Functional and Antigenic Similarities Between a 94-kD Protein of *Schistosoma mansoni* (SCIP-1) and Human CD59

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Summary

Schistosomiasis is a parasitic disease affecting ~200 million people, primarily in the third world. *Schistosoma mansoni*, one of the causative agents of this disease, parasitize the human mesenteric and portal blood systems while successfully evading host immune responses. During parasite penetration into the mammalian host and shortly afterwards, the larvae rapidly convert from being sensitive to being resistant to C-mediated killing. Treatment of the C-resistant parasitic forms with trypsin renders the parasite susceptible to C attack, thus indicating the presence of C inhibitory protein(s) on the parasite surface. We describe here an intrinsic schistosome C inhibitory protein (SCIP-1) that exhibits antigenic and functional similarities with the human C-inhibitor CD59. Like CD59, SCIP-1 is capable of inhibiting formation of the C membrane attack complex (MAC), probably by binding to C8 and C9 of the C terminal pathway. In addition, SCIP-1 is apparently also membrane-anchored via glycosyl phosphatidylinositol as it can be specifically released with phosphatidylinositol-specific phospholipase C. Soluble SCIP-1, partially purified from Nonidet P-40 extracts of schistosome tegument is capable of inhibiting hemolysis of sensitized sheep erythrocytes and of rabbit erythrocytes by human C. Anti-human CD59 antibodies block this activity of SCIP-1 and in addition, upon binding to intact parasites, render them vulnerable to killing by human and guinea pig C. SCIP-1 is located on the surface of C-resistant forms of the parasite, i.e., 24-h cultured mechanical schistosomula and in vivo-derived adult worms as revealed by immunofluorescence and immunogold electron microscopy studies. These results identify one of the mechanisms schistosomes use to escape immune attack.

Schistosoma mansoni is a blood fluke that successfully parasitizes pulmonate snails and mammalian hosts (1). Efficient evasion of the mammalian host's C system is therefore essential for parasite survival. During penetration of the mammalian host's skin, the young schistosomula are sensitive to the cytolytic action of the C system. These young larvae rapidly transform and undergo many morphological changes, resulting in a form that is highly resistant to C attack (reviewed in 2). The later developmental stages of the parasite, i.e., the lung and adult worms, found in the lung and mesenteric veins, respectively, also show refractoriness to C killing (3, 4). The freshly transforming schistosomula use several evasion strategies to resist C damage. First, the schistosomula shed their glycocalyx coat which is a strong activator of the alternative C pathway (5, 6). Consequently, the schistosomula become weak activators of the alternative C pathway. Second, due to this release of the C activating glycocalyx, there is consumption of C components at a site distant to the parasite's cell membrane (7). A 28-kD schistosomular serine protease, which is released from the acetabular glands of transforming cercaria and is expressed later on the surface membrane of schistosomula and adult worms, probably plays a dual role in C resistance. The protease accelerates the release of the glycocalyx (8), and cleaves certain C proteins (9–11). By degrading C components in plasma or on the cell surface, such a protease may protect the parasite from C opsonization and lysis. Another protective mechanism employed by *S. mansoni* is the use of C regulatory proteins. Initial studies performed by us indicated the existence of surface proteins which, upon removal by trypsin or pronase, render the parasite susceptible to C attack (12). Recently, our results have indicated that schistosomula and adult worms express two C inhibitors, one acting at the C3

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step (reference 10 and Parizade, Arnon, and Fishelson, manuscript in preparation), and one acting at the C8/C9 step of the C cascade (see below).

Five plasma C proteins (C5, C6, C7, C8, and C9) participate in formation of the membrane attack complex of C (MAC)[1] on activating surfaces (13). This process may be restricted on homologous cells by several membrane proteins, such as homologous restriction factor (HRF, C8-binding protein, MAC-inhibiting protein) (14–16) and CD59 (17–23). CD59 is an 18–20-kD membrane protein widely distributed in many human tissues (24, 25) and released in a soluble form in human urine (17). Two nonmammalian proteins with partial homology to human CD59 have been described. One is an adhesion molecule of *Entamoeba histolytica* (27) and the second one is a gene product of *Herpesvirus saimiri* (28).

In this report we describe a schistosome C inhibitory protein type 1 (SCIP-1) that is a 94-kD protein functionally and antigenically related to human CD59. We demonstrate that several properties of CD59, such as the capacity to inhibit formation of C MAC and the binding of human C8 and C9 components (23, 29, 30) are manifested by SCIP-1 as well, and that both molecules are, apparently, external glycosyl phosphatidylinositol-anchored membrane proteins (31). Furthermore, antibodies directed to human CD59 bind to SCIP-1 and render the parasite susceptible to C-mediated killing.

Materials and Methods

Parasites and Parasite Extracts. A Puerto Rican strain of *S. mansoni* was maintained in *Biomphalaria glabrata* snails and outbred ICR mice. Cercaria were collected after a 90-min shedding period and mechanically transformed by passage between two connected syringes (32). They were washed immediately with ice cold defined synthetic medium (DSM) composed of a 1:1 mixture of RPMI 1640 and nutrient F12 (GIBCO BRL, Gaithersburg, MD) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine (Kibbutz Beth Haemek, Israel), and 20 mM Hepes buffer, pH 7.2. The tails and bodies were separated by centrifugation through a 61% Percoll (Pharmacia LKB, Uppsala, Sweden) solution. The schistosomula were washed several times in DSM and cultured for 24 h in DSM at a concentration of 20,000/well in a 24-well plate (Falcon Labware, Oxnard, CA) in a humidified, 6% CO_2 incubator. Lung stage worms of *S. mansoni* were obtained by lung/heart perfusion of ICR mice infected with 3,000 cercaria by subcutaneous injection 6 d earlier. The perfusion medium was DSM supplemented with 10 U/ml Heparin (Sigma Chemical Co., St. Louis, MO). The perfused lungs and hearts were washed several times, finely minced, and incubated for 3 h at 37°C. The suspension was filtered through a Nitex-132 mesh nylon sieve (Tetko, Rolling Meadows, IL) and the lung worms recovered by centrifugation at 120 *g* for 3 min. Adult worms (AW) of *S. mansoni* were isolated by perfusion of the hepatic portal system of Syrian gold hamsters infected 6 wk earlier with 1,000 cercaria by the ring method (33). The perfusion medium was DSM supplemented with 500 U/ml Heparin.

NP-40–released Material (NPRM) Preparation. One million 24-h-old schistosomula or 2,000 AW were incubated in 1 ml NP-40 (1%) (Sigma Chemical Co.) in PBS for 2 h on ice. The schistosomula and AW remained intact and were removed by centrifugation and the supernatant was further cleared by centrifugation in an Airfuge (Beckman Instruments, Inc., Fullerton, CA) at 10^5 *g*. The resulting supernatant was mixed and gently agitated with SM-2 beads (Bio-Rad Laboratories, Richmond, CA) according to manufacturer's instructions, for removal of the NP-40. The resulting NPRM was stored in aliquots at −70°C.

Trypsin-released Material (TRM) Preparation. 3×10^5 24-h-old schistosomula or 1,000 AW were treated with 100 μg/ml or 500 μg/ml trypsin (TPCK-treated; Sigma Chemical Co.), respectively, in DSM for 30 min at 24°C. The parasites were removed by centrifugation and soybean trypsin inhibitor (Sigma Chemical Co.) was added (twice the concentration of trypsin) to the supernatant for inhibiting further trypsin activity.

Sera and Antisera. Normal human serum (NHS) was obtained from healthy donors. C7- and C8-deficient human sera (C7d-NHS and C8d-NHS) were prepared from two donors who are genetically deficient in C components C7 and C8, respectively (34). Normal rabbit serum (NRbS) and guinea pig serum (GPS) were prepared from healthy animals and C4-deficient GPS (C4d-GPS) from genetically C4-deficient guinea pigs. Animals were provided by the Animal Breeding Center of the Weizmann Institute of Science. All sera were aliquoted and kept at −70°C until used. Anti-CD59 monospecific polyclonal Abs were prepared in rabbits by three subcutaneous injections of 20 μg purified human urine CD59 at 2-wk intervals. $F(ab')_2$ fragments and rat mAb YTH 53.1 anti-human CD59, were prepared as described elsewhere (17). Abs to schistosomular TRM (anti-TRM Abs) were obtained as previously described (12).

Labeling Procedures. Schistosomula were surface iodinated using the lactoperoxidase technique (35) and $Na^{125}I$ (Amersham International, Amersham, UK). Freshly transformed schistosomula were metabolically labeled in methionine-free DME (GIBCO BRL) (20,000 schistosomula/ml in a 24-well tissue culture plate) supplemented with [^{35}S]methionine (50 μCi/ml) (Amersham International, 1 Ci/μmole) for 24 h. Protein A (Sigma Chemical Co.) and purified human C9 (36) were iodinated by the Iodogen method (37) using Iodogen reagent (Pierce Chemical Co., Rockford, IL) and $Na^{125}I$ (Amersham International).

C9 Deposition on Schistosomula. Schistosomula (15,000) were incubated in 100 μl NHS supplemented with ^{125}I-C9 for various times at 37°C (12 μg ^{125}I-C9 were introduced to 1 ml of NHS; 20% of native C9). The schistosomula were washed four times with DSM and boiled for 10 min in reducing sample buffer (50 mM Tris, pH 6.8, 10% glycerol, 4% SDS and 5% β-mercaptoethanol) containing protease inhibitors (10 μg/ml Soybean trypsin inhibitor; 5 mM PMSF; 1 mM Diisopropyl fluorophosphate; 10 mM EDTA). The schistosomula were then sonicated in a bath sonicator (Heat System; Ultrasonic, Inc., Farmingdale, NY) at 4°C for 3 min, three times, with attenuated vortexing. The resulting extracts were applied on a 2.5–10% gradient acrylamide gel for SDS-PAGE analysis (38).

Hemolytic Assays. Sensitized sheep erythrocytes (EAs) were prepared as previously described (39) with rabbit hemolysin (DIFCO, Detroit, MI). The EAs were washed twice with GVB^{2+} (veronal-buffered saline, pH 7.4, containing 0.1% gelatin, 0.5 mM $MgCl_2$, 0.15 mM $CaCl_2$, and 0.02% NaN_3), brought to a concentration

[1] *Abbreviations used in this paper:* AW, adult worm; C4d-GPS, C4-deficient GPS; C7d-NHS, C7-deficient NHS; C8d-NHS, C8-deficient NHS; DSM, defined synthetic medium; EAs, sensitized sheep erythrocytes; ECL, enhanced chemiluminescence; E_R, rabbit erythrocytes; GPS, normal guinea pig serum; MAC, membrane attack complex of C; NHS, normal human serum; NPRM, NP-40 released material; NRbS, normal rabbit serum; PI-PLC, phosphatidylinositol specific phospholipase C; SCIP-1, schistosome C inhibitory protein type 1; TRM, trypsin released material.

of 10^9/ml and stored at 4°C. To test the effect of parasitic extracts on the classical pathway of C, 1.5×10^7 EAs were incubated at 37°C for 30 min in 100 μl GVB^{2+} containing a dilution of NHS (usually 1:200) giving 50% cell lysis, with or without parasite TRM or NPRM. Cell lysis was stopped by adding 1 ml of cold GVB and centrifugation at 500 *g* and percent hemolysis was determined by measuring spectroscopically the supernatant at 412 nm. Activity of the C alternative pathway was assayed using fresh rabbit erythrocytes (E_R). 1.5×10^7 E_R were incubated for 30 min at 37°C in 100 μl GVB^{2+} containing 5 mM $MgCl_2$, 10 mM EGTA, and 6 μl NHS. Cell lysis was stopped by adding 1 ml of cold GVB containing 10 mM EDTA. Percent hemolysis in absence or presence of parasite TRM or NPRM was determined as described above. To measure the effect of parasitic extracts on the preformed C5b-7 complex, EAs (10^9/ml) were incubated first with 400 μl of 1:20 diluted C8d-NHS for 15 min at 37°C and washed twice with cold GVB^{2+}. 1.5×10^7 EAC5b-7 were incubated with or without parasite NPRM for 15 min at 24°C, washed twice with cold GVB^{2+} containing 10 mM EDTA, and incubated with C7d-NHS (diluted 1:2,000 in GVB), as the source of C components C8 and C9, for 30 min at 37°C.

Killing of Parasites by C. 200 schistosomula or lung worms (in 20 μl) were transferred into wells of 96-well microtiter plates (Nunc, Roskilde, Denmark) and 10 AW were transferred into polystyrene tubes (12 × 75 mm; Falcon Labware). The parasites were then mixed with 100 μl of NHS, GPS (diluted 1:2), or C4d-GPS (diluted 1:2) and incubated for 18 h at 37°C in a humidified incubator with 6% CO_2. Heat inactivated (30 min at 56°C) sera served as control. Mortality was determined under a stereoscopic microscope based on lack of parasite motility and granular and opaque appearance. Experiments were run in duplicates. Percent mortality in control cultures (Cn) was subtracted from that of experimental (E) and percent net mortality was calculated according to the formula: % net mortality = $(E - Cn)/(100 - Cn) \times 100$.

Antigenic Competition Assay. A 96-well microtiter plate (MicroTest Flexible Assay Plate; Falcon Labware) was coated with schistosomular NPRM (15 μg/100 μl PBS/well) for 3 h at 24°C. The plate was washed and uncoated areas were blocked with PBS-0.5% gelatin for 1 h at 24°C. Polyclonal anti–human CD59 antiserum, as such or preincubated with the competing substance, was added at various dilutions in PBS to the microtiter plate for 2.5 h at 24°C and for 18 h at 4°C. After three washes with PBS, ^{125}I-Protein A (120,000 cpm/well) in PBS-0.1% gelatin was added for 3 h at 24°C. After an additional three washes, the plate was dried and wells were cut and counted in a Packard γ-counter (Packard Instruments, Chicago, IL). Competition was effected by preincubation of the anti-CD59 antiserum with purified human urine CD59 (200 ng/well), human E ghosts (prepared according to reference 40) (4.8 μg/well), schistosomular NPRM (15 μg/well), or with buffer alone as control, for 2 h at 37°C.

Immunofluorescence. Schistosomula (3,000–5,000) or AW (10–20) were washed in DSM containing 0.5% BSA and 0.1% γ globulin (Fraction II; Sigma Chemical Co.) several times and incubated with anti-CD59 antiserum (diluted 1:1 in the above medium) for 30 min at 37°C. After extensive washes, the parasites were further incubated with FITC-conjugated anti–rabbit IgG Ab (Bio-Yeda, Rehovot, Israel) diluted 1:10 in PBS for 30 min on ice. After additional washes, the schistosomula were fixed in 3% paraformaldehyde (BDH Laboratory Supplies, Poole, UK) for 1 h on ice. AW were observed unfixed. Parasites were examined and photographed using a fluorescence microscope (Photomicroscope III, Zeiss, Oberkder, Germany) and a Kodak 400 ASA film.

Immunogold Labeling and Electron Microscopy. 24-h-old schistosomula were washed with PBS and fixed with a mixture of 3% paraformaldehyde and 0.1% glutaraldehyde (Polysciences Inc., Warrington, PA) in PBS for 2 h at 4°C. Cryosections were prepared as previously described (41). Immunolabeling was performed at room temperature according to the method of Tokuyasu and Singer (42), using rabbit anti-CD59 antiserum or NRbS (control) diluted 1:200 in PBS and gold-conjugated protein A (15 nm; Zymed Laboratories Inc., S. San Francisco, CA). Standard procedures for fixation, dehydration, and embedding in EPON 812 of the schistosomula were employed. Electron micrographs were obtained using an electron microscope (EM410; Philips Technologies, Cheshire, CT) at 80 kV.

Protein Determination, SDS-PAGE, and Western Blotting. Protein concentration was measured by using Bio-Rad's protein assay dye reagent (Bio-Rad Laboratories) according to manufacturer's instructions. Electrophoresis was performed on acrylamide gels under reducing or nonreducing conditions according to Laemmli (38). Molecular weight markers of Pharmacia LKB were used: phosphorylase B (94,000), BSA (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), and α-lactalbumin (14,400). Nitrocellulose membranes (0.2 μm; Schleicher & Schuell, Inc., Keene, NH) were used for Western blotting according to Towbin (43). Membranes were blocked with 3% BSA in Tris-buffered saline (TBS-BSA). Thereafter, anti-CD59-reactive bands were detected with polyclonal anti–human urine CD59 Ab (diluted 1:300) or mAb YTH 53.1 (20 μg/ml), followed by ^{125}I-Protein A (200,000 cpm/ml). Membranes were dried and exposed to an X-ray film (Curix PP-2; AGFA Corp., Orangeburg, NY). In some experiments, the enhanced chemiluminescence reagent (ECL; Amersham, International) was used according to manufacturer's instructions. Detection of the positive bands in these blots was performed with horseradish peroxidase–conjugated goat anti–rabbit IgG Ab (Sigma Chemical Co.) and the ECL detection reagents.

Immunoprecipitation. Immunoprecipitation was carried out according to Kessler (44) with some modification. Briefly, [^{35}S]methionine metabolically labeled schistosomula or AW NPRM (40 μl) was mixed with 460 μl of 50 mM Tris HCl, pH 7.4, containing 5 mM EDTA, 150 mM NaCl, and 0.5% NP-40 and 40 μl of anti–human CD59 antiserum or NRbS for 1 h on ice. After an additional incubation of 10 min on ice with 25 μl of 10% (wt/vol) formalin-fixed *Staphylococcus aureus* (Sigma Chemical Co.), the bacterial adsorbent was washed three times with the latter buffer containing 5% (wt/vol) sucrose. After a final wash with 50 mM Tris HCl, pH 7.4, and 150 mM NaCl, the immunoprecipitates were resuspended in sample buffer (50 mM Tris, pH 6.8, 10% glycerol, 4% SDS, and 5% β-mercaptoethanol), boiled for 5 min, and subjected to SDS-PAGE analysis (38). The gel was stained with coomassie blue (Sigma Chemical Co.), destained, treated with DMSO and 2,5-diphenyloxazole (PPO) (Sigma Chemical Co.), and dried. Autoradiography was performed by exposure of Curix RP-2 AGFA X-ray films to the dried gel at −70°C.

Immunoaffinity Adsorbtion. ^{125}I-NPRM prepared from surface-labeled schistosomula, was mixed with noniodinated schistosomular NPRM at a 1:2 ratio and passed through a 2-ml affinity column composed of anti-CD59 Ig coupled to cyanogen bromide-activated Sepharose (Pharmacia LKB) or NRb-Ig-Sepharose. The unbound proteins were washed away extensively with 20 ml TBS (10 mM Tris, 150 mM NaCl) and then with 10 ml of TBS supplemented with 400 mM NaCl. The column was washed again with 20 ml of TBS and eluted with 0.1 M glycine-HCl, pH 2.5 containing 0.01% NaN_3. The pH of the eluted fractions (250 μl/tube) was neutralized with 25 μl of 1.5 M Tris, pH 8.8. The eluted fractions were dialyzed for 18 h at 4°C against GVB^{2+} and tested for their

capacity to inhibit C-mediated E hemolysis. Active fractions were pooled, concentrated in a Speed Vac (Hetovac VR-1; Heto High Technology of Scandinavia, Birkerod, Denmark), and further analyzed by SDS-PAGE and autoradiography.

Binding of SCIP-1 to Human C8 and C9. 5 μg of human C8 (a kind gift from Dr. J. M. Sodetz, University of South Carolina, Columbia, SC) and C9 were electrophoresed under nonreducing conditions on 7.5% acrylamide gels and transferred to nitrocellulose membranes (0.2 μm, Schleicher & Schuell). After blocking the membrane for 2 h at 24°C in TBS, pH 7.6 containing 5% skimmed milk (Marvel Cadbury, Bournville, UK) and 0.1% Tween-20 (Sigma Chemical Co.), it was incubated with schistosomular NPRM diluted 1:4 in blocking buffer containing protease inhibitors (10 mM PMSF [Sigma Chemical Co.], 10 mM EDTA [BDH Laboratory Supplies] and 10 μg/ml aprotinin [Sigma Chemical Co.]) for 2 h at 37°C. The membrane was extensively washed and bound SCIP-1 was detected by using anti-human CD59 antisera and the ECL method as described for Western blotting.

Anti-CD59 Abs were eluted from membrane strips (0.5 × 1 cm) containing C9, SCIP-1, and bound Ab molecules with 0.2 M glycine-HCl, pH 2.6, for 1 h at room temperature, according to Ritter (45). The specificity of the eluted Ab to SCIP-1 was examined by Western blotting of schistosomular extracts.

Results

Identification of C Inhibitory Proteins on the Surface of C-resistant Schistosomes. Treatment of resistant schistosumula with trypsin renders them susceptible to complement killing (12). The trypsin's sensitizing effect on five different life cycle stages of *S. mansoni* was examined (Table 1). Freshly transformed (0 h), 4- and 24-h-old schistosomula, lung worms, and AW were treated with trypsin at various concentrations and their susceptibility to guinea pig C was then measured. Freshly transformed schistosomula showed high susceptibility to C even without any treatment and trypsin had hardly affected their sensitivity to C. 4- and 24-h-old schistosomula and AW became, upon trypsinization, susceptible to C-mediated killing. Trypsin concentrations of 250–500 μg/ml produced maximal (100%) killing of the schistosomula and up to 65% killing of AW. Trypsin treatment had almost no effect on the interaction of lung worms with GPS. We have previously suggested that schistosomula and AW express on their surface trypsin-sensitive C inhibitory proteins (CIPs) (12).

The presence of a C inhibitor on schistosomula was further indicated in experiments analyzing activation of the terminal C components. Successful activation of the C cascade culminates in generation of the MAC and polymerization of C9 (13). The polymerized C9 (poly C9) has an apparent M_r of ~10^6 and is resistant to boiling in reducing SDS-PAGE sample buffer. One way to determine the extent of C activation is to analyze poly C9 formation after inclusion of ^{125}I-C9 in the serum used as a C source (46). We have demonstrated formation of poly C9 on the surface of freshly transformed schistosomula incubated for 20 min with NHS (Fig. 1). Poly C9 formation was markedly reduced on 24 h schistosomula but trypsinization led to successful polymerization of C9 on the same larvae (Fig. 1).

The schistosomal TRM inhibited both classical and alternative C pathways as demonstrated by its effect on the lysis of EAs and E_R by NHS. As shown in Fig. 2, TRM from AW (AW/TRM) and schistosomula (S/TRM) inhibited both EAs lysis (90 and 80%, respectively) and E_R lysis (89 and 36%, respectively), indicating inhibition of the two C lytic pathways. In contrast, trypsinization of cercaria has not produced any inhibitory activity to sheep E hemolysis by NHS (not shown). NP-40 extracts of AW (AW/NP-40) inhibited EAs lysis (83% inhibition) as well. As shown here and in

Table 1. *Trypsin-induced Killing of Schistosomes by Complement*

	Percent mortality*				
	Schistosomula				
Trypsin‡	0 h	4 h	24 h	Lung worms	Adult worms
μg/ml					
0	87	2	1	16	3
50	100	42	51	4	17
100	100	55	78	27	18
250	ND	71	100	23	44
500	ND	100	ND	28	65

* Killing was performed in GPS diluted 1:2 in DSM and percent mortality was calculated.
‡ Trypsinization was carried out for 30 min at 24°C in DSM.

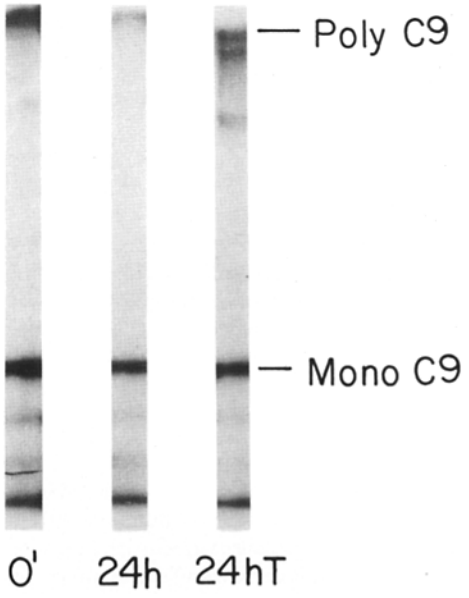

Figure 1. C9 deposition on schistosomula. Freshly transformed schistosomula (0'), 24-h-old schistosomula (24 h) and trypsinized 24-h-old schistosomula (24-hT), were incubated with NHS supplemented with ^{125}I-C9 for 20 min at 37°C. Parasites were then washed in DSM, resuspended in reducing sample buffer, boiled for 10 min, and sonicated. The resulting extracts were applied on a 2.5–10% gradient gel for SDS-PAGE analysis. A representative autoradiogram is shown.

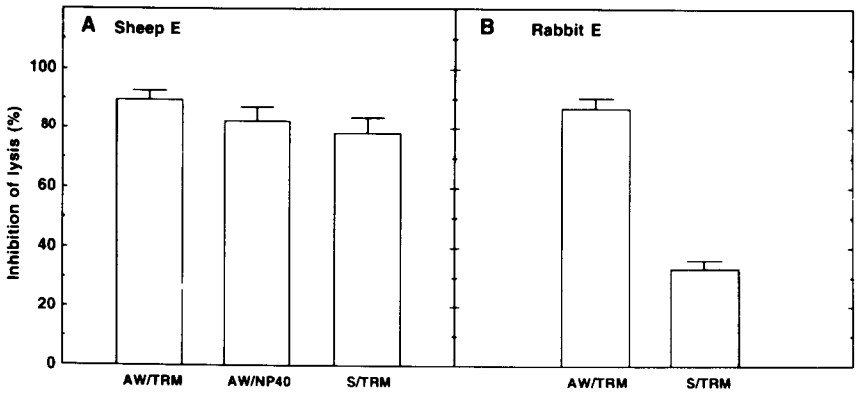

Figure 2. Inhibition of C-mediated hemolysis by membrane extracts of *S. mansoni*. 1.5 × 10^7 EAs (*A*) or E_R (*B*) in GVB^{2+} were mixed with NHS diluted 1:80 in the absence or presence of membrane extracts and incubated for 30 min at 37°C. Unlysed Es were removed by centrifugation and percent lysis was determined. Percent inhibition of lysis relative to control without parasite extracts was calculated. Results represent means ± SE of seven experiments.

following experiments, NP-40 solubilized the AW membrane components with the inhibitory activity.

Inhibition of the Membrane Attack Complex (MAC) of C. To directly assess whether schistosomula and AW express an inhibitor of MAC formation, we have examined the ability of parasite NP-40 extracts (NPRM) to inhibit E lysis after formation of C5b-7 complexes. This was performed by preincubation of EAC5b-7 with schistosomular or AW NPRM, followed by addition of C7d-NHS as the source of C components C8 and C9. As shown in Fig. 3 *A*, E lysis was inhibited by NPRM in a dose-dependent manner. Heat treatment (50°C and above) of the NPRM completely abolished its inhibitory effect (Fig. 3 *B*). Furthermore, polyclonal monospecific anti-human CD59 Abs blocked the NPRM C inhibitory effect (Fig. 3 *C*). Preincubation of the NPRM with 1.0 mg of anti-CD59 Ig decreased the extent of inhibition by 77% (from 94.4 to 23%). No significant decrease of the NPRM-inhibitory effect was observed when the NPRM was incubated with 1.0 mg of normal rabbit immunoglobulin. These results establish that a protein found in NPRM extracts of schistosomes, is capable of inhibiting formation of C MAC, and is therefore termed SCIP-1, schistosome C inhibitory protein type-1. SCIP-1 activity may be blocked specifically by anti-human CD59 Abs, indicating an antigenic similarity to human CD59.

Induction of Schistosomacidal Activity with Anti-CD59 Abs. The finding that schistosomular NPRM inhibited the terminal C pathway and that this C inhibitory effect could be blocked specifically by anti-CD59 Abs led us to test whether the anti-CD59 Abs could block the MAC inhibitory molecule on the intact parasite surface and render the parasite sensitive to C killing. Incubating C resistant 24-h-old schistosomula with anti-CD59 Abs indeed sensitized the schistosomula to killing by GPS (Fig. 4 *A*). To exclude the possibility that the anti-CD59 Ab simply activated classical C pathway, 24-h-old schistosomula were treated with $F(ab')_2$ fragments of the anti-CD59 Abs that are not expected to induce classical C pathway activation. On addition of GPS, increasing levels of mortality were observed (up to 48%) (Fig. 4 *B*). Abs directed to the 28-kD proteases, which are known to bind to the surface of 24-h old schistosomula (11) served as nonrelevant control Ab and produced up to 18% mortality of

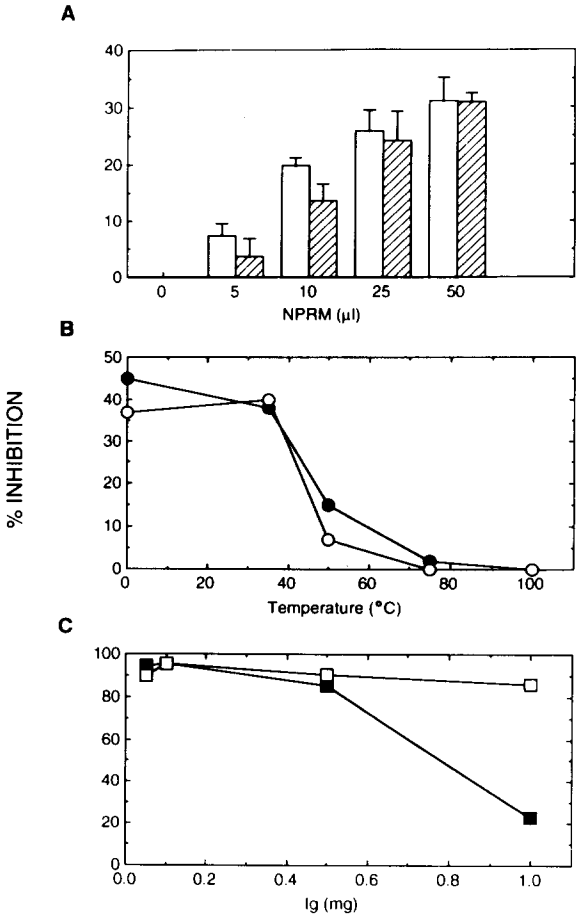

Figure 3. Inhibition of the C terminal pathway by parasitic NPRM. (*A*) EAC5b-7 (1.5 × 10^7) were treated with different amounts of schistosomular (□) or AW NPRM (▨) for 15 min at 24°C. Next, C7d-NHS was added and incubation continued for 30 min at 37°C. Percent lysis was measured and percent inhibition relative to control without any NPRM was calculated. Results are mean of three experiments. (*B*) Heat inactivation of the inhibitor of MAC formation. Schistosomular (○) or AW NPRM (●) (50 µl) were heated for 5 min at various temperatures and tested for their capacity to inhibit MAC formation as explained in *A*. (*C*) Neutralization of the MAC inhibitor with anti-CD59 Abs. Rabbit anti-CD59 Ig (■) or normal rabbit (NRb) Ig (□) were mixed with schistosomular NPRM (20 µg) for 1 h at 24°C and then for 3 h on ice. 1.5 × 10^7 EAs and NHS (1:200 final dilution) were then added and incubation continued for 30 min at 37°C. Percent inhibition was calculated.

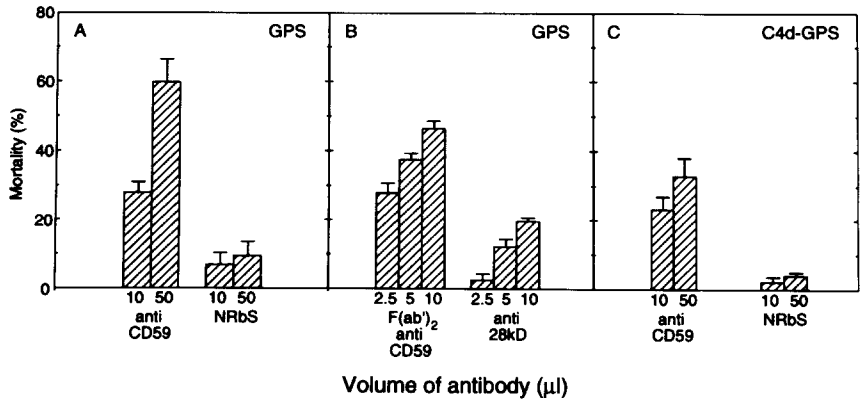

Figure 4. Antibodies to human CD59 induce killing of schistosomula by GPS. 24-h-old schistosomula were treated with increasing volumes of undiluted rabbit anti-CD59 antisera (*A* and *C*), NRbS (*A* and *C*), $F(ab')_2$ fragments of the rabbit anti-CD59 Ab (*B*), or anti-28 kD protease antisera (*B*), for 30 min at 24°C. The schistosomula were then washed and killing was induced by GPS (*A* and *B*), or C4d-GPS (*C*) for 18 h at 37°C. Percent net mortality was calculated relative to controls with heat-inactivated GPS. Results represent means ± SD of three experiments.

schistosomula (Fig. 4 *B*). Anti-28kD antibodies probably activated directly the classical complement pathway. Schistosomular killing with C4d-GPS which has no classical C pathway activity, was also induced by the anti-CD59 antiserum (Fig. 4 *C*). $F(ab')_2$ fragments of the anti-CD59 Ab-induced killing of schistosomula by human C as well (Fig. 5). These results demonstrated killing of the schistosomula via the alternative pathway of guinea pig and human C, effected by blocking the activity of SCIP-1, with anti-human CD59 Abs.

Antigenic Competition Between human CD59 and SCIP-1. Additional evidence supporting antigenic-cross-reactivity between SCIP-1 and human CD59 was obtained by competing the two for binding to anti-human CD59 Abs. The assay was performed as explained under Materials and Methods. As shown in Table 2, preincubation of the anti-CD59 antiserum (diluted 1:480) with human urine CD59 or E ghosts, reduced its binding to schistosomular NPRM by ~50%. Binding of the antiserum diluted 1:960 and blocked with E and urine CD59 to NPRM was reduced by 65 and 74%, respectively. Preincubation of the antiserum with schistosomular NPRM completely abolished its binding to the NPRM attached to the microtiter plate. These results indicate that SCIP-1 shares antigenic epitopes with human CD59, but probably contains unique parasitic epitopes as well.

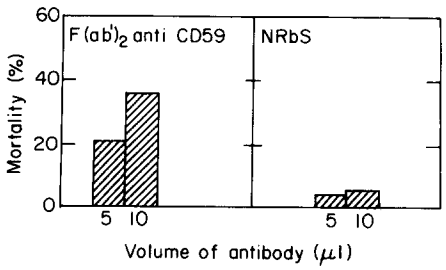

Figure 5. Killing of schistosomula by anti-CD59 Abs and NHS. 24-h-old schistosomula were treated with $F(ab')_2$ fragments of rabbit anti-CD59 Abs or NRbS as control for 30 min at 24°C and then with NHS for 18 h at 37°C. Concentration of $F(ab')_2$ was 50 μg/ml and NRbS and NHS were used undiluted. Results are of an experiment representative of three independent experiments.

Localization of SCIP-1 in Schistosomula and AW. The localization of SCIP-1 in the C-resistant parasitic stages was first studied using intact parasites, rabbit anti-human CD59 Abs, and the immunofluorescence technique (Fig. 6). Freshly transformed schistosomula, which are sensitive to C attack, had almost no SCIP-1 on their surface, whereas 24-h-old schistosomula and 6-wk-old AW were stained nicely on their surface with the anti-CD59 Abs. Additional evidence confirming the presence of SCIP-1 on the surface of 24-h-old schistoso-

Table 2. *Human CD59 and SCIP-1 Compete for Binding to Anti-CD59 Ab*

1/Ab dilution*	Buffer alone	Human erythrocyte ghosts	Pure human urine CD59	Schistosomular NPRM
60	1,911‡	1,025	1,021	70
120	1,625	985	827	65
240	1,365	607	746	72
480	1,085	512	525	59
960	957	332	249	57
1,920	214	168	179	63

* Rabbit anti-CD59 Abs were preincubated at varying dilutions with buffer alone, human erythrocyte ghosts (4.8 μg), purified human urine CD59 (200 ng), or schistosomular NPRM (15 μg). The blocked Abs were then added to microtiter plate wells precoated with 15 μg schistosomular NPRM and incubated for 20 h. Ab binding was quantitated with ^{125}I-Protein A.

‡ Results are expressed as cpm of ^{125}I-Protein A bound to Abs in each well. Results represent a mean of triplicates in three different experiments. SD <2%.

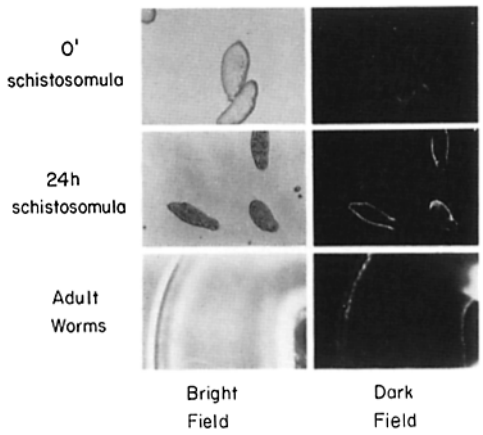

Figure 6. Immunofluorescence staining of schistosomula and AW with anti-CD59 Ab. Freshly transformed schistosomula (0'), 24-h-old schistosomula and 6-wk-old in vivo derived adult worms were stained with rabbit anti-human CD59 Ab, and then with FITC-conjugated goat anti-rabbit IgG and observed under a fluorescence microscope. Bright and dark field representative exposures are shown.

mula was obtained by using cryosectioning and the immunogold labeling technique (Fig. 7). The electron micrographs revealed exclusive presence of SCIP-1 on the outer membrane of the schistosomular tegument (Fig. 7, *B* and *C*). No gold particles could be detected within the tegument and the underlying muscle layers. Furthermore, control sections treated with NRbS were clearly devoid of gold particles (Fig. 7 *D*).

Identification of the Molecular Size of SCIP-1. Anti-human CD59 polyclonal and monoclonal Abs were used to identify SCIP-1 by immunoprecipitation and Western blotting techniques (Fig. 8). 24-h-old schistosomula and AW were metabolically labeled with [^{35}S]methionine and extracted with NP-40. As shown in Fig. 8 *A*, a 94-kD band was immunoprecipitated from both schistosomular and AW NPRM. Some of the 94-kD protein was also immunoprecipitated nonspecifically (Fig. 8 *A*, lane *5*), possibly due to weak binding of this protein to the bacterial adsorbent. A 94-kD protein was also identified by Western blotting using polyclonal anti-human urine CD59 Abs and a mAb YTH 53.1 raised against

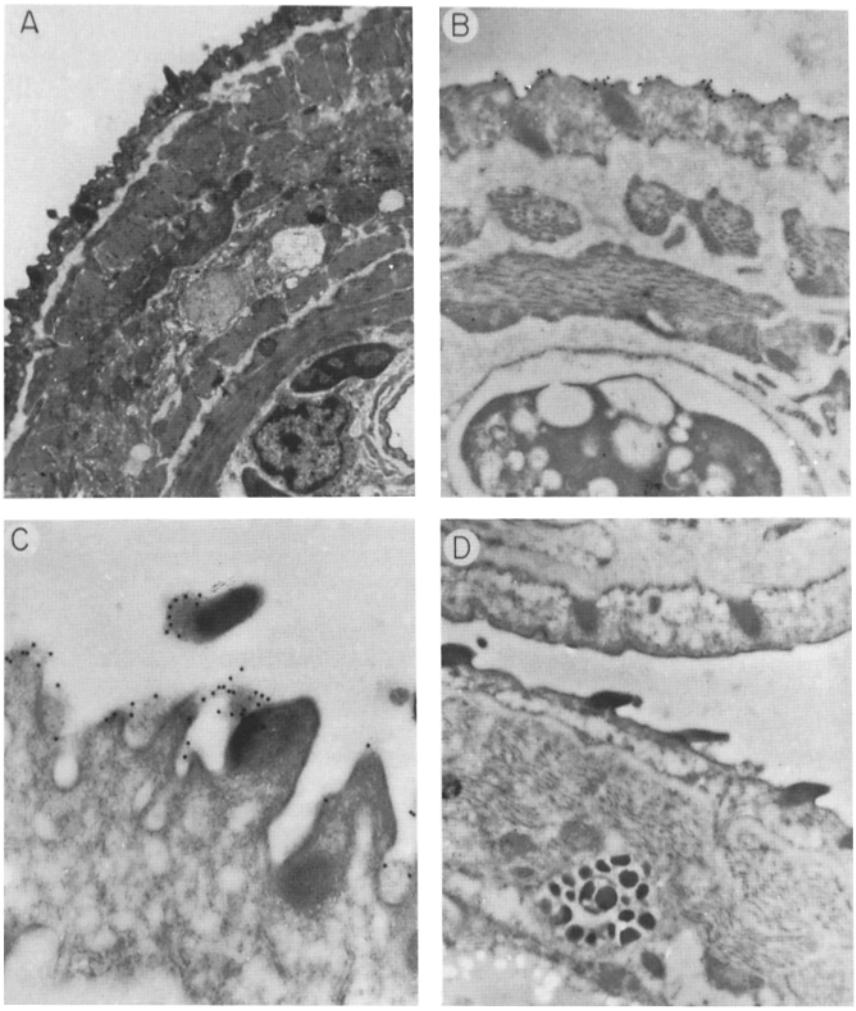

Figure 7. Electron micrographs of 24-h-old schistosomula immunolabeled with anti-human CD59 Ab. Epon section is shown in *A*. Cryosections were treated with rabbit anti-human CD59 antiserum (*B* and *C*) or with NRbS (*D*) and then with gold-conjugated protein A. Sections are shown at different magnifications: (*A*) 6,900, (*B*) 17,000, (*C*) 34,000, (*D*) 14,900.

human CD59 (17) (Fig. 8 *B*). In all cases, a major 94-kD protein band was detected, although occasionally an additional band of 120 kD was observed. The same protein pattern was obtained with schistosomular and AW TRM. The mobility of the 94-kD protein on SDS-PAGE gel remained unchanged under reducing conditions (data not shown). We have concluded that this 94-kD protein is SCIP-1.

Partial Purification of SCIP-1. Since the polyclonal anti-human CD59 Abs bind well to SCIP-1 and are capable of blocking its C inhibitory effect, they served to affinity purify the protein. IgG was partially purified from the anti-CD59 antiserum (by precipitation in 50% ammonium sulphate) and coupled to cyanogen bromide-activated Sepharose beads. 24-h-old schistosomula were surface labeled with ^{125}I and NPRM was prepared. A mixture of 1/3 [^{125}I]NPRM and 2/3 unlabeled NPRM was loaded onto the Ab column, and after extensive washing the bound protein was eluted with glycine-HCl, pH 2.5, and the pH was neutralized. The eluted fractions were dialyzed for 18 h against GVB^{2+} and examined for C inhibitory activity (not shown). A pool of the active eluted fractions was analyzed by SDS-PAGE (not shown) and autoradiography (Fig. 9 *C*). The same procedure was carried out using a nonrelevant affinity column containing normal rabbit Ig bound to Sepharose. Major bands of 94 and 82 kD were observed in the material eluted from the anti-CD59–Sepharose column (Fig. 9 *C*, lane *1*). It is possible that the 82-kD band observed in Fig. 9 *C*, lane *1*, is a cleavage product of the 94-kD protein. The corresponding fractions of the normal rabbit IgG–Sepharose eluate were devoid of any protein (Fig. 9 *C*, lane *2*). The effect of the columns eluates on lysis of EA and E_R by NHS was next examined. The material eluted from the NRb-IgG–Sepharose column had almost no effect on EA and E_R lysis. In contrast, the proteins eluted from the anti-CD59–Sepharose column, reduced EA lysis from 65 to 25% (Fig. 9 *A*), and E_R lysis from 55 to 20% (Fig. 9 *B*).

Binding of SCIP-1 to Human C8 and C9. Human CD59 binds the C components C8 and C9, thus restricting formation of the MAC (29). We tested whether SCIP-1 can bind to the same C components (Fig. 10). Purified human C8 and C9 were electrophoresed under nonreducing conditions and transferred to a nitrocellulose membrane. After blocking, the membrane was treated with schistosomular NPRM (supplemented with protease inhibitors), washed, and further treated with anti-human CD59 Abs. As shown in Fig. 10 *B*, SCIP-1 bound to C8 (β subunit) and to C9 but not BSA. Other results have demonstrated that excess soluble C9 can block binding of SCIP-1 to electroblotted C9 (not shown). To further establish the identity of the C8/C9-binding protein and the 94-kD SCIP-1, the Abs bound to the C9-binding protein were eluted from the nitrocellulose membrane with acidic buffer and reexamined by Western blotting on schistosomular NPRM. As shown in Fig. 10 *D*, the eluted Abs specifically recognized in schistosomular NPRM the 94-kD SCIP-1.

Release of SCIP-1 with PI-PLC. Human CD59 is anchored to cell membranes via a glycosyl phosphatidylinositol anchor, and can be removed with PI-PLC (17, 47, 48). We tested

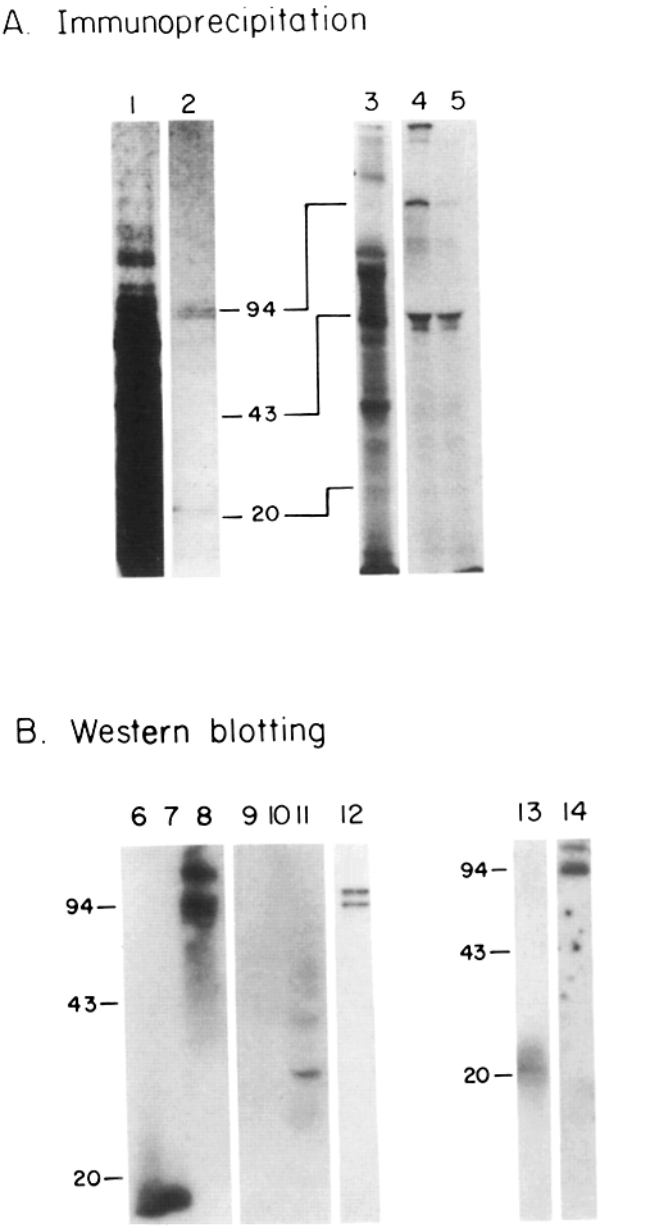

Figure 8. Immunodetection of SCIP-1 in schistosomular and AW extracts. (*A*) Immunoprecipitation of [^{35}S]methionine-labeled NPRM. Lane *1*, total proteins labeled in schistosomular NPRM. Lane *2*, schistosomular NPRM immunoprecipitated with anti-human CD59 Abs. Lane *3*, total proteins in labeled AW NPRM. Lane *4*, AW NPRM immunoprecipitated with anti-human CD59 Abs. Lane *5*, AW NPRM immunoprecipitated with NRbS. All immunoprecipitates were analyzed on SDS-PAGE under reducing conditions (lanes *1–2*, 5–15% gradient gel; lanes *3–5*, 12% gel). (*B*) Western blotting analysis. Lanes *6* and *9*, pure human urine CD59 (200 ng); Lanes 7 and *10*, normal human E ghosts; Lanes *8*, *11*, and *12*, schistosomular NPRM. Lanes *6–8* were treated with polyclonal anti-human urine CD59 Abs followed with ^{125}I-Protein A. Lanes *9–11* were treated with NRbS followed with ^{125}I-Protein A. Lane *12* was treated with rat mAb YTH.53.1 followed by peroxidase goat anti-rat IgG conjugate and developed using ECL reagents. Lane *13*, normal human E ghosts. Lane *14*, AW TRM. Lanes *13* and *14* were treated with anti-CD59 Ab and ^{125}I-Protein A. All Western blots were carried out after SDS-PAGE on 12% gels under nonreducing conditions.

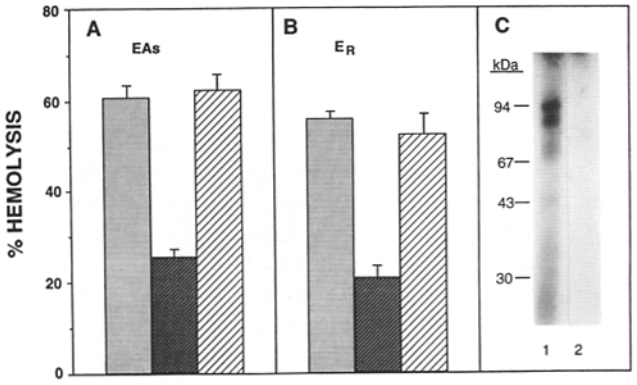

Figure 9. Affinity purification of the SCIP-1. 24-h-old schistosomula were labeled with ^{125}I and extracted with NP-40. A mixture of [^{125}I] NPRM (1/3) + unlabeled NPRM (2/3) was loaded onto an affinity column of anti-CD59 Ig-Sepharose or normal rabbit Ig-Sepharose. The bound proteins were eluted with glycine-HCl, pH 2.5, and tested for their capacity to inhibit the classical (EAs lysis) or alternative (E_R lysis) pathways of C. The pools of eluted anti-CD59 Sepharose active fractions (▨) and the correspondent NRbS Sepharose eluted fractions (▨) were tested on EAs (*A*) or E_R (*B*). Also shown is a control of buffer alone (□). The pools of eluted active fractions were further subjected to SDS-PAGE unreduced on a 12% gel and to autoradiography (*C*). Lane *1*, anti-CD59–Sepharose eluate; lane *2*, NRbS-Sepharose eluate.

whether SCIP-1 is similarly anchored to the parasite's membrane. 24-h-old schistosomula were treated with *Bacillus thuringiensis* PI-PLC (2.7 U/ml in TBS) (Oxford Glycosystems, Ltd., Abingdon, UK), and the released material was subjected to electrophoresis and Western blotting analysis. As shown in Fig. 11, anti-CD59 Abs detected in the PI-PLC–released material a 94-kD major protein and a higher molecular mass band (Fig. 11, lane *2*). No protein could be detected in the supernatants derived from schistosomula incubated in TBS alone (Fig. 11, lane *1*). Also shown is the detection of human urine CD59 (Fig. 11, lane *3*) and SCIP-1 in NPRM of 3 h old schistosomula (Fig. 11, lane *4*) by the anti-CD59 Abs. These results suggest that SCIP-1 is PI-anchored to the schistosomular surface.

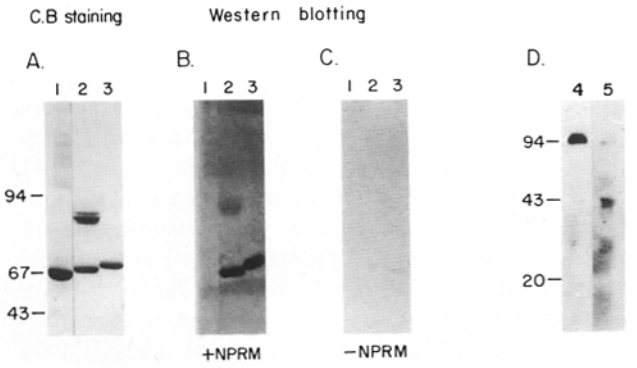

Figure 10. SCIP-1 binding to C8 and C9. Purified human C8 and C9 and BSA (5 μg each) were subjected unreduced to SDS-PAGE (7.5% gel) and stained with coomassie blue (*A*) or transferred to nitrocellulose membranes (*B* and *C*). The membranes were blocked with milk and overlayed with (*B*) or without (*C*) schistosomular NPRM for 2 h at 37°C. After extensive washes, anti-CD59 antisera (1:50) was added for 1 h at 24°C, followed by horseradish peroxidase-conjugated goat anti–rabbit IgG for 45 min. Detection was carried out with the ECL kit. Molecular masses: lane *1*, BSA, 67 kD; lane *2*, human C8 α,γ subunit, 86 kD, β subunit, 68 kD; lane *3*, human C9, 71 kD. (*D*) Antibodies were eluted from a nitrocellulose strip of C9-bound NPRM and Ab-treated membrane and treated by Western blotting on schistosomular NPRM. After blocking, the membrane containing the NPRM was overlayed with the eluted Ab for 18 h at 24°C, washed, and Ab binding was detected using the peroxidase-conjugated goat anti–rabbit IgG and the ECL kit (lane 4). NRbS, instead of the eluted Abs, served as a negative control (lane *5*).

Discussion

Pathogenic parasites employ multiple strategies to evade the host's complement system (7) and in general, the immune response (49). *S. mansoni* can successfully infect its permissive mammalian hosts and resides for years within their blood system in direct contact with the C proteins. The mechanisms whereby this parasite avoids C-mediated damage has intrigued us and others for several years now, and it has become apparent that a combination of several physical, biochemical, and immunological barriers protects the parasite from C. We have hereby described a novel schistosome C inhibitor, SCIP-1, which targets its inhibitory action at the terminal C proteins C8 and C9 and thereby prevents formation of the C MAC.

We have previously shown that treatment of schistosomula of *S. mansoni* with trypsin or pronase sensitizes them to killing by the alternative C pathway (12). This finding is confirmed now with adult worms, although higher trypsin concentrations are required for sensitization of AW than of schistosomula (Table 1). Lung stage worms are much less affected by trypsin (Table 1). This latter result is in agreement with previous data indicating that the tegument of lung worms is resistant to immune attack (50). Adherence of eosinophils, mediated by Ab or C receptors, which is lethal to young 3-h-

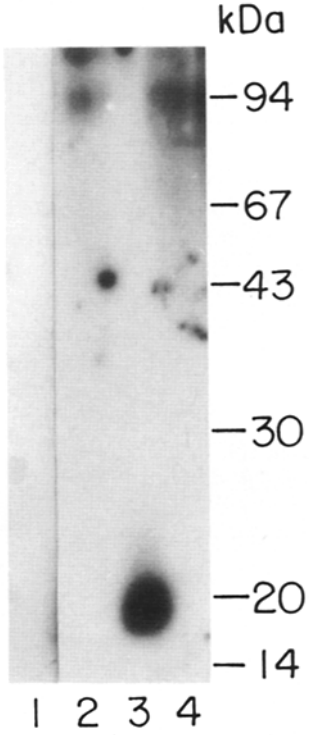

Figure 11. Removal of SCIP-1 with PI-PLC. Samples of 24-h-old (100,000) schistosomula were either untreated (lane *1*) or treated with PI-PLC (2.7 U/ml) in TBS for 1.5 h at 37°C (lane *2*). After treatment, the schistosomula were removed and supernatant applied unreduced to a 12% SDS-PAGE gel. Also shown are pure human urine CD59 (200 ng) (lane *3*) and NPRM prepared from 3-h-old schistosomula (lane *4*). Proteins separated on the gel were transferred to nitrocellulose paper and detected using anti-CD59 antisera (diluted 1:300) followed by ^{125}I-Protein A (8×10^5 cpm/ml). An autoradiogram is shown.

old schistosomula, does not occur when 4-d-old lung worms are used as targets (51). It has been claimed that the lung worms have low surface antigenicity and do not promote effector mechanisms involving recognition and direct surface damage (50). It may be suggested now that lung worms either lack C activating substances on their surface, or express potent trypsin-resistant C inhibitors.

The finding that trypsinization has markedly enhanced the generation of complement MAC (formation of poly C9) on schistosomula (Fig. 1), has implied that the schistosomula contain an external MAC inhibitory protein. In agreement, we could demonstrate that TRM and NPRM from shistosomula and AW can inhibit C-mediated hemolysis by both classical and alternative pathways. Other results have clearly indicated that the inhibitor can block MAC formation even after generation of the C5b-7 complex (Fig. 3), namely at the C8 and C9 level. CD59 is a known mammalian protein capable of binding to C8 and C9 and preventing MAC formation (17, 23, 29). It is present on erythrocytes, leukocytes and many other cell types (24). We have undertaken to determine whether the schistosomula express a CD59-like protein. Results have shown that indeed, antibodies directed to human CD59 bind to intact schistosomula and AW and potentiate schistosomacidal activity of guinea pig and human C (Figs. 4 and 5). Furthermore, anti-CD59 Ab could block the C inhibitory activity of schistosomular NPRM. Immunogold labeling of cryosections of schistosomula have identified the CD59-like protein exclusively on the tegumental outer membrane (Fig. 7). Based on immunoprecipitation and Western blotting analysis of schistosomular and AW extracts, we have concluded that the parasite CD59-like molecule is a 94-kD protein with few, if any, disulfide bonds. It was named SCIP-1, abbreviated for schistosome C inhibitory protein type 1. The C-inhibitory activity of SCIP-1 is highly sensitive to heat treatment.

Additional evidence illustrating functional similarity between human CD59 and SCIP-1 is their binding to C8 and C9 (23) (Fig. 10 *B*). It has been postulated that upon this binding of CD59, insertion of C9 into membranes and interaction with C5b-8 complexes is inhibited (23, 29, 30). Specific binding of human CD59 to C8 and C9 was detected only when these human C proteins were adsorbed to either plastic or nitrocellulose, suggesting that a conformational change that accompanies surface adsorption exposes a CD59-binding site that is normally buried in these serum proteins (52). Similar to CD59, SCIP-1 binds to nitrocellulose-fixed C8 and C9, but the fact that soluble C9 can compete for binding of SCIP-1 to membrane bound C9, suggests that SCIP-1 recognizes native epitopes of C9. In addition, SCIP-1 binds primarily to the β-subunit of C8, whereas CD59 binds to the α subunit of C8 (52).

Another feature common to CD59 and SCIP-1 is their attachment to membranes via a linker sensitive to PI-PLC (31) (Fig. 11). The fact that PI-PLC treatment sensitizes schistosomula to killing by human complement (Ghendler, Y., and Z. Fishelson, unpublished data) further emphasizes the contribution of SCIP-1 to complement resistance in *S. mansoni*. The structure of the lipid anchor of SCIP-1 has not been studied yet and cannot be compared with that of CD59 (48). SCIP-1 is much larger than CD59 (18–20 kD), yet high molecular weight proteins (80 kD) have also been immunoprecipitated with anti-CD59 Abs from human cell lysates (17, 22, 53). Although remote, we still have to consider the possibility that SCIP-1 is composed of two or more molecules held together by SDS- and mercaptoethanol-resistant bonds.

Paramyosin is a schistosome 94-kD protein that was recently described as a possible C regulator (54). Paramyosins of *S. mansoni* and of *Taenia solium* are capable of binding complement C1, and inhibiting the classical C pathway. The possible relationship between paramyosin to SCIP-1 was therefore assessed. By Western blotting analysis, no cross-reaction between the two proteins was detected (Parizade, M., R. Arnon, and Z. Fishelson, unpublished data). Anti-CD59 Abs do not recognize paramyosin and anti-paramyosin Abs do not bind to SCIP-1. Furthermore, it has been reported that paramyosin binds C1 exclusively and does not inhibit the alternative C pathway (54). In contrast, SCIP-1 binds C8 and C9, thus inhibiting both classical and alternative C pathways. Moreover, SCIP-1 is located on the parasite surface, whereas paramyosin is an intramuscular protein.

Other parasites have also been reported to evade C destruction by specifically reacting with components of the terminal C pathway. The *E. histolytica* 260-kD galactose adhesion molecule has limited sequence identity to human CD59 and exhibits a C5b-9 inhibitory activity (27). This adhesion molecule is capable of binding the terminal C components C8 and C9 and confers upon reconstitution C5b-9 resistance on sensitive amoeba (27). A gene encoding a protein with 48% identity in its amino acid sequence to human CD59 has recently been found in the genome of *H. saimiri* (28). The site of expression and biological activity of this CD59-like protein have not been determined yet. The *T. cruzi* amastigotes express probably a surface protein that inhibits the lytic activity of C5b-9 complex (55). The lipophosphoglycan (LPG) of *Leishmania major* is thought to inhibit proper membrane insertion of C5b-9 complexes (56, 57). It has also been proposed that elongation and compositional changes of the LPG on C resistant promastigotes, sterically interfere with the access of the C5b-9 macromolecules to the parasite membrane, thereby preventing channel formation and lysis by MAC (58).

Human CD59 is reported to have other functions in various tissues, in addition to its role in restricting assembly of the cytolytic C5b-9. On human endothelial cells, CD59 was suggested to attenuate the capacity of C5b-9 to evoke thrombogenic responses (59). On human blood platelets, CD59 normally serves to attenuate the procoagulant responses of these cells exposed to activated complement proteins (60). More thoroughly investigated is the role of CD59 on human T cells. It has been suggested that CD59 on these cells serves as a physiological signaling receptor. mAbs to CD59 have been reported to initiate signal transduction events in T cells (61, 62) and CD59 was found to be associated with a tyrosin kinase implicated in T cell signaling (63). CD59 was shown to interact with CD2 and contribute together with CD58 to T cell activation and adhesion (64, 65). Whether any of these human CD59 functions is also expressed by SCIP-1 is yet to be determined.

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