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# Stage-specific schistosome antigens

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One of the central ideas in schistosome immunity is represented by the concept of concomitant immunity. This term describes "a situation in which the host is resistant to reinfection but cannot at the same time rid itself of an established parasite population" (Smithers and Terry, 1976). The most obvious (although not exclusive) interpretation of such a phenomenon is that the newly invading schistosomula and the established adult worms are antigenically different. Thus, the search for stage specific antigens can be seen as part of a strategy aimed at directing the immune response against those parasite stages which appear to be most vulnerable, i.e. the immature forms.

The impressively long survival of adult schistosomes in immunologically hostile hosts makes this stage of the parasite a rather unattractive candidate as a possible target of immune intervention. Yet, there are instances in which mature schistosomes appear much less invulnerable to immune mechanisms than we usually think they are. A classical example is the very efficient killing of adult mouse schistosomes transferred to "anti-mouse" monkeys (Smithers et al., 1969). Hyperimmune monkeys, i.e. animals in which a condition of "sterile immunity" had been induced by repeated infections, were also capable of causing extensive (possibly lethal) damage to transplanted monkey adult worms (Hockley and Smithers, 1970). Even more interesting is the less artificial situation in which rhesus monkeys infected with an adequate number of parasites have been shown to spontaneously eliminate – possibly by immune mechanisms – the adult, egg-laying schistosomes of a primary infection (Cheever and Powers, 1972). Thus, the prospect of "immune therapy" should be kept into the realm of theoretical possibilities, although its practical implementation appears extremely difficult at the present stage of our knowledge.

The idea that immature schistosomula are likely to represent the most suitable target of immune intervention received considerable support from a large body of in vitro experiments performed during the last several years (reviewed in: Capron et al., 1982; Smithers and Doenhoff, 1982). Newly trans-

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formed schistosomula were shown to be susceptible to the cytotoxic effects of antibody and complement (Clegg and Smithers, 1972), antibody and neutrophils (Dean et al., 1974), antibody and eosinophils (Butterworth et al., 1977; Capron et al., 1978), antibody and macrophages (Capron et al., 1975), antibody and platelets (Joseph et al., 1983). A general feature of these in vitro effector mechanisms is that only very young schistosomula are susceptible of being killed, whereas older forms obtained from the lungs 5 days after infection are always refractory, and even parasites aged 24–28 h in vitro or in vivo are almost insensitive to the cytotoxic mechanisms. Such a sensitivity pattern appeared to be in agreement with the concomitant immunity concept and it suggested that very early schistosomula were the target of immune elimination in previously infected hosts. These ideas stimulated us, as well as other investigators, to focus our attention on the molecules expressed on the surface of newly transformed schistosomula.

Using the technique of surface labeling by lactoperoxidase-catalyzed iodination, we detected a protein of approximately 18 kDa which became preferentially labeled in 3 h schistosomula obtained either by mechanical transformation or by in vitro skin penetration (Liberti et al., 1986). The level of radioactivity bound by this protein was at least one order of magnitude higher than that of any other protein. The surface location of the 18 kDa component was confirmed by its susceptibility to proteolytic digestion on intact live schistosomula. Stage specificity was observed in vivo as the protein was not labeled in lung schistosomula and in adult worms, and it was also observed upon in vitro culture where the protein ceased to be the most heavily labeled component after about 24 h. The 18 kDa protein was not precipitated by infected sera of either mouse, rat or human origin, but it was precipitated by an antiserum obtained in rabbits repeatedly injected with 3 h schistosomula or by an antiserum raised against the material eluted from the 18 kDa region of polyacrilamide gels loaded with 3 h schistosomula. It is possible that the short time in which the protein is exposed on the surface of schistosomula in the course of a normal infection may not be sufficient to stimulate the host immune system, but it is conceivable that an adequate pre-immunization with this major larval surface protein may be able to block the infection. Isolation of the 18 kDa protein in sufficient amounts to test this hypothesis has not yet been completed due to the finding that another protein of very similar $M_r$ is present in 3 h schistosomula.

In more recent years, the concomitant immunity model (i.e. the induction of resistance by means of a previous chronic infection) has been complemented by the irradiated cercaria model (Hsü et al., 1969; Minard et al., 1978; Bickle et al., 1979). In the latter system, resistance is induced by one or more infections with highly irradiated cercariae. Since these larvae never develop to egg-laying adults, the serious complications caused by host pathology are eliminated, while the levels of resistance obtained (around 80%) are even higher than those obtained after chronic infection. There is good evidence about the immunolog-

ical basis of this resistance, as T- or B-suppressed mice are unable to become resistant (Sher et al., 1982). There is also evidence for an involvement of activated macrophages (James et al., 1984), while at the same time passive transfer of serum alone has been shown to be effective in transferring resistance (Mangold and Dean, personal communication).

It is clear that, in the irradiated vaccine model, resistance is induced by the immature schistosome, and it is reasonable to assume that the effect of irradiation may be just that of arresting schistosomulum development in a way that enhances host exposure to the most immunogenic parasite stage(s). Thus, it would be useful to know exactly which immature stage is immunogenic and which stage is the target of the irradiated vaccine. From immunizations of mice with various irradiated stages of *S. mansoni,* a general trend seems to emerge suggesting that the earlier the stage of immunizing larvae, the better the resistance obtained (Sher and Benno, 1982; Dean et al., 1981). Considerable effort has been devoted to determining the stage which is the target of immune elimination in vaccinated mice. In this context, it is interesting to remember that even in a primary infection about half the penetrating larvae are eliminated before the liver stage. It is conceivable that this "normal attrition" may have features in common with "immune attrition".

An important advance in the techniques used to follow schistosome migration has been the recent introduction of macroautoradiography, which enables the detection of individual parasites in various body compartments (Georgi, 1982). The results obtained with this technique support the idea that nearly all penetrating schistomes reach the lungs, both in normal and in irradiated cercaria-immunized animals (Mangold and Dean, 1983; Dean et al., 1984; Wilson et al., 1986). Migration out of the lungs is a slower process in immune than in normal animals, but in both cases the number of schistosomes disappearing from the lungs is significantly higher than the number appearing in the liver. In other words, there is a "loss" of radiolabeled parasites during their transit from lung to liver. At 3 weeks, the number of foci detectable in immune animals is the same as in controls, although in the former hosts a higher proportion of parasites is still in the lungs (Dean and Mangold, personal communication). After week 3, the total number of foci remains constant in normal mice and is very close to the number of adult parasites recoverable by portal perfusion. In mice immunized with irradiated cercariae, on the other hand, the total number of detectable foci continues to decrease after week 3, mainly because foci disappear from the lungs and fail to appear in the liver or in other compartements. The results of these experiments indicate that the major site of immune elimination is most likely to be the lung. This is in agreement with the recent finding that serum from mice vaccinated with irradiated cercariae can passively transfer resistance if injected about one week after infection (Mangold and Dean, personal communication). Thus, there are strong suggestions that the target antigen(s) of irradiated cercaria-induced resistance may reside in the lung schistosomulum.

We have examined the surface of lung schistosomula using the technique of lactoperoxidase-catalyzed iodination. As previously mentioned, the 18 kDa antigen present in 3 h schistosomula does not become labeled in lung forms (Liberti et al., 1986). The most heavily labeled band in 6-day-old schistsomula has an $M_r$ which is definitely higher. This is true in parasites recovered from the lungs of mice infected 6 days previously as well as in parasites kept in culture for a few days. Exposure of the whole schistosomulum to trypsin modifies the electrophoretic migration of the labeled band, thus supporting its protein nature and its surface location. In view of the remarkable size similarity between the most intensely labeled component of 3 h schistosomula and the most intensely labeled component of lung forms, we have entertained the possibility that the two proteins might be structurally related. Peptide maps of the two components labeled in vivo dit not show any similarity. In addition, rabbit antisera raised against whole 3 h schistosomula or against the 18 kDa surface protein of the early larvae failed to precipitate the major surface labeled component of lung forms. Thus, it appears that during schistosomulum development a low $M_r$ surface protein is replaced by a different protein of a similar size.

There is general agreement about the idea that schistosomula tend to lose antigens in developing from newly penetrated larvae to lung forms (see Pierce et al., 1986 for references). It is interesting, however, that there are also biochemical studies showing the appearance of new antigens in a way similar to what we have observed. Thus, Simpson et al. (1984) described the appearance, after 48 h of in vitro culture, of a 45 kDa doublet and of an 11 kDa antigen. Payares et al. (1985) described a 25 kDa antigen which is present on the surface of lung forms but not on young schistosomula. Additional proteins have been identified on the surface of lung schistosomula after removal of "masking" components (Aronstein and Strand, 1983; Harn et al., 1985). The possible relationships between the surface antigens observed by us and those reported by other investigators remain to be determined.

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