Schistosomiasis

Immunologic Properties of Developing Schistosomula

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ONE OF THE MOST FASCINATING events in the life of the schistosome is its transformation from an aquatic free-living cercaria to a schistosomulum—a larval parasite adapted for life within the vertebrate host (Figure 1). At the morphologic level this transition is striking. The cercaria, upon penetrating the skin of the host, loses its forked tail, sheds its external glycocalyx, and develops a surface membrane consisting of two lipid bilayers, a tegumental structure shared by all all blood-dwelling trematodes.[1,2] In addition, more subtle changes occur in the organism, allowing it to survive in its new physiologic environment and, as will be discussed in this review, enabling it to withstand attack by the host immune system.

That the host mounts a vigorous immune response against schistosome infections has been well documented.[3,4] Perhaps the key manifestation of the response is the concomitant immunity displayed by laboratory animals bearing adult schistosomes. These animals (mice, hamsters, monkeys) become resistant to challenge with cercariae even though the adult worms responsible for stimulating the immunity continue to thrive inside the blood vessels of the host. The paradox of concomitant immunity can be explained by postulating that invading schistosomula are susceptible to immune rejection, whereas established adult worms are not. Alternatively, one could explain the phenomenon by arguing that immunity acts in a site-specific manner, such that adult worms living in the mesenteric veins (or veins of the bladder in the case of *S haematobium*) are somehow protected from immune attack.

That schistosomula during their development *in vivo* or *in vitro* lose their susceptibility to immune damage has been demonstrated by several laboratories.[5-9] If one considers the killing of schistosomula mediated by antibody-dependent mechanisms, the loss in susceptibility to immune damage occurs rapidly, that is, within hours after the transformation of the organisms from cercariae. A striking demonstration of this transition in susceptibility was obtained in studies in which immune serum was injected into mice at various times before and after intravenous challenge with newly transformed schistosomula of *Schistosoma mansoni*.[5] It was found (Figure 2) that several hours after their entry into the host, schistosomula undergo a rapid, nearly linear decline in their ability to be rejected by passively transferred immune serum and by 24 hours are essentially refractory to antibody-dependent killing. A similar conclusion was reached in related studies in which killing by antibody plus complement[6] or antibody plus eosinophils [9] was followed *in vitro*. In these experiments schistosomula were observed to lose their susceptibility to immune damage within the first 24 hours of culture.

What mechanisms account for this dramatic adaptation of the parasite to the host immune response? The problem is complicated by the fact that schisto-

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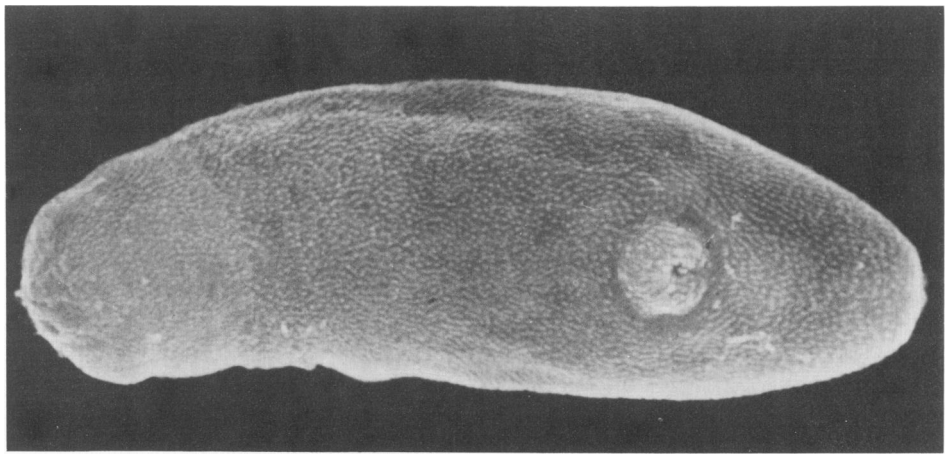

Figure 1—Scanning electron micrograph of a 3-hour-old schistosomulum prepared by the skin penetration method.[29,30] (× 1200) (Courtesy of J. Samuelson and J. Caulfield)

somula during the first several days after their transformation from cercariae undergo a wide variety of changes that could account for or underlie the organisms' resistance to immune attack (Table 1). These changes include the reduced ability to induce cellular reaction in immune mouse lung,[10] a loss in the capacity to activate complement by the alternate pathway,[11,12] and an increase in motility ("wriggle power").[13] In addition, during the development of schistosomula to the lung stage, the tegument of the parasite has been shown to undergo a series of major changes. These changes include an increase in total surface area,[14] a reduction in concanavalin A binding sites,[15] and a redistribution of intramembraneous particles within the lipid bilayers of the membrane.[16]

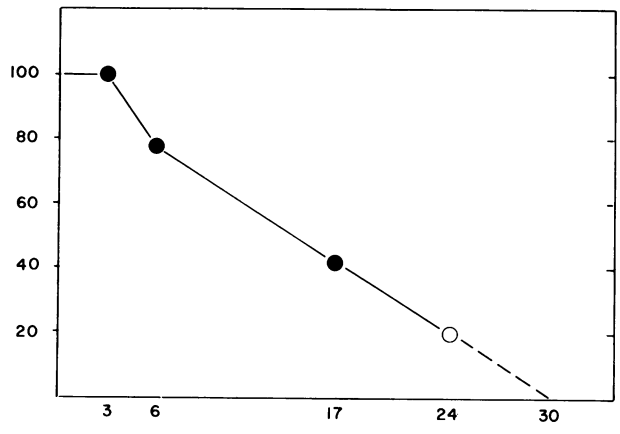

Figure 2—Susceptibility to antibody dependent rejection in mice versus age of schistosomulum in host.[5] The plot shown is a transformation of data obtained from experiments in which chronic immune serum was administered to mice at various times before and after intravenous challenge with 500 freshly transformed schistosomula. The survival of the parasites was then measured by recovery of the larvae from the lungs 5 days later and compared with the recovery from mice injected with normal instead of immune mouse serums. (Reproduced with the permission of the *American Journal of Tropical Medicine and Hygiene*)

However, probably the most intriguing change undergone by developing schistosomula is the surface acquisition of host molecules.

The phenomenon of host molecule acquisition by schistosomes was first described by Smithers and Terry and their colleagues,[17] who demonstrated the presence of antigens of host origin on the surface of adult worms. These workers speculated that by coating themselves with host molecules schistosomes might escape damage by the immune response. In later experiments the group at Mill Hill led by Smithers identified the A, B, H, and Lewis glycolipid blood group substances as one class of host molecule acquired by the worm.[18]

In more recent work from this laboratory, we have shown that products of the major histocompatibility complex (MHC) are present on the tegument of 4–6-day-old schistosomula recovered from mouse lungs[19] as well as 21-day-old larvae perfused from mouse liver (B. F. Hall and A. Sher, unpublished observation). That the presence of these MHC-encoded alloantigens on the schistosome surface is a general phenomenon has been confirmed in recent experiments in which it has been shown that schistosomula recovered from the lungs of inbred Strain 2 and Strain 13 guinea pigs react by indirect immunofluorescence with the appropriate 13 anti-2, 2 anti-13 alloantiserums (Table 2). Such antiserums are known to be specific for the I region of the guinea pig MHC.[20] Although in our original work mouse MHC antigens (K, I, and D region products) were detected by means of their serologic reactivity,[19] in later experiments it was possible to confirm the presence of K and D region gene products on lung-stage schistosomula by studying the adherence of alloreactive cytotoxic lymphocytes (CTL) to the larval surface.[21,22]

As substances which play a key role in self–non-self-discrimination,[23,24] MHC glycoproteins might

be considered as an ideal component for a schistosome molecular disguise. That host molecules are indeed responsible for preventing the interaction of effector antibodies with the surface of the worm was suggested by the observation that whereas skin-stage schistosomula bind antiparasite antibodies and lack mouse host molecules, lung-stage parasites possess a dense coat of host molecules and bind little if any antiparasite antibody.[25,26] This inverse correlation between the presence of host molecules on schistosomes and their ability to bind antibody was shown, however, to be incomplete. Thus, in the examination of developmental stages other than lung worms (ie, schistosomula recovered from skin, adult worms), parasites were found that possessed host molecules yet also bound antibodies directed against worm antigens.[26]

Further evidence against the hypothesis that schistosomes evade the immune response by masking parasite antigens with host molecules has been obtained by G. Moser in recent experiments employing haptenated schistosomula.[27] In this work skin-stage schistosomula and lung-stage schistosomula were surface-labeled with trinitrophenyl (TNP) groups and then tested for their susceptibility to killing by anti-TNP antibody plus complement or human eosinophils. Whereas TNP-labeled skin-stage worms were readily killed by these antibody-dependent effector mechanisms, TNP-lung-stage worms, which in separate experiments were shown to bind the same if not more anti-TNP antibody per unit surface area than TNP-skin-stage parasites, were found to be resistant to damage by the same hapten-specific killing reactions.[27] Thus, even when the host molecule disguise of lung-stage schistosomula is bypassed by superimposing haptenic groups on the worm surface, parasites of that stage cannot be killed by immunologic reactions to which newly transformed worms are normally susceptible. This apparently "innate" change in susceptibility to immune damage occures soon after the transformation of cercariae into schistosomula. One can study the kinetics of the transition by injecting newly transformed schistosomula into mouse lungs and recovering them from the host at various times thereafter. The parasites are next labeled with TNP and then tested for their susceptibility to killing by anti-TNP antibody plus complement. The results of such an experiment are shown in Figure 3. It can be seen that, depending on the concentration of trinitrobenzene sulfonic acid (12.5 and 25 mM) used in labeling the parasites, schistosomula lost their susceptibility to killing by anti-TNP antibody plus complement 17 and 40 hours after recovery from mouse lung, respectively. The kinetics resemble those obtained in the *in vivo* passive transfer study discussed previously (Figure 2).

Table 1—Some Developmental Differences Between Lung-Stage and Skin-Stage Schistosomula

1. Increased motility[13]
2. More highly involuted surface with increased total area[14]
3. No longer able to activate C via the alternate pathway[11,12]
4. Elicit less cellular reaction in mouse lung[10]
5. Altered distribution of intramembranous particles in freeze-fractured tegument[16]
6. Less concanavalin A receptors per unit surface area[15]
7. Possess host molecules on their surface[25,26]

The TNP-worm experiments just described argue that developing schistosomula lost their susceptibility to immune damage because of an intrisic change in the tegument of the parasite that occurs independently of the masking of parasite antigens by host molecules. Evidence that an additional mechanism may be involved in the evasion of the immune response by schistosome larvae has been obtained recently by J. Samuelson and his colleagues.[28] These workers studied the binding of antibodies in immune serum (from heavily infected rates) to *in vitro* cultured schistosomula during the first few hours after their transformation from cercariae. By means of indirect immunofluorescence and microfluorimetry, they demonstrated an age-dependent loss of bound immunoglobulin or schistosomula treated with antiparasite immune serum and fluorescein-conjugated anti-rate IgG (Figure 4). This decrease in fluorescence apparently reflects the spontaneous loss of parasite antigens from the worm surface, since larvae incubated *in vitro* and then reacted with immune serum and fluoresceinated antiimmunoglobulin also emit less fluorescence than newly transformed schistosomula reacted with the same reagents (Figure 4). In other experiments it was shown that the loss in surface antigens does not occur at 4C and therefore is

Table 2—Expression of MHC Products on Schistosomula Recovered from Guinea Pigs

Worms Tested	Immunofluorescent staining* of worms with following antiserums: 2 anti-13	13 anti-2
3-hour-old	–	–
Strain 2	–	+
Strain 13	+	–

* Schistosomula were obtained either immediately after transformation (3 hours old) or 5 days after intravenous injection into either Strain 2 or Strain 13 guinea pig lungs. The worms were then tested for their reactivity by indirect immunofluorescence[28] with alloantiserums (kindly donated by Drs. M. Dorf and B. Benacerraf) produced by cross-immunizing Strain 2 and Strain 13 guinea pigs. +, denotes 20/20 worms showing strong tegumental staining with fluoresceinated rabbit antiserum directed against guinea pig IgG.

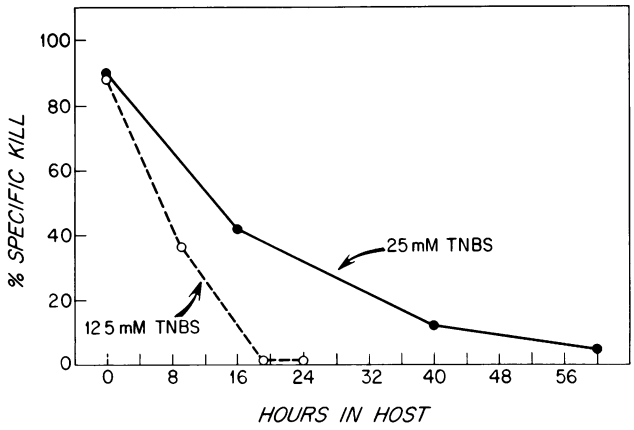

Figure 3—Loss of susceptibility to killing by anti-TNP antibody plus complement by schistosomula recovered from mouse lungs at various times after intravenous injection. The schistosomula, recovered from the lungs of CF_1 mice at the times indicated, were surface-labeled with trinitrobenzene sulfonic acid (TNBS)[27] at either of the two concentrations noted. They were then incubated with a 1:8 final dilution of hyperimmune rabbit anti-TNP antibody in the presence of 25% fresh guinea pig serum for 18 hours at 37 C. The percentage of dead larvae in the cultures was then determined by morphologic examination and dye exclusion.[27] "Percent specific kill" refers to the percentage of dead organisms in cultures containing anti-TNP serum minus the percentage observed in control cultures containing normal rabbit serum instead of anti-TNP serum. Each point is the mean of duplicate determinations.

probably dependent on the metabolism of the parasite.[28] In addition, acceptor sites for the third component of complement (C3) (deposited on the parasite surface as a consequence of activation of the alternate pathway[11,12] and binding sites for concanavalin A[15] were shown to be shed from newly transformed

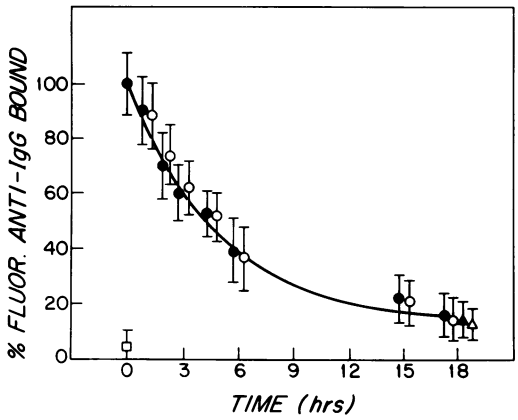

Figure 4—Binding of fluoresceinated anti-rat IgG to the surface of schistosomula preincubated with immune serum from rats infected for 6 weeks with 1000 *Schistosoma mansoni* cercariae each. ●, schistosomula labeled and then cultured in medium containing 20% normal rabbit serum. O, schistosomula labeled and then cultured in medium without serum. ▲, schistosomula cultured in medium containing 20% rabbit serum for 18 hours and then labeled. △, schistosomula cultured in medium without serum for 18 hours and then labeled. □, zero time control with heat-inactivated normal rat serum. Values are expressed as a percentage of zero time labeling. (Reproduced with permission of the *Journal of Immunology*)

schistosomula with kinetics indistinguishable from those observed in studying the turnover of surface antigen.[28] Since schistosomula grown in the same media (RPMI-1640 plus serum) have been shown to undergo morphologic differentiation *in vitro*,[14] it is unlikely that the surface molecules are lost and not replaced because of a defect in biosynthesis caused by inappropriate culture conditions. Finally, it is also clear that the loss in antigenicity undergone by cultured schistosomula is not due to the masking of the parasite surface by host molecules, since the same decrease in antibody binding is observed when the larvae are cultured in serum-free medium (Figure 4).[28]

In summary, the *in vitro* experiments described above suggest that schistosomula during their early development shed and do not replace surface antigens recognized by antibodies from heavily infected hosts. The nature of the parasite membrane after the occurrence of this dramatic change his not been investigated. Either the lost macromolecules are not replaced or else are replaced with other surface molecules not recognized by host antibody. In any case, it would appear that the surface of the parasite at this stage, even in the absence of associated host molecules, is poorly recognized by both the effector and affector arms of the immune system.

That post-skin-stage schistosomula undergo a loss in immunogenicity as well as antigenicity is supported by recent experiments from this laboratory (unpublished) employing heavily irradiated schistosomula in the induction of protective immunity in mice. In this work, irradiated lung-stage schistosomula (recovered from syngeneic donors) have been shown to induce significantly less immunity to cercarial challenge than comparable numbers of irradiated newly transformed larvae. Further evidence that lung-stage schistosomula are poorly immunogenic comes from recent studies (A. Sher, S. James, and A. Abbas, manuscript in preparation) measuring the *in vitro* induction of anti-TNP antibody responses by TNP-labeled larvae. In these experiments, TNP-labeled fresh schistosomula have been shown to stimulate excellent TNP-specific plaque-forming cell responses, whereas TNP-conjugated lung-stage schistosomula consistently fail to induce responses significantly above background even when added to the cultures in high numbers. Thus, it would appear that in addition to being itself poorly immunogenic, the surface of lung-stage schistosomula is an environment that somehow reduces the immunogenicity of nonparasite antigens introduced into it. It is tempting to speculate[9] that this loss in immunogenicity results from the association of either parasite antigen or hapten with worm-associated MHC molecule. How-

ever, at present there is no information supporting an immunoregulatory hypothesis of this type.

In summary, the data discussed here suggest that the evasion of the immune response by developing schistosomula involves multiple overlapping mechanisms (Figure 5) and as such may be thought of as an example of "overkill" in a biologic process. Within 24 hours after transformation from the cercaria, at a time immediately before its exit from the dermis and its entry into the bloodstream, the schistosomulum undergoes at least three different changes that render it less susceptible to immune attack: 1) the development of a tegument intrinsically resistant to immune damage; 2) a loss in surface antigens recognized by antibodies from parasitized hosts; 3) the acquisition of a coat of host molecules. In addition, preliminary experiments suggest that a fourth factor may play a role in the escape of post-skin-stage schistosomula from immune attack—a decrease in parasite immunogenicity. The simultaneous deployment of these multiple mechanisms, each of which in itself is presumably sufficient to protect the parasite against destruction by the immune system, may reflect the long evolutionary history of the schistosome and its need to confront different immune effector mechanisms utilized by different vertebrate hosts.

The finding that schistosomes employ a sophisticated combination of mechanisms for evading the immune response has obvious implications for the design of methods of vaccination against the infection. It can be seen clearly from the kinetic data reviewed in this paper (Figures 2, 3, and 4) that in order to be effective against invading parasites, antibody-dependent immunity must attack schistosomula in the skin essentially immediately (within a few hours) after transformation. In order to achieve such an early contact with the larvae, rapid extravasation of the protective antibodies into the dermis may be necessary. Thus, as has been suggested previously,[31] efficient vaccination may require the induction of a state of immediate hypersensitivity in addition to effector antibodies directed against schistosomulum surface antigens.

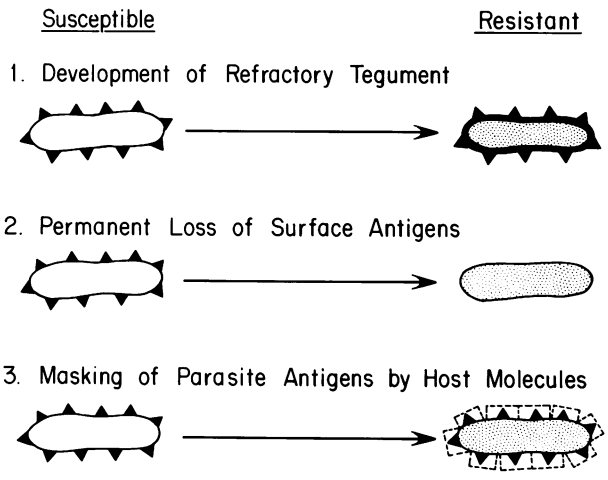

Figure 5—Schematic representation of the three mechanisms discussed in this article that limit the susceptibility of developing schistosomula to immune attack.

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