A Blood Fluke Serine Protease Inhibitor Regulates an Endogenous Larval Elastase

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Background: Schistosoma mansoni skin degradation is mediated by a serine protease, cercarial elastase (SmCE). A parasitic serpin, SmSrpQ, covalently binds to SmCE and is released into the host as the parasite penetrates the skin. SmSrpQ regulation of SmCE represents an important mechanism by which the parasite balances tissue penetration and host tissue damage.

The larvae of Schistosoma mansoni invade their mammalian host by utilizing a serine protease, cercarial elastase (SmCE), to degrade macromolecular proteins in host skin. The catalytic activity of serine and cysteine proteases can be regulated after activation by serpins. SmSrpQ, one of two S. mansoni serpins found in larval secretions, is only expressed during larval development and in the early stages of mammalian infection. In vitro, 35S-SmSrpQ was able to form an SDS-stable complex with a component of the larval lysate, but no complex was detected when 35S-SmSrpQ was incubated with several mammalian host proteases. Formation of a complex was sensitive to the protease activity of serine and cysteine proteases. For example, cowpox virus serpin, CrmA, is involved in protease injury to the worm (10). Additionally, serpins have been identified in parasites. One serpin identified in Clonorchis sinensis, CsSERPIN, is localized in the secretion glands of the parasite. Fluorescence immunohistochemical analysis of simulated infection showed co-localization of SmCE and SmSrpQ in host tissue suggesting a post-translational regulation of parasite protease activity during skin transversal. The results of this study suggest that cercarial elastase degradation of skin tissue is carefully regulated by SmSrpQ.

Schistosomes are digenetic trematodes (flukes) that cycle between a mammalian host and a fresh water molluscan intermediate host. Schistosoma mansoni, S. japonicum, and S. hematobium are the three main etiological agents of human schistosomiasis, a widespread parasitic infection that affects an estimated 207 million individuals (1). 15,000 deaths per year are attributed to schistosome infection and humans lose 1.7 million disability-adjusted life years, a mark of the high disease burden caused by these parasites (2). Infected individuals are treated with praziquantel (Biltricide), which is only effective against early larvae (schistosomules) and adult worms (3, 4). If left untreated, the disease can progress and persist in human hosts for decades (5), even in the face of a host strong immune response. Mammalian S. mansoni infection begins with larval degradation of extracellular matrix and cell-cell contacts in the epidermis and dermis, followed by breach of the vascular endothelium, migration of the schistosomula to the lungs, prolonged residence of adults in the hepatic portal system, and egg passage through the intestinal wall (see Fig. 2a). Proteomic analysis of S. mansoni larval secretions has identified several parasite proteins that could function in host immune evasion and/or promotion of parasite survival (6–9). One class of proteins that was identified and whose role in the pathobiology of S. mansoni has yet to be elucidated is that of a superfamily of macromolecular serine protease inhibitors (serpins)

2 The abbreviations used are: serpin, serine/cysteine protease inhibitor; SmSrpQ, S. mansoni serpin Q; SmCE, S. mansoni cercarial elastase; Z, benzyloxy-carbonyl; Z-AAPF-CMK, Z-Ala-Ala-Pro-Phe-chloromethyl ketone; Z-Ala-Ala-Pro-Leu-chloromethyl ketone; Z-AAPV-CMK, Z-Ala-Ala-Pro-Val-chloromethyl ketone; qPCR, quantitative PCR.

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the reproductive glands of the liver fluke (13). CsSERPIN has strong chymotrypsin inhibitory activity (13) and is highly up-regulated in the metacercaria (14). With respect to schistosomes, a 56-kDa serpin-like protein was isolated from adult worm extracts of S. mansoni (15). Several studies also identified peptides or protein sequences in schistosome larval secretions with homology to serpins (2, 6, 7, 16). Using antisera raised against proteins in larval secretions, Harrop et al. (16) published the partial sequence of a serpin (clone 8, AAB86571) with homology to leukocyte elastase inhibitor. The cognate protease and biological function of these parasitic serpins remains largely speculative. Here we present evidence that the complete clone 8 protein, hereafter called SmSrPQ (Smp_062080), is a serpin involved in regulating the activity of a parasite-derived protease within the host.

**EXPERIMENTAL PROCEDURES**

**Parasite Material—**S. mansoni (Puerto Rican isolate) specimens were maintained in the laboratory by using Biomphalaria glabrata as the intermediate snail host and golden hamsters (Mesocricetus auratus) as the mammalian host. Cercariae were harvested from B. glabrata by light induction and washed according to a previously published protocol (17). Cercariae used for schistosomula production were washed twice in RPMI and mechanically sheared using a 22-gauge needle to remove their tails. They were then cultured for 24–48 h in Basch Schistosoma culture medium 169 with 10% fetal calf serum and penicillin/streptomycin to produce schistosomula. Eggs were collected from three hamster livers and harvested as previously described (18) in which livers were homogenized in 2× saline solution and the eggs were cleared of mammalian tissue. Miracidia were hatched from the eggs by slowly removing the saline solution and replacing it with hypotonic solution. Lung schistosomula were dissected from hamster lungs, and adult worms were perfused from hamster livers 3 days and 6 weeks post infection, respectively. Cercarial/egg/adult/snail hepato-pancreatic lysates were prepared by freeze/thawing parasites or snail tissue once in an ethanol/dry ice bath followed by homogenization and sonication using the Sonifer 250 (Branson) at 30% output for 30 s. Lysates were centrifuged at 16,000 rpm in the Centrifuge 5415D (Eppendorf, Germany), and supernatants were collected.

**Northern Blots and qPCR—**Total RNA samples were collected by homogenizing and sonicating tissue/parasites in TRIzol (Invitrogen) according to manufacturer’s instructions. RNA was resuspended in water, and treated with 5 units of DNase I (New England Biolabs) for 1 h at 37 °C. Samples were then heat-inactivated and cleaned using the RNeasy Mini Kit (Qiagen). Concentrations of final total RNA samples were determined using NanoDrop 3300 (Thermo Scientific). For quantitative PCRs and cloning, cDNA was synthesized using the SuperScript III kit (Invitrogen) according to the manufacturer’s instruction using oligo(dT) and 50 ng of total RNA per reaction. Control reactions containing no reverse transcriptase were also carried out. cDNA reactions were diluted 1:4, and 5 μl/reaction was used for subsequent qPCR reactions. All qPCR reactions were carried out using a Roche Light Cycler 480 SYBR green master mix and the Applied Biosystems 7300 Real-Time PCR system. Primers were designed to amplify a 250-bp fragment with an amplification program 95 °C for 10 min, 45 cycles of 95 °C for 30 s, 55 °C for 60 s and 30 s at 72 °C (forward primer: 5′-GGT TTT ATG GAG ATA TAG TAG AAG AAC AGA GTC ATT CG-3′; reverse primer: 5′-GGT TGA TAG TGA TTG AGA CGA CGA AAA GAG TTC TTG ATT TT-3′). Reactions were also carried out in triplicate with cytochrome oxidase (forward primer: 5′-TAC GGT TGG TGT CAC AG-3′; reverse primer: 5′-ACG GCC ATC ACC ATA CTA GC-3′) as an internal standard. For Northern blots, 5 μg of total RNA was denatured in formaldehyde, and formamide was then loaded onto a 1.1% agarose/formaldehyde gel and run for 90 min at 72 V. After electrophoresis, RNA was transferred to a polyvinylidene membrane (Bio-Rad) and cross-linked to the membrane using Stratalinker (Stratagene). 32P-labeled DNA probes were generated using a RediPrime II DNA labeling kit (GE Life Science) according to the manufacturer’s instructions. Blots were hybridized overnight at 42 °C in 50% formamide, 5× SSC, 4× Denhardt’s solution, 0.1% SDS, 0.1% sodium pyrophosphate.

**Recombinant Protein Expression and Antibody Production—**cDNA from cercariae, prepared as described above, was used to subclone SmSrPQ into pET28a+ for Escherichia coli overexpression using forward primer 5′-GAG CTC ATG GAT GTA TTA CAA TCC CTT AAA AC-3′ and reverse primer 5′-GGC GCC GCT TAA TAT TGA TCT ATT GGA TTT G-3′. The amplicon was first cloned into TOPO-TA pCR2.1 (Invitrogen), digested with SacI and NotI, and directionally ligated to pET28a+ (Novagen). Plasmids were used to transform Xjb (DE3), an autoclaving E. coli strain (Zymo Research). Induction was carried out according to the manufacturer’s instruction. Inclusion bodies were collected and solubilized in 6 M guanidine solution (6 M guanidine, 100 mM Tris, 500 mM NaCl, 20 mM imidazole, pH 8.0). Hexa-His-tagged SmSrPQ was bound to Ni2+-agarose beads (immobilized metal affinity chromatography), washed with 8 M urea buffer (8 M urea, 100 mM Tris, 500 mM NaCl, 25 mM imidazole), and eluted with 8 M urea solution with 500 mM imidazole. Purified protein was excised from SDS-PAGE gel and analyzed by mass spectrometry or used for the immunization of 8-week-old BALB/c mice. Pre-bleeds were collected, and mice received two boosts every 4 weeks. Excised protein gel slices were homogenized in PBS, and TiterMax Gold (Sigma-Aldrich) was used as an adjuvant. Rabbit anti-SmCE antibodies were previously generated (19).

**Western Blots—**10 μg of crude lysates was incubated at room temperature for 10 min in 20 mM Tris (pH 7.5) and 25 mM NaCl. Samples were boiled under reducing conditions, resolved by SDS-PAGE analysis, and transferred to PVDF membranes. Blots were blocked overnight with 5% milk in TBS-T (Tris-buffered saline with 0.25% Tween 20). Blots were incubated for 1 h at room temperature with mouse anti-SmSrPQ antibodies (1:2000) or rabbit anti-SmCE antibodies (1:2000), washed with TBS-T, and then incubated for 1 h with horseradish peroxidase-conjugated IgG (GE Life Science, 1:2000). Membranes were extensively washed and visualized with ECL Western detection reagents (GE Life Science). Protein concentrations were determined by Bradford assay (20) using the Bio-Rad Pro-
tein Assay and BSA as a standard. Readings were done using Spectramax Plus 384 (Molecular Devices).

Collection of Material from Newly Transformed Schistosomula—Freshly collected cercariae were transformed into schistosomula in RPMI by passing the larvae through a 22-gauge needle 10 times, and tails were removed. Schistosomula were cultured in 4 ml of schistosomula culture media containing 2% BSA at 37 °C. After each incubation period of either 30 min or 1 h, the parasites were removed washed, gently centrifuged, transferred into fresh media, and visually inspected under a microscope to ensure viability and intact morphology. At each time point all media were collected, including the wash media. Plates were scraped as previously described (6) to remove any adherent secreted granules. These were collected and pooled with the media. 1 mM PMSF was added to prevent proteolytic cleavage of proteins. Samples were lyophilized and resuspended in water for Western blot analysis.

Confocal Imaging—Parasites were fixed overnight in cold acetone at −20 °C then air-dried as previously described (21) prior to blocking overnight in blocking buffer (PBS, 0.5% Triton X-100, 5% BSA, and 5% milk). Primary antibody was diluted 1:2000 in blocking buffer and incubated overnight at 4.0 °C, followed by three 4-h washes in wash buffer (PBS, 0.5% Triton X-100); secondary antibody (Invitrogen, Alexa Fluor) was diluted 1:2000 in blocking buffer, incubated overnight, and washed extensively. Images were taken using the Zeiss LSM 510 confocal microscope. All confocal images were taken with the gain and offset values set from negative controls using pre-bleed serum to ensure a low background signal.

Ex Vivo Skin Invasion and Immunohistochemistry—Human skin sections were removed from the abdomen of a recently deceased human male in accordance with Internal Review Board-approved necroscopy practices. Skin samples were washed with RPMI, and the subcutaneous fat was removed. Cercarial infection was carried out by placing skin sections over warm RPMI-filled wells as previously described (17) and placing the infective cercariae over the skin. After a 2-h incubation at 37 °C, skin sections were removed and fixed in 10% phosphate-buffered formalin (Fisher Scientific), embedded in paraffin, and sectioned. Fluorescence staining was carried out by blocking the sections in 20% normal goat serum in PBS with 0.5% Triton X-100. Antibodies were diluted in PBS with 2% normal goat serum and 0.5% Triton X-100. Primary antibodies were diluted 1:100 and incubated for 2 h. Secondary antibodies (Alexa Fluor) were diluted 1:250 and incubated for 1 h. Slides were mounted in Vectamount with DAPI (Vectashield). Images were taken using the Zeiss LSM 510 confocal microscope.

35S-labeled in Vitro Cell-free SmSrPQ Expression and Inhibitor Assays—Reactions were carried out using the TNT Quick Coupled Transcription/Translation System (Promega, Madison, WI) according to the manufacturer’s instruction. Briefly, the pET28a/SrPQ plasmid was linearized downstream of the SrPQ ORF using NotI, and the transcription/translation reaction was incubated at 30 °C for 90 min. A similar pET28a clone of human α1-antitrypsin was constructed and used as a positive control. Inhibition reactions were generally performed in 20 mM Tris, 25 mM NaCl, pH 8.0, at room temperature. Cercarial lysate was incubated for 10 min at room temperature with Z-AAPF-CMK, Z-AAPL-CMK, Z-AAPV-CMK, E-64, or PMSF prior to incubation with35S-SrPQ for 10 min at room temperature. CMK inhibitors were purchased from Bachem (Torrance, CA), whereas PMSF was purchased from Sigma-Aldrich.

RESULTS

qPCR and Northern Blot Analysis of SmSrPQ Transcript Levels—The full-length amino acid sequence of clone 8 was completed and renamed SmSrPQ. The predicted P1-P1’ reactive center loop specificity was determined to be Leu-Ser based on the alignment with α1-antitrypsin (Fig. 1, triangle). qPCR analysis revealed that SmSrPQ is expressed in the late schisto-
some sporocysts when cercariae are completing development in the intermediate snail host, and in aquatic free-swimming cercariae. Transcript levels remained high in cercariae-derived schistosomula after 24 h of *in vitro* culturing (Fig. 2b). These life stages correspond to the early skin invasion stages of infection (Fig. 2a). Peak transcript levels were seen in the cercarial stage. There was less transcript signal in early daughter sporocysts. Because sporocyst RNA samples were isolated from snail hepatopancreas, these samples contained high amounts of snail RNA as well as parasite-derived RNA. To verify this expression profile especially in the life stages where snail RNA might mask the qPCR signal of schistosome transcripts, Northern blot analysis was performed (Fig. 2c). DNA probes detected peak transcripts in late sporocysts. Transcripts were also detected in cercariae and daughter sporocysts. SmSrpQ was not detected in adult worms. Northern blot analysis also did not detect any expression in egg/miracidia total RNA extracts.

**Functional Expression of SmSrpQ**—The amino acid sequence of SmSrpQ contains all the components necessary to function as an inhibitory serpin (22). To confirm its ability to act as a suicide substrate inhibitor and form a complex with proteases, radiolabeled His₆-tagged SmSrpQ (supplemental Fig. S1a) was produced using a cell-free transcription/translation reaction. The reaction yielded the expected 49-kDa-tagged serpin protein (Fig. 3a). The recombinant serpin formed a complex with a component of the cercarial lysate, producing a product of ~68 kDa (Fig. 3a, black arrow). This complex formed under all three temperatures tested. A lower molecular weight band was also detected consistent with the cleaved serpin (Fig. 3a, double arrow). 3⁵⁸-S-SmrPQ only bound to a protease species found in the late sporocyst and cercaria stages. 3⁵⁸-S-SmrPQ did not bind to any components of the lysate from early daughter sporocysts, uninfected snail, or adult worms (Fig. 3b). 3⁵⁸-S-SmrPQ was not able to form a complex with neutrophil elastase and chymotrypsin (Fig. 3c). In contrast, 3⁵⁸-S-α₁-antitrypsin was able to form a complex with chymotrypsin and neutrophil elastase under the same conditions (Fig. 3c, black arrow). When these reactions were carried out for longer and under more physiological conditions, 3⁵⁸-S-SmrPQ was still unable to complex with neutrophil elastase and chymotrypsin (Fig. 3d). 3⁵⁸-S-SmrPQ was also unable to form an SDS-stable complex with pancreatic elastase, cathepsin G, and recombinant human chymase (data not shown).

To confirm that the serpin-protease complex was formed via the enzyme active site, the crude cercarial lysate was preincubated with PMSF, a small molecule chemical inhibitor that irreversibly modifies the active site serine of serine hydrolases (Fig. 4). At 400, 200, and 100 μM PMSF uncleaved 3⁵⁸-S-SmrPQ (double arrow, lanes 3, 4, and 5, respectively) was detected. In the methanol control (lane 2), where both cleaved and complexed 3⁵⁸-S-SmrPQ are seen, the presence of uncleaved serpin correlated with a corresponding decrease in the amount of complex detected (black arrow), as evidenced by a decrease in band intensity.

To examine the active site P1 specificity of the serpin-targeted protease, the lysate was preincubated with tetrapeptide chloromethyl ketone (CMK) inhibitors, and the formation of the serpin-protease complex was assessed. Complex formation was highly sensitive to Z-AAPF-CMK. 25 μM Z-AAPF-CMK reduced the level of complex formation to 61% of the band intensity seen in the DMSO control reaction (Fig. 4b). Z-AAPL-CMK also had an effect at 200 μM and 100 μM, whereas preincubation with Z-AAPV-CMK had no effect on complex formation (Fig. 4c).

**Western Blots of SmSrPQ and SmCE during Early Life Stages**—The predicted specificity of the reactive center loop of SmSrPQ, the sensitivity to PMSF, and the specific effects of the CMK inhibitors were all consistent with the previously reported biochemical characteristics of SmCE (23). SmCE is the major protease found in larval stages. To confirm the presence of a protein complex between endogenous SmCE and SmSrPQ, Western blots were performed on the crude lysates from different life stages. The lysates were incubated at 25 °C for 10 min to allow a complex to form. Western blots of uninfected snails and adults detected no SmSrPQ (Fig. 5a, lanes 1 and 5, respectively). Although, SmSrPQ protein (double arrow) was detected in the daughter sporocysts, no complex (black arrow) was seen. Late sporocysts and cercarial lysates contained both unbound SmSrPQ and complexed serpin (Fig. 5a, lanes 3 and 4, respectively). The size of the complex was approximately the same size and in the same life stages as the 68-kDa complex detected with the *in vitro* translated 3⁵⁸-S-SmrPQ. Protein levels of SmSrPQ were higher in the cercarial stage than in late sporocysts.

Daughter sporocysts exclusively contained the higher molecular weight, inactive zymogen of SmCE (Fig. 5b, black star). The activated, lower molecular weight SmCE protein (Fig. 5b, open arrowhead) was found in the lysates of late sporocysts and cercariae. SmCE has been observed to form a dimer under certain conditions, which would account for the 50-kDa central band observed with the α-SmCE antibody in the cercarial lysate (Fig. 5b, lane 4). The higher molecular weight band corresponding to a complex between SmCE and SmSrPQ was detected in late sporocysts and cercarial lysate (Fig. 5b, black arrow).

**Localization of SmSrPQ within the Parasite**—Given that SmCE and SmSrPQ are produced by the parasite at similar time points and SmCE appears to be active in late sporocysts and cercariae, the question was raised: does SmSrPQ prevent parasitic tissue damage from SmCE activity within the parasite? To address this question, localization studies of the SmSrPQ and SmCE in cercariae, 24-h schistosomula, and 3-day-old lung stage schistosomula were performed to identify the location and any interaction of SmSrPQ and SmCE. *S. mansoni* larvae contained several secretory glands that release material onto and into the host during skin invasion: the post-acetabular glands, the pre-acetabular glands, and the head glands (Fig. 6a). Factors needed for parasite binding and penetration are packaged in acetabular secretory granules and exit from extended cellular processes called ducts. These cell processes extend along the body of the larva to allow release of secretions into the environment at the head of the parasite (24). Confocal images show that SmSrPQ was located in the post-acetabular glands and their corresponding ducts, in one set of pre-acetabular glands, and the head gland of the cercaria (Fig. 6b, red). In 24-h schistosomula, residual SmSrPQ is seen in similar regions of the parasite (Fig. 6c, red) except that no serpin protein was detected.
FIGURE 2. SmSrPQ mRNA levels during *S. mansoni* developmental life cycle. *a*, the life cycle of *S. mansoni*. In addition to the free swimming miracidium and cercaria, shown are the stages in the molluscan intermediate host: mother sporocysts and daughter sporocysts (early and late), as well as the mammalian stages: schistosomula and adults. *b*, qPCR of SmSrPQ during the parasitic life cycle: early daughter sporocysts (35 days post snail infection, lane 1), late sporocysts (8 weeks post infection, lane 2), free swimming cercariae (after exiting the snail, lane 3), transformed cercariae after 24-h incubation at 37 °C (schistosomula, lane 4), and fully matured paired adult worms removed from hamster host (lane 5). Reactions were performed in triplicate and normalized to *S. mansoni* cytochrome oxidase expression. *c*, Northern blot analysis of 5 μg of mRNA. In addition to daughter sporocysts (lane 1), late sporocysts (lane 2), cercariae (lane 3), and adults (lane 4), shown are the egg/miracidia (lane 5) stage not shown in the qPCRs. 28S RNA was used as a loading control.
in the head gland. In the cercaria SmCE was located in a separate set of pre-acetabular glands and their corresponding ducts (Fig. 6b, blue). In the 24-h schistosomula, SmCE was found as residual protein in the pre-acetabular glands (Fig. 6c, blue). SmCE was neither detected in the head gland nor in the post-acetabular glands. Lung stage schistosomula isolated from infected hamsters had virtually no SmSrpQ and SmCE (Fig. 6d, blue).

SmSrpQ and SmCE Release and ex Vivo Skin Co-localization—Lacking co-localization within the parasite, intracellular inhibition of SmCE activity by SmSrpQ seemed unlikely. To test if the parasite temporally regulated the release of SmSrpQ and SmCE, newly transformed schistosomula were placed in Basch

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DISCUSSION

SmSrpQ is a serpin originally identified in the secreted products of invading *S. mansoni* larvae (cercariae) (16). Northern blot and qPCR analyses showed that SmSrpQ is expressed from the initiation of cercarial development in daughter sporocysts until 24-h schistosomula. This suggests that SmSrpQ plays a role in the early stages of infection when cercariae/schistosomula invade host skin and enter dermal blood vessels. No transcripts were found in either adult worms or the egg/miracidium stage, nor was SmSrpQ protein detected in lung stage schistosomula. Despite advances in RNAi construct delivery and induction in the larval stages of *S. mansoni* (25), problems remain in terms of effectively knocking down genes of choice to levels that would interfere with their function (26) and in delivering constructs to the parasite in the intermediate snail host (27); hence, this study employed a biochemical approach to investigate the role of SmSrpQ in *S. mansoni* pathogenesis.

Recombinant SmSrpQ was unable to bind host proteases, namely, neutrophil elastase (Fig. 3cd) and cathepsin G (data not shown), but was able to complex with a component of the larval lysate. PMSF, a serine protease inhibitor, blocked serpin-protease complex formation (Fig. 4a).

Invasion of human skin by *S. mansoni* requires the disruption of the epidermal cell layer, the basement membrane, and the extracellular matrix of the dermis. Larval invasion of human skin produces degradation products of host desmosomes, complement factors, immunoglobulin, as well as epidermal cell lysis products (28). Extracellular matrix and cell-cell junction degradation has been attributed to serine protease activity, particularly to SmCE (19, 23, 29, 30). SmCE is one of the most abundant proteins in cercarial secretions (7). A synthetic tetrapeptide library screen of purified native SmCE showed that the
S1 pocket of the protease prefers a Leu, Phe, or Met substrate side chain (19). The primary sequence of SmSrPQ predicts a Leu-Ser in the P1-P1’ position in the reactive center loop (Fig. 1, triangle).

Z-AAPF-CMK is an effective inhibitor of cercarial invasion and a known inhibitor of SmCE (17). When cercarial lysates were preincubated with Z-AAPF-CMK, a marked reduction in complex formation with SmSrPQ was observed (Fig. 4b). This is in contrast to the complete lack of effect on complex formation following preincubation of the lysate with Z-AAPV-CMK (Fig. 4c), which does not inhibit SmCE but does inhibit neutrophil elastase (31). Western blots of lysates confirmed that complex formation only occurs when active SmCE is present (Fig. 5b). In early daughter sporocyst lysates, where only the SmCE zymogen form was present, no complex with SmSrPQ was detected.

SmSrPQ could potentially function as a cytosolic serpin responsible for preventing internal damage in the event of accidental SmCE release inside the parasite secretion glands. This would be a similar function to monocyte/neutrophil elastase inhibitor, an intracellular serpin of cells of the myeloid lineage (32). This potent inhibitor of neutrophil elastase, cathepsin G, and chymase (33) lacks a signal sequence and functions to protect granulocytes from intracellular release of protease found in azurophilic granules (33). Localization of SmSrPQ versus SmCE (Fig. 6, b and c) suggests it is unlikely that SmSrPQ interacts with SmCE inside the parasite. SmSrPQ is in the head gland, post acetabular, and a disparate set of pre-acetabular glands, whereas SmCE resides in the adjacent set of pre-acetabular glands. The complex formation seen by Western blots (Fig. 5) is likely due to the break down of this spatial/cellular barrier between these two proteins upon lysis of the parasite. Electron-
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micrograph localization in frozen thin sections showed SmCE in both pre- and post-acetabular glands (29), but subsequent studies using confocal imaging have not detected SmCE in the post-acetabular glands (34).

The co-localization of SmSrPQ and SmCE (Fig. 7e) in human skin invasion assays suggests that the parasite is regulating the extracellular matrix-degrading activity of SmCE inside the host. In vitro cultivated schistosomula release both proteins simultaneously, but this release in vivo may be regulated by host and or invasion cues not simulated by culture conditions. Nevertheless, these results confirm that SmSrPQ is not retained inside the parasite. It is released by the larva in a similar fashion to SmCE. Fluorescent images of the parasite indicate that SmSrPQ is not localized to the tegument but is released into the skin of the host. The potential role of SmSrPQ as an external regulator of SmCE is consistent with the function of serpins like α1-antitrypsin in lung tissue. Patients with an α1-antitrypsin deficiency suffer from severe early onset emphysema. This condition results from an imbalance in neutrophil elastase proteolytic activity in bronchial and alveolar tissue (35). During inflammation, leukocytes release extracellular matrix-degrading enzymes, such as neutrophil elastase, that may inadvertently cause tissue damage (36). α1-Antitrypsin and, to a lesser extent, α2-macroglobulin regulate elastase activity and limit the extracellular matrix damage. Like schistosome cercariae, inflammatory cells such as macrophages and monocytes can secrete both proteases and the corresponding inhibitors (36).

Inflammatory damage to host skin tissue, called cercarial dermatitis, can be observed with the accidental infections of humans by avian schistosomes that are not adapted to a human host, such as Trichobilharzia regenti. Initial infection with T. regenti larvae causes an acute inflammatory response characterized by edema, thickening of the skin, and a large infiltration of mast cells, neutrophils, and other leukocytes (37) leading to the killing of most parasites within the dermal tissue. S. mansoni is a schistosome more adapted to the human host. Infection experiments using human skin (38) or a mouse model (39) have shown a limited immune response to invading larvae with no significant parasite killing, resulting in parasites being able to enter the host blood vessels. Dermatitis can occur after a heavy exposure to cercariae, but symptoms subside after 48 h and the rare cases are confined to non-sensitized individuals, e.g. tourists (40).

S. mansoni effectively invades intact skin and has developed multiple mechanisms to evade the host immune response and establish an infection that can debilitating but do not kill the host. The tight post-translational regulation of SmCE by SmSrPQ may represent an adaptation to limit host tissue damage to the region of larval invasion and prevent severe inflammation due to excessive or uncontrolled damage to host cells/tissue.

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