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HETEROLOGOUS RESISTANCE IN SCHISTOSOMIASIS

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Humans and animals infected with parasitic helminths develop varying degrees of immunity to both homologous and heterologous reinfection, but this immunity is incomplete and sterile immunity as in infections with viruses or bacteria is not observed. The immunity achieved in many cases is, nevertheless, significant and evidenced by resistance to infection and/or diminished pathology or disease. Nelson (1974) defined zooprophylaxis as "the prevention or amelioration of disease in man as a result of previous exposure to heterologous infections of animal origin." He suggested that man's constant exposure to occult and mainly non-pathogenic zoonotic infections is of major importance in relation to his general well being and of his survival, particularly in the tropics.

There is abundant experimental evidence of cross resistance between many different viruses, bacteria, fungi, protozoa, and helminths. The classical example has been the protective effect of cowpox in humans against subsequent infection with smallpox where antigenic cross reactivity is responsible for the heterologous resistance observed. In other cases, conversely, non-specific mechanisms have proved to be involved in cross-protection. Heterologous resistance against schistosomes due to non-specific factors has been reviewed by Mahmoud (1982).

Heterologous immunity (or more appropriately, resistance) has been demonstrated in several parasite/parasite interactions involving schistosomes. Moreover, some adjuvants and defined molecules have been shown to protect animals against challenge infection with schistosome cercarie. Both of these approaches are the subject of this review. Other reviews include Dean (1983), Hillyer (1984), Haroun & Hillyer (1986), and Christensen et al. (1987).

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Infections inducing heterologous resistance against schistosomes

Protozoan infections – Both partial resistance to infection with *Shistosoma mansoni* and diminished pathology has been observed in mice previously infected with *Toxoplasma gondii.* Mahmoud, et al. (1976) found that mice infected with *T. gondoii* 4 weeks to one day before infection with *S. mansoni* developed up to 35% less worms than controls, but infection with *T. gondoii* 4 weeks after infection with *S. mansoni* had no effect on the schistosome infection. Subsequent work showed that toxoplasmosis infection for 2-20 weeks markedly suppressed granulosa formation around *S. mansoni* eggs, and that mice with combined infections had markedly smaller hepatic granulomas and lower mean portal pressures than those infected with *S. mansoni* alone. Moreover, although the prevalence of esophageal varices in the mice with schistosomiasis alone was 60%, there was no visible collateral circulation in the animals with both infections (Mahmoud et al., 1977). Thus, under specific circumstances, infection with this intracellular protozoan not only induces partial resistance to infection with a parasitic trematode, but also reduces disease. On the other hand, only, marginal protection was obtained using mice previously infected with *Trypanosoma cruzi.* Kloetzel et al. (1973) examined concomitant infection of albino mice with *Trypanosoma cruzi* and *S. mansoni* and found that mice infected with *T. cruzi* for 5 or 15 days and challenged percutaneously with *S. mansoni* cercariae had reductions in schistosome worms recovered over controls, although the results as presented did not appear to be statistically significant. Those infected with *T. cruzi* for 16 or 63 days and challenged subcutaneously with *S. mansoni* cercariae clearly had no reduced schistosome worm burden recoveries, suggesting lack of protection. Moreover, *T. cruzi* counts were greatly enhanced by concomitant infec-

tion with *S. mansoni* with ensuing heavy mouse mortality.

Nematode infections – *S. mansoni* antigens react serologically with antibodies to *Trichinella spiralis*. Using this as a basis, Jachowski & Binghan (1961) infected mice with *T. spiralis* to determine whether this infection would influence the worm burden from a superimposed infection with *S. mansoni* 5 weeks later. No protection was seen in two experiments in which the challenge with *S. mansoni* was light (50 cercariae); significant protection in the form of 46% *S. mansoni* worm burden reduction was observed in one experiment in which the challenge with *S. mansoni* was heavier (200 cercariae).

Cestode infections – There are apparently no reports on the effect of cestode infections on challenge infection with schistosomes (Hillyer, 1984; Chrstensen et al., 1987). This neglected field requires attention since there is a great deal of heterologous cross-reactivity and cross-resistance among the cestodes (Capron et al., 1968; Harrison &Parkhouse, 1985) and heterologous cross-reactivity among the cestodes and the trematodes (Capron et al., 1968).

Trematode infections – A great number of studies have been published on heterologous resistance among the trematodes, particularly among different schistosome species. Resistance to heterologous schistosome infection can be reflected in reduced establishment of worms after challenge infection, in reduced total tissue egg counts, and often in reduced tissue egg counts per worm pair. In fact, a wide variety of schistosome species have been shown in the murine model to induce resistance against challenge infection to many heterologous schistosome species (Christensen et al., 1987).

Moreover, the work of Hillyer and colleagues and Christensen and colleagues has clearly demonstrated that infection with *F. hepatica* in the murine model induces high levels of resistance to challenge infection with *S. mansoni* and vice versa (Hillyer, 1984; Christensen et al., 1987). The mechanisms of these heterologous resistance patterns are yet to be determined.

Heterologous resistance in schistosomiasis using defined molecules

When Dean published his review on immunity to schistosomes in 1983, there wasn't a single published article on a defined schistosome antigen which had been shown to protect experimental animals against challenge infection with cercariae. Since that time, numerous monoclonal antibodies with the ability to passively transfer protection, and several protective antigens have been identified (Simpson & Cioli, 1987). Some of the more recent reports on adjuvants and protective defined molecules which may have relevance for heterogous resistance are summarized below.

Non specific heterologous resistance to challenge with *S. mansoni* cercariae has been induced with bacterial adjuvant *Mycobacterium bovis* strain BCG. James (1987) reports that in the case of BCG, the method of antigen presentation is critical to induction of resistance. Thus, the same antigen preparations that are protective when administered intradermally with BCG are of no protective benefit when given intravenously or intramuscularly. Schistosome larval or adult worm antigens injected intradermally with BCG induce in mice significant levels of protection against challenge infection with *S. mansoni* cercariae. The immunized mice display delayed hypersensitivity, lymphocyte proliferation, lymphokine production, and demonstrate activated (larvacidal) macrophages at the site of specific antigen challenge *in vivo*. These immunized mice produce surprisingly little or no antibody against parasite surface membrane antigens and a surprisingly restricted antibody response to soluble antigens, recognizing an internal 97 kDa protein which is located in areas just interior to the tegumental and gut syncytia in the adult schistosome. Clones of cDNA expressing products reactive with antibody against the native protein have been identified, and a portion of the gene encoding the 97 kDa protein has been sequenced. The deduced amino acid sequence indicates that the molecule is paramyosin, an alpha-helical coiled-protein that forms the core structure for myosin filaments in all invertebrates. This molecule has been purified by affinity chromatography and found to induce both T cell reactivity and protection when injected intradermally with BCG (James, 1987; James & Sher, 1986). The question remains whether a paramyosin-like immunogen is found in other parasitic trematodes which cross-react and cross-protect against schistosomes.

Cross-reacting antigens between schistosomes and other organisms have also been shown to

induce the production of anti-schistosome antibodies and to induce protective immunity.

For example, a 28 kDa *S. mansoni* antigenic protein (P28) containing epitopes cross reactive with *S. haematobium* and *S. japonicum,* and with the bovine schistosome, *S. bovis,* was identified, isolated and shown to be protective in rats, mice, and hamsters. The complementary DNA enconding P28 was cloned and expressed in *Escherichia coli,* and the recombinant protein induced similar levels of protection as the purified native protein in rats, mice, and hamsters (Capron et al., 1987). Its cross reactivity with other schistosomes suggests a possible role of this antigen in protection against other human and bovine schistosome species. P28 is also interesting because it is an enzyme (glutathione transferase), and is not an integral membrane protein but rather excreted by the parasite and transiently expressed at the surface of schistosomula pointing to the essential role that could be played by excretory and secretory antigens in the induction of immunity.

Grzych et al. (1987) demonstrated that *S. mansoni* shares a protective carbohydrate epitope with the hemocyanin (KLH) of an ancestral marine mollusk named *Megathura crenulata* (keyhole limpet). The corresponding epitope is found on a 38 kDa glycoprotein schistosomulum antigen, a 115 kDa molecule in the excretory/secretory products of *S. mansoni* adult worms, on high molecular weight components of cercariae and eggs, and in the snail intermediate host, *Biomphalaria glabrata.* Rats immunized with KLH develop antibodies which immunoprecipitate the 38 kDa schistosomulum antigen, and are significantly protective against challenge with *S. mansoni* cercariae.

Subcellular fractions of 2 parasitic trematodes, *Fasciola hepatica* and *Paragonimus westermani,* have been shown to induce in mice resistance to challenge infection with *S. mansoni* (Hillyer, 1984; Hillyer & Serrano, 1983). The protective *F. hepatica* antigens were those which bound to antibodies to *S. mansoni* and purification resulted in an antigenic complex designated $Fh_{SmIII(M)}$ (Hillyer & Cervoni, 1978). $Fh_{SmIII(M)}$ is composed of multiple antigenic polypeptides ranging in MW from 66 to 12 kDa, the two most prominent being of 12 (Fh P12) and 14 kDa. When gels are overloaded the 12 and 14 kDa polypeptides fuse forming a single broad band. The 12 kDa antigen appears to be a pure polypeptide since it is degraded by proteinase K to lower MW peptides which still retain their antigenicity by immunoblot. Treatment with either endoglycosidase H, neuraminidase or dithiothreitol has no effect on its mobility in SDS-PAGE, or in its recognition by antibody, suggesting the absence of carbohydrate moieties or disulphide bonds in relation to its antigenic determinants. This molecule is clearly a protective antigen being capable of inducing up to 77% protection against cercarial challenge in mice. The serum of calves known to be partially resistant to *F. hepatica* infection recognize an *in vitro* translation product of similar MW as Fh P12 which, if found to be identical, makes for recombinant DNA technology a feasible approach for the large scale manufacture of the vaccine (Hillyer & Taylor, 1988). The 14 kDa antigen is a cytochrome c-like molecule which exhibits cross-reactivity in ELISA with cytochrome c from *Saccharomyces cerevisiae,* pigeon breast muscle, and rabbit heart. Its protective nature still needs to be ascertained (Hillyer et al. 1988a, c).

The mechanism of immunity induced by Fh P12 is unknown. Since fascioliasis induces eosinophilia, our initial working hypothesis was that it could be an antibody-dependent eosinophil-mediated cytotoxicity, but experiments with serum from rabbits and cows immunized with $Fh_{SmIII(M)}$, or the serum of humans with fascioliasis failed to induce killing of *S. mansoni* schistosomula via this mechanism (Hillyer et al., 1987). Thus a DTH-type mechanism as report by James (1987) needs to be investigated.

In summary, the latter half of this decade has resulted in the identification of several protective schistosome molecules with surprising heterospecifity suggesting that the schistosome vaccine which will undoubtedly be developed may be active against more that one species of trematode.

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