Novel Mechanisms of Immune Evasion by *Schistosoma mansoni*

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The interaction of Schistosoma mansoni *with its host's immune system is largely affected by multiple specific and non-specific evasion mechanisms employed by the parasite to reduce the host's immune reactivity. Only little is known about these mechanisms on the molecular level. The four molecules described below are intrinsic parasitic proteins recently identified and studied in our laboratory.*

1. m28 - *A 28kDa membrane serine protease. m28 cleaves iC3b and can thus restrict attack by effector cells utilizing complement receptors (especially CR3). Treatment with protease inhibitors potentiates killing of schistosomula by complement plus neutrophils.*

2. Smpi56 - *A 56kDa serine protease inhibitor. Smpi56 binds covalently to m28 and to neutrophil's elastase and blocks their proteolytic activity.*

3. P70 - A *70kDa C3b binding protein. The postulated activity of P70 includes binding to C3b and blocking of complement activation at the C3 step.*

4. SCIP-1 - *A 94kDa schistosome complement inhibitor. SCIP-1 shows antigenic and functional similarities to the human 18kDa complement inhibitor CD59. Like CD59, SCIP-1 binds to C8 and C9 and blocks formation of the complement membrane attack complex. Antibodies directed to human CD59 bind to schistosomula and potentiate their killing by complement.*

The structure and function of these four proteins as well as their capacity to induce protection from infection with S. mansoni *are under investigation.*

Key words: immune evasion - complement - protease - inhibitor - CD59

Cercariae of *Schistosoma mansoni* are well adapted to survive for several hours in fresh water while searching for a compatible host. However, their heavy "armor" (the glycocalyx) and strong "engine" (the tail) will hamper their survival inside the host (McLaren 1980). Therefore, the penetrating larvae have to get rid of their glycocalyx and tail as soon as they are within the host. The larvae that migrate from the skin to the lungs and then to the liver and mesenteric veins, keep developing, transforming and adapting to their new habitat. Eventually, the mature worms reside within the mesenteric veins for years, in continuous contact with the host's blood. The developing and mature worms must face multiple challenges imposed on them by the host's defense mechanisms. Innate and induced immunity, mediated by complement, antibodies and effector cells (neutrophils, macrophages monocytes, eosinophils and lymphocytes) combine in an effort of the host to reject the intruders. However, to escape from those hosts' effector mechanisms, the larvae and worms have developed multiple mechanisms of immune evasion . Several review articles (Pearce & Sher 1987, Damian 1989, Fishelson 1989, 1991a, Capron 1992) have summarized the state of the art in this rapidly developing field of research. Recently, our research has led us to identify four new proteins of *S. mansoni* which may contribute to its immunoresistance. Only these four proteins will be described here.

PROTEOLYSIS OF COMPLEMENT PROTEINS (M28)

Earlier studies (Gazzinelli & Pellegrino 1964, Auriaut et al. 1981, 1982, Landsperger et al. 1982, Keene et al. 1983, Bogitsh & Dresden 1983, Chappell & Dresden 1987, McKerrow & Doenhoff 1988) indicated that cercariae, schistosomula and adult worms produce several proteases expressing a wide range of substrate specificities. Some of the functions of these proteases are: assistance in penetration through the skin by the cercaria (McKerrow et al. 1991), degradation of the cercarial glycocalyx (Marikovsky et al. 1988a), cleavage of host's immunoglobulin (Auriaut et al. 1981) and food (hemoglobin) digestion (Chappell & Dresden 1987).

Cercariae of *S. mansoni* produce and store in their acetabular cells a 28-kDa serine protease

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(Fishelson et al. 1992). Upon skin invasion, the cercariae release this protease and utilize it to digest epidermal and dermal connective tissue proteins and to facilitate penetration (Cohen et al. 1991, McKerrow et al. 1991, Fishelson et al. 1992). The released 28-kDa protease, present in a soluble form in cercarial secretions, was purified and characterized (Marikovsky et al. 1988b). Anti-protease antibodies raised in rabbits (Marikovsky et al. 1988b) were used to localize the 28-kDa protease in the acetabular cells of cercariae and on the surface of schistosomula (Marikovsky et al. 1990a). Binding of these antibodies to the surface of lung-stage and adult worms was also detected by immunofluorescence (Ghendler, Parizade, Arnon and Fishelson, manuscript in preparation).

The possibility that the 28-kDa ecto-protease (m28) contributes to the immune evasion of *S. mansoni* was examined. Incubation of schistosomula with human serum leads to activation of the complement system and binding of several complement proteins to the surface of the larvae. We have demonstrated binding of C3 (Marikovsky et al. 1990b) and C9 (Parizade et al. 1994) to the schistosomula; bound C3b and iC3b as well as polymerized C9 were identified. Bound C3b is known to facilitate formation of the membrane attack complex (MAC) of complement and thus polymerization of C9 and target cell lysis (Muller-Eberhard 1988). On the other hand, iC3b serves as an acceptor for the leukocyte complement receptor type 3 (CR3; CD11b,CD18), thus promoting leukocyte adhesion to iC3b-bearing cells and leukocyte-mediated lytic or inflammatory events (Lambris 1989, Fishelson 1991b). Neutrophils, eosinophils and macrophages kill complement-opsonized schistosomula much better than non-opsonized schistosomula (Anwar et al. 1979, Ramalho-Pinto et al. 1979).

Purified human C3, C3b, iC3b and C9 can be cleaved by the 28-kDa soluble (cercarial secretion) or membrane (schistosomular) protease (Parizade et al. 1990, Ghendler et al. manuscript in preparation). Of these four substrate molecules, iC3b was found to be the most sensitive. We have, therefore, speculated that by cleaving iC3b molecules deposited on their surface, the schistosomula protect themselves from iC3b-mediated leukocyte-dependent killing. Indeed, treatment of schistosomula with the protease inhibitor phenylmethanesulfonyl fluoride or soybean trypsin inhibitor rendered schistosomula more sensitive to complement-mediated neutrophil-dependent killing (Ghendler et al. manuscript in preparation).

INHIBITION OF NEUTROPHILS' ELASTASE (SMPI56)

Proteases released from activated leukocytes can be harmful to pathogenic microorganisms. To avoid the action of these proteases, bacteria and parasites produce protease inhibitors (Suquet et al. 1984, Martzen et al. 1990, Shepherd et al. 1991, Bode & Huber 1992). Recently, we have identified the presence of a serine protease inhibitor in tegumental detergent extracts from adult worms of *S. mansoni* (Ghendler et al. 1994). The protease inhibitor was found to be a 56-kDa protein capable of specifically binding to the 28-kDa serine protease of *S. mansoni* and to pancreatic and neutrophil elastases and inhibiting their activity. The protein was named Smpi56, for '*S. mansoni* protease inhibitor of 56-kDa'. Our results indicated that Smi56 forms a covalent bond with the reactive serine of the 28-kDa protease and elastase. Smpi56 showed no reactivity with trypsin, chymotrypsin, proteinase K or urokinase.

By using biotinylated-elastase and streptavidin-agarose, Smpi56 was isolated from crude worm extract in a single step (Ghendler et al. 1994). Rabbit antibodies prepared against Smpi56 could immunoprecipitate the 56-kDa protease inhibitor and a 74-kDa complex of protease-protease inhibitor.

Part of the Smpi56 cDNA was isolated from a *S. mansoni* adult worm cDNA library. Analysis of its nucleotide sequence has identified a concensus sequence of a reactive center present in members of the serpin family of serine protease inhibitors (Ghendler et al. manuscript in preparation). The cDNA sequence of a postulated serpin of *S. haematobium* was deposited in GenEmbl by Blanton et al. (1994). Alignment of Smpi56's and *S. haematobium* serpin's cDNAs and their deduced protein sequences shows about 80% homology at the nucleotide level and 73% identify at the amino acid level between the two serpins.

INHIBITION OF COMPLEMENT C3 DEPOSITION (P70)

Trypsin-treated schistosomula are more sensitive to complement than control schistosomula (Marikovsky et al. 1990b). Trypsinization enhances deposition of C3 on treated schistosomula, suggesting that trypsin removes an inhibitor of C3 deposition. Known mammalian membrane proteins acting as inhibitors of C3 deposition, such as the complement receptor type 1 (CR1, CD35), decay accelerating factor (DAF, CD55) and membrane cofactor protein (MCP, CD46), bind to the C3b fragment of C3 or to the C3 convertases (Lambris 1989, Fishelson 1991b). It has been previously suggested that schistosomula of *S. mansoni* express a receptor for C3b on their surface (Santoro 1982).

Immunoadsorption of a detergent extract or trypsin-released material from schistosomula and adult worms over a C3b-Sepharose column permitted us to identify a 70-kDa C3b binding protein (Parizade, Arnon and Fishelson, manuscript

in preparation). In addition, our results have clearly demonstrated in the trypsin-released material an activity inhibitory to C3 deposition on antibody-coated sheep erythrocytes. It is conceivable that the 70-kDa C3b binding protein is the regulatory protein limiting C3 deposition on schistosomula and adult worms of *S. mansoni*.

INHIBITION OF COMPLEMENT MAC FORMATION (SCIP-1)

Complement resistant 24 hr-old schistosomula do not permit formation of the complement membrane attack complex (MAC) on their surface (Parizade et al. 1994). The MAC is formed on trypsinized schistosomula. Detergent extracted proteins from schistosomula and adult worms inhibit lysis of sheep erythrocytes, even if added after C5b-7 has been deposited on them (Parazide et al. 1994).

CD59 is an 18-20-kDa membrane protein that has a broad tissue distribution in man (Davies et al. 1989, Meri et al. 1991). It is found on blood, epithelial and endothelial cells, linked to the cell membrane via a glycosyl phosphatidylinositol (GPI) anchor (Davies et al. 1989, Ratnoff et al. 1992). CD59 inhibits MAC assembly by binding to the complement components C8 and C9 (Meri et al. 1990, Rollins et al. 1991).

The MAC inhibitor present on schistosomula and adult worms of *S. mansoni* was identified as a CD59-like molecule by using polyclonal and monoclonal antibodies directed to human CD59 (Parizade et al. 1994). It is a 94-kDa protein synthesized by the parasite and attached to the surface of schistosomula probably via a GPI linker. This CD59-like protein was named 'schistosome complement inhibitory protein type-1' or SCIP-1 (Parizade et al. 1994).

Like CD59, SCIP-1 binds to human C8 and C9 and inhibits MAC formation. Blocking of the protective activity of SCIP-1 on intact schistosomula with polyclonal anti-CD59 antibodies permitted efficient killing of the schistosomula by human and guinea pig complement.

CONCLUSIONS

The surface of schistosomula and adult worms of *S. mansoni* is covered with numerous proteins, most of which play an essential role in the survival of the parasite within its host. Some of these proteins confer on the parasite protection from the host's immune system. Four intrinsic membrane proteins, which probably contribute to the immune evasiveness of *S. mansoni*, have been described above: (1) a 28-kDa serine protease capable of cleaving the complement proteins iC3b, C3b and C9 (Parizade et al. 1990); (2) a 56-kDa serine protease inhibitor (Smpi56) which can block activity of neutrophil's elastase (Ghendler et al. 1994); (3) a 70-kDa C3b binding protein, probably inhibiting C3 deposition on the parasite (Parizade et al. 1990); and (4) a 94-kDa C8/C9 binding protein (SCIP-1) which is related functionally and antigenically to human CD59 (Parizade et al. 1994). It is reasonable to assume that blocking the activity of these and other immune evasion molecules *in vivo* will assist an infected host in combatting the parasite. Two additional schistosomal proteins recently described which may affect complement activation on the surface of the parasite are: 1. the 94-kDa paramyosin which binds to complement C1 (Laclette et al. 1992), and 2. a 130-kDa C3 binding protein (Silva et al. 1993). As suggested (Fishelson 1991a), one of these new immunoregulatory molecules may perhaps be an "Achilles' Heel" of *S. mansoni*. It is, therefore, important to examine whether any of them may be applied as vaccine to control schistosomiasis.

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