2hc), and this value was subtracted to generate the data shown. The results are expressed as mean ± S.E. (pmol/oocyte/h) of 13–42 oocytes from 2 to 6 independent experiments. Statistical comparison of means was carried out using one way analysis of variance and Tukey as post-test when p < 0.05.

1-Ariginine was selected to monitor the concentration dependence of substrate transport in oocytes expressing SPRM1lc together with either SPRM1hc or h4F2hc. For this...
study, oocytes were injected with 1 ng of each cRNA and evaluated, as above, for γ-Arg transport (at 0.1, 1, 10, 100, and 1000 μM) after 24 h, in uptake buffer containing Na⁺.

**Bioisotopic Labeling of Oocyte Proteins and Immunoprecipitation**—Non-injected or cRNA-injected oocytes were incubated in ND96 supplemented with 1 mCi [35S]methionine for 48 h at 16 °C. Oocytes were then washed twice with ND96 and lysed in a buffer containing 50 mM Tris/HCl, pH 8.0, 120 mM NaCl, 0.5% Nonidet P-40; and protease inhibitors. Extracts were vortexed and centrifuged for 10 min at 12,000 rpm at 4 °C. The supernatant was separated from the pellet by yolk granules and stored at −70°C. Aliquots of 2 × 10⁶ cpm were rotated for 16 h at 4 °C with 1 μg of anti-SPRM1hc antibody (21) prebound to protein G/protein A-agarose. The beads were washed five times with 20 mM Tris/HCl, pH 8.0; 100 mM NaCl, 1 mM EDTA, 500 mM LiCl, 0.5% Nonidet P-40; and five times with 20 mM Tris/HCl, pH 8.0; 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40. The resulting immunoprecipitates were heated in SDS-sample buffer (with or without reducing agent (β-mercaptoethanol)) for 3 min at 95 °C.

**Anti-SPRM1hc Antibody Production**—The following peptide comprising SPRM1hc amino acid residues 615–633 was synthesized: NH₂-SDPQGSGQRLKSDGQPM-COOH by Genemed Synthesis, Inc. San Francisco. This sequence is indicated in bold script in Fig. 1B. A cytoine residue was added at the amino terminus to facilitate conjugation of the peptide to bovine serum albumin (BSA). Approximately 500 μg of the peptide-BSA conjugate in Freund’s Complete Adjuvant was used to immunize two New Zealand White rabbits subcutaneously. The rabbits were boosted with 100 μg of peptide alone in Incomplete Freund’s Adjuvant 20, 40, and 60 days later. Ten days following this, serum was recovered from both rabbits, pooled and anti-SPRM1hc antibodies were affinity-purified as above, for L-Arg transport (at 0.1, 1, 10, 100, and 1000 μM) in the presence of 120 mM NaCl, 0.5% Nonidet P-40; and protease inhibitors. The sera were extensively searched for homologs of SPRM1hc in 7-H9262/L9262 parasites using the TRIzol method (Invitrogen), following the manufacturer’s instructions. Next, 1 μg of total RNA, pre-treated with TurboDNase (Ambion, TX), was reverse-transcribed to cDNA using random hexamers and Superscript reverse transcriptase (Invitrogen). qRT-PCR was performed using cDNA derived from 50 ng of total RNA and primer sets/reporter probes labeled with 6-carboxyfluorescein (FAM), custom synthesized by Applied Biosystems (Foster City, CA). For SPRM1hc the following primers and probe were used: SPRM1HC3PM-HCP3F, 5'—GGTTTGGCTTCCAGGTCTCTG-3' and SPRM1HC3PM-HCP3R, 5'-CGTTTTCTCATTTAACCTGGAACCA-3' with the FAM-labeled probe, SPRM1HC3PM-HCP3M1, 5'-FAM-CCTCCAGGACCTTCTC-3'; for the endogenous control S. mansoni triosephosphate isomerase (SmTPI) gene the following primers and probe were used: SMTPI-TPI3F, 5'-CTGCTGATGATTGACATCCCC-3' and SMTPI-TPI3R, 5'-TTCATACAGTTTCTGATGTGAC-3' with the FAM-labeled probe, SMTPI-TPI3M1, 5'-FAM-CCATACAGTTTCTGATGTGAC-3'. Probe positions were designed to span exon/exon boundaries to minimize detection of any contaminating genomic DNA. All samples were run in triplicate and underwent 45 amplification cycles on a 7500 ABI PRISM® Sequence Detection System Instrument. For relative quantification, the ΔΔCt method was employed (29).

**RESULTS**

The **SPRM1hc Gene and cDNA**—Prior studies showed that the schistosome permease SPRM1hc is a member of the glycoprotein associated family of amino acid transporters (gpaATs) and requires association with a heavy chain glycoprotein belonging to the SLC3 family to function in *Xenopus* oocytes. The ESTs and the nearly complete genomes of *S. mansoni* and *S. japonicum* were extensively searched for homologs of h4F2hc or rBAT, representing the two classes of the SLC3 fam-
ily of glycoproteins known to associate with gpaATs. Only a single putative SLC3 family member was identified that we named SPRM1hc (schistosome permease 1 heavy chain). A complete cDNA encoding SPRM1hc was obtained from adult schistosome mRNA by RT-PCR, which permitted characterization of the gene structure. A map of the SPRM1hc gene is shown in Fig. 1A. The gene is 6.56 kb, only one-third of which is coding sequence and possesses 9 introns and 10 exons. The first 6 introns are small (32–42 bp); the remaining 3 vary from 1.02 to 1.95 kb. All introns possess conventional GT:AG intron donor:acceptor sites and are composed of 60–75% A/T residues (range 54–63%). Exon size varies from 120–370 bp with 58% AT (range 54–63%). Searching schistosome genome and EST databases for additional homologs of SPRM1hc was unsuccessful. The GenBank accession number of the S. mansoni SPRM1hc cDNA reported here is EF204542.

FIGURE 1. The SPRM1hc gene and predicted protein. A, diagrammatic representation of the SPRM1hc gene. Open rectangles, numbered 1–10, represent exons. The position of a poly(A) addition site is indicated (right). K represents kilobase pairs. B, alignment of SPRM1hc predicted amino acid sequence with homologs. GenBank accession numbers of these proteins are: SPRM1hc, EF204542; SPRM1hc, EF204543; rBAT (human), AAH93626; h4F2hc (human), NP_001013269; Ce BAT (or ATG1, C. elegans) CAB02316. Identical residues are indicated by shading. TM indicates the predicted transmembrane domain. β1–8 and α1–8 indicate domains comprising the TIM barrel. The arrowhead indicates a conserved cysteine that is postulated to be involved in SPRM1hc cross-linking to its light chain partner (SPRM1lc). Arrows at position Asp78, Glu332, and Asp408 highlight residues that may comprise a catalytic triad. The C-terminal peptide indicated in bold (615IDQPVGSQRVYLKDSDGQPM633) was synthesized and used to generate anti-SPRM1hc antibodies.
Amino Acid Uptake in Schistosome Parasites

The predicted SPRM1hc protein is 640 amino acids long, with a molecular weight of 71.645 daltons and a pI of 5.05. A comparison of the SPRM1hc protein with homologs from other species is shown in Fig. 1B. The complete coding DNA of the homolog from *S. japonicum* was determined (designated SjPRM1hc, GenBank™ accession number EF204543) and, relative to an earlier *S. japonicum* data base entry (GenBank™ accession number AAW26021.1), revealed several polymorphisms or sequencing errors (not shown). SmPRM1hc nucleotide and protein sequences are 80 and 79% identical to SjPRM1hc respectively. Not surprisingly, considerably lower sequence similarity is evident between the SPRM1hc protein and homologs from other organisms e.g. humans or *Caenorhabditis elegans* (all ~20%). GenBank™ accession numbers of the related permease heavy chain sequences compared in this work are as follows: rBAT, AAH93626; h4F2hc, NP 001013269; Ce BAT (ATG1) CAB02316.

All of these heavy chain proteins are predicted to possess a single transmembrane (TM) domain. In addition, the schistosome proteins are also predicted to display what is called a TIM barrel (because the domain was first identified in triosephosphohexose isomerase enzymes). TIM barrels assume a (~8) conformation. The positions of the α-helices and β-sheet motifs within the TIM domain of SPRM1hc shown in Fig. 1B were predicted by Network Protein Sequence Analysis (30). Both schistosome proteins also possess a potential catalytic triad (at position Asp^278, Glu^332, and Asp^408, arrows Fig. 2). A conserved cysteine residue (Cys^89, arrowhead, Fig. 1B) is predicted to cross-link this protein with its light chain partner (SPRM1lc) in the plasma membrane, as was demonstrated for the human transporter (21). A membrane topology model of the complex heterodimeric amino acid transporter SPRM1hc with SPRM1lc (collectively called SPRM1), is shown in Fig. 2. This model of the SPRM1hc/SPRM1lc heterodimer is based on the 12-transmembrane domain topology proposed by TMpred analysis for SPRM1lc (19) and on the fact that the extracellular cysteine residue involved in the disulfide bridge with the 4F2hc heterologous heavy chain is located in the loop between predicted transmembrane helices 3 and 4 (21).

Expression and Functional Characterization of SPRM1hc in Xenopus Oocytes—Amino acid uptake characteristics were studied in *Xenopus* oocytes expressing SPRM1lc in combination with either SPRM1hc or the human homolog h4F2hc (Fig. 3A). It is clear that oocytes injected with RNA encoding SPRM1hc, together with RNA encoding its schistosome light chain partner (SPRM1lc), import several amino acids, most notably phenylalanine, arginine, and lysine. The amino acids alanine, glutamine, histidine, tryptophan, and leucine are also transported (Fig. 3A). No major differences were observed between transport rates measured in the presence (+, Fig. 3A) or absence (−, Fig. 3A) of Na^+^−. It is not clear whether the small differences in the pattern of amino acid uptake observed when SPRM1hc is replaced in the assay with h4F2hc are biologically relevant (Fig. 3A). Injected alone, RNA encoding neither SPRM1hc nor SPRM1lc nor h4F2hc promotes increased amino acid uptake when compared with control, uninjected oocytes. Fig. 3B shows that the concentration dependence of L-Arg transport is similar for oocytes expressing SPRM1hc + SPRM1lc as for those expressing h4F2hc + SPRM1lc. The bars represent the mean ± S.E. from three independent experiments (n = 24 oocytes). The K_0.5_ for SPRM1hc/SPRM1lc is 82 ± 32 μM and for h4F2/SPRM1lc is 51.4 ± 19 μM.

Immunoprecipitation experiments using anti-SPRM1lc antibodies were undertaken to determine whether the SPRM1hc protein was complexed with SPRM1lc in *Xenopus* oocytes, as hypothesized. Oocytes were injected with cRNAs, incubated with [35S]methionine, and immunoprecipitated were resolved by SDS-PAGE. A representative autoradiograph result is shown in Fig. 3C. In oocytes injected with heavy and light chain cRNAs, both SPRM1lc (arrowhead, lc) and SPRM1hc (arrowhead, hc) can be detected under reducing conditions (+, lane 4, Fig. 3C), following immunoprecipitation with anti-SPRM1lc antibodies. When this immunoprecipitate is resolved in the absence of reducing agent (−), the high molecular weight SPRM1hc/SPRM1lc complex is detected (arrow, lc/hc, lane 8). This demonstrates that the proteins form reduction-sensitive heterodimers.

Developmental Expression of SPRM1hc—Membrane extracts of several schistosome life cycle stages were resolved by SDS-PAGE and the gel was stained using Coomassie Blue (Fig. 4A, left panel). An equivalent gel was subjected to Western blotting analysis using affinity purified anti-SPRM1hc antibodies and results are shown in Fig. 4A, right panel. One prominent band (arrow, Fig. 4A), running at near the predicted size of SPRM1hc, is detected in all of the life stages examined, namely; eggs, cercariae, schistosomula, and adult male and female worms, when extracts are resolved in the presence of reducing agent. The
mixed sex adult membrane preparation (Fig. 4A, left-most lanes) was resolved either in the presence (+) or absence (−) of reducing agent, dithiothreitol. In the absence of this reagent, the protein has reduced mobility (>100 kDa, arrowhead) presumably representing both SPRM1 components, SPRM1hc complexed with SPRM1lc. Anti-SPRM1lc antibodies recognize a moiety of the same size in membrane extracts resolved without dithiothreitol (20). In some preparations an additional, unidentified faint band, running at ~90 kDa, can be detected.

Developmental expression of SPRM1hc was also measured at the mRNA level by qRT-PCR and these data are shown in Fig. 4B. Of the various life stages tested, the relative expression of SPRM1hc is low only in cercariae, perhaps reflecting the fact that this life stage does not import nutrients from the environment. Relative expression is high in schistosomula and remains high in adult males and females. Eggs also have a comparably high expression of SPRM1hc, which may indicate that miracidia within the eggs continue to import amino acids, even as they traverse host tissues while exiting the host.

**Localization of SPRM1hc in Adult Tissues**—SPRM1hc is widely distributed throughout adult male and female worms as determined by immunolocalization (Fig. 5A). Parasite muscle and parenchymal tissue stain clearly with anti-SPRM1hc antibodies. Fig. 5B shows a higher magnification image of the periphery of an adult male section where the exterior of the tegument (i) shows clear staining (arrow). Localization of SPRM1hc by immunogold electron microscopy (Fig. 5C) confirms that the protein is distributed on the host-interactive tegumental membranes. Arrowheads in Fig. 5C point to some of the tegumental immunogold particles. Parasites treated with secondary antisera alone demonstrate no tissue staining (data not shown). A recent proteomic survey has been completed that identified schistosome proteins which are available for biotinylation on living adult worms (38). SPRM1hc was one of the few proteins identified in the study, confirming our localization of this protein at the host-interactive surface of schistosomes.

**DISCUSSION**

*S. mansoni* adults are parasitic worms that live in the mesenteric venules of their vertebrate hosts. Whereas the parasites possess a mouth and a gut, many nutrients such as glucose and amino acids can be imported directly across their body surface (or tegument) from the host blood stream (4–6). Nutrient uptake into the tegument must be facilitated by specific membrane transporter proteins located in the tegumental membranes. For instance, the apical tegument glucose transporter protein SGf7P4 probably facilitates glucose uptake from the vasculature into the parasites (14, 15). Similarly, the uptake of at least some amino acids into these parasites is likely mediated by the amino acid permease SPRM1lc in the tegumental membranes (19, 20). Previous work showed that in *Xenopus* oocytes, SPRM1lc does not mediate amino acid uptake unless the protein is chaperoned to the plasma membrane by a heavy chain partner protein (19). The human protein h4F2hc can act as this partner to functionally associate with SPRM1hc; this demonstrates a high level of evolutionary conservation for this interaction. In this work, we cloned and characterized the endogenous schistosome
Amino Acid Uptake in Schistosome Parasites

FIGURE 4. Developmental expression of SPRM1hc. A, SPRM1hc protein expression determined by Western analysis. Membrane preparations from different parasite life stages (E, egg; C, cercariae; S, 24 h cultured schistosomula; Δ, adult female (7-week-old); δ, adult male (7-week-old)) were resolved by SDS-PAGE under reducing conditions; Adult, mixed populations of both males and females, prepared in the presence (+) or absence (−) of reducing agent were resolved by SDS-PAGE. The left panel shows a Coomassie Blue-stained gel, and the right panel shows a Western blot of an equivalent gel probed with anti-SPRM1hc antibodies. The arrow indicates the position of SPRM1hc, the arrowhead indicates the position of a higher molecular weight complex (likely SPRM1hc/SPRM1lc) seen when reducing agent is omitted. M, molecular mass markers, numbers represent kDa. B, SPRM1hc gene expression determined by qRT-PCR. The following developmental stages were examined: egg, cercariae, schistosomula (15-day cultured), adult males (7-week-old), and adult females (7-week-old). The relative expression level in adult males was arbitrarily set at 100.

heavy chain partner which we designate SPRM1hc (Schistosome Permease 1 heavy chain).

Transcriptome analysis of S. mansoni revealed just one EST with strong sequence similarity to members of the amino acid transporter heavy chain family (26), and this was designated SPRM1hc. By comparing this expressed sequence with the available S. mansoni genome assembly a complete coding sequence for SPRM1hc was identified. The putative protein encoded by the sequence is 640 amino acids long and displays a domain structure that is conserved among members of the heterodimeric amino acid transporter (HAT) family (31). In particular, much of the protein (encompassing residues 103–445) is predicted to assume a (βα)6 conformation (otherwise known as a TIM barrel) and this has been described most prominently in members of the α-amylase enzyme family (32–34). However, unlike vertebrate transporters heavy chain proteins, like h4F2hc and rBAT, SPRM1hc appears to possess a catalytic triad that is conserved in amylase proteins. This suggests that SPRM1hc may possess some enzymatic activity in addition to its role in facilitating amino acid transport. The functional significance of the hydrolase-like extracellular domain structure of SPRM1hc remains elusive as do the equivalent domains of the corresponding mammalian heteromeric amino acid transporter heavy chains 4F2hc and rBAT, even though the three-dimen-

FIGURE 5. Immunolocalization of SPRM1hc in adult parasites. A, cross section through a male/female couple showing widespread staining with anti-SPRM1hc antibodies. B, higher magnification image of the peripheral tissue of an adult male (t, tegument; m, muscle). The arrow indicates the outer tegmental membrane. C, electron micrograph of the adult tegument showing immunogold labeling of SPRM1hc. Arrowheads indicate gold particles at the host/parasite interface. Numbers above scale bars represent microns.
In view of the fact that all heterodimeric amino acid transporters appear to function as obligatory amino acid exchangers, it is likely that the schistosome heterodimeric transporter SPRM1 also functions in this manner. In this scenario, another, as yet unidentified, transporter would likely directionally import some of the SPRM1 amino acid substrates into the tegumental cytoplasm. SPRM1 would then exchange these molecules, to permit the import of its other amino acid substrates.

To investigate the importance of SPRM1hc in parasite development, attempts were made to silence the expression of the gene using RNAi. Using conditions that resulted in the potent suppression of other schistosome genes, the consistent and significant suppression of SPRM1hc was not observed in this study (36). Our inability to suppress SPRM1hc may reflect differences in the stability or accessibility of its mRNA to the cellular machinery of suppression, as seen in other systems (37).

SPRM1hc is detected in all life stages examined and this is consistent with its role in amino acid uptake into cells, an important function that would likely be required by all parasites. In order for SPRM1 to import amino acids across the body surface of intravascular schistosomes, the complex must exist in the tegumental membranes. Previously, we showed that SPRM1hc can be detected in many adult tissues including the tegument (20). SPRM1hc is likewise widely expressed in the adult parasites and electron microscopy demonstrates that the protein localizes in the tegumental membranes, including at the host/parasite interface. This surface localization of SPRM1hc has been confirmed by other researchers using proteomics to identify the entire protein composition of the tegumental membranes (38, 39). In tegumental extracts, SPRM1hc is detected by proteomics only in the membrane fraction (39).

The heavy chain subunits of vertebrate heterodimeric amino acid transporter proteins, SLC3 glycoproteins, comprise two related families one exemplified by h4F2hc and the other by rBAT (32). Schistosomes are members of the phylum Platynematidae, the earliest branch of the Bilateria. Genome and transcriptome analysis indicates that a single heavy chain heterodimeric amino acid transporter family (represented by SPRM1hc) exists in these parasites. Thus SPRM1hc represents the most primordial member of the SLC3 family of glycoproteins that has been identified to date. While SPRM1hc has only about 20% of its amino acids conserved with h4F2hc or rBAT, remarkably, it can be functionally interchanged effectively with h4F2hc when dimerized with SPRM1lc. Clearly the functional role of this protein family is highly conserved across vast evolutionary distances.

Earlier work implicated the presence of at least 5 different amino acid uptake systems in adult male schistosomes (5). SPRM1 is a functional amino acid transporting heterodimer that exhibits transport characteristics akin to one of the described systems and is localized in the host-exposed tegumental membrane. This makes it most likely that SPRM1 represents the molecular basis for one of these amino acid transport systems. This surface localization of SPRM1, a complex that is likely providing an essential function (amino acid uptake) for the parasites, suggests that it will make a viable target for immunological intervention. Further, the low level of overall sequence similarity between SPRM1hc and human homologs suggests that targeting this molecule as a vaccine would not likely elicit a deleterious, autoimmune response.

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Amino Acid Uptake in Schistosome Parasites

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