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HUMAN ANTIBODY RESPONSES TO *SCHISTOSOMA MANSONI:* DOES ANTIGEN DIRECTED, ISOTYPE RESTRICTION RESULT IN THE PRODUCTION OF BLOCKING ANTIBODIES?

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After treatment young Kenyan schoolchildren are highly susceptible to reinfection with Schistosoma mansoni. *Older children and adults are resistant to reinfection. There is no evidence that this age related resistance is due to a slow development of protective immunological mechanisms, rather, it appears that young children are susceptible because of the presence of blocking antibodies which decline with age, thus allowing the expression of protective responses.*

Correlations between antibody responses to different stages of the parasite life-cycle suggest that, in young children, antigen directed, isotype restriction of the response against cross-reactive polysaccharide egg antigens results in an ineffectual, or even blocking antibody response to the schistosomulum.

Immunity to schistosomiasis in man

Until recently, because of the difficulties involved in studying immunity to schistosome infections in man, there has been no clear demonstration that immunologically mediated resistance to reinfection occurred in man. The problems associated with studying human immunity are well known. It is difficult to estimate rates of reinfection when existing adult worms are dying at unknown and possibly variable rates; it is difficult to distinguish between immunity and lack of exposure as reasons for lack of reinfection; and as immunity in experimental animals has been found to be incomplete, it must be assumed that immunity in man may also be only partial, although this does not preclude its importance in reducing both transmission and disease. An inevitable consequence of this inability to measure immunity in man has been the inability to identify which of the many readily demonstrable immune responses directed different schistosome antigens, are really a help or a hindrance to the human host's defence against reinfection. Detailed knowledge of this kind is important if we are to successfully manipulate the immune system by vaccination, in such a way that it will resist a parasite which has evolved to thrive in an apparently hostile immunological environment.

However, there is now a growing body of evidence in favour of the existence of human immunity to reinfection with schistosomes. The methods used to obtain this evidence have involved treating patients in order to eradicate existing adult worm populations (and accepting the danger that treatment itself may affect the immune response); and then trying to obtain some measure of subsequent exposure of individuals to reinfection, while monitoring the actual rate of reinfection as judged by parasite egg output in the faeces. Serum samples taken during the course of such studies can be used subsequently to test whether any immunological parameter correlates with resistance to reinfection.

Such studies have shown that heavy exposed young children remain susceptible to reinfection with *Schistosoma mansoni* after chemotherapeutic cure. In contrast, older individuals, who have been treated, exhibit a progressively declining incidence of reinfection with age, which cannot be attributed to reduced contact with infective water. This age-related resistance to reinfection has been found to be dependent on previous experience of infection. Individuals

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who were previously uninfected show a steady incidence of new infections that is independent of age (A. E. Butterworth et al., 1985, *Trans. R. Soc. Trop. Med. Hyg. 79:* 393-408; R. F. Sturrock et al., 1987, *Trans. R. Soc. Trop. Med. Hyg., 81:* 303-314). A similar age-dependent resistence to reinfection occured in *S. haematobium* infected patients (H. A. Wilkins et al., 1987, *Trans. R. Soc. Trop. Med. Hyg., 82:* 397-404).

The conventional interpretation of such an acquired, age-dependent resistance to reinfection would be that it is a manifestation of acquired immunity in these infected people. However, if this is a correct interpretation, it is clearly a very slow development of immunity, which may take years to become effective. There are two possibilities which would account for this; one is that under conditions of natural exposure and infection, protective immune responses simply take a long time to develop. There is no evidence to support this explanation; in fact, both susceptible and resistant children appear to have the same range of potentially protective immune response (A. E. Butterworth, et al., 1985). The second possible explanation for this very slow development of immunity is that this susceptibility to reinfection seen in younger children is due to the presence of ineffectual isotypes which act as blocking antibodies, preventing the expression of protective immune mechanisms, but declining with age.

Blocking antibodies

There are several strands of evidence which suggest that blocking antibodies may have a significant influence on immunity to *S. mansoni:*

a) The first demonstration of blocking antibodies was in the rat by J. M. Grzych et al. (1984, *J. Immunol., 133:* 999-1003) who found that an IgG2c monoclonal antibody, which reacted with the schistosomulum surface, but which did not mediate immune killing, inhibited killing by an IgG2a monoclonal antibody, both *in vivo* and *in vitro.*

b) The presence of IgM blocking antibodies in human infection serum has been directly demonstrated by J. Khalife et al. (1986, *J. Exp. Med., 164:* 1626-1640). These workers found that the IgM fraction of human infection serum did not mediate the killing of schistosomula in the presence of eosinophils *in vitro.* In contrast, IgG fractions from the same serum showed enhanced killing when compared with unfractionated serum. Preincubation of schistosomula with the IgM fraction inhibited the killing mediated by both whole serum and the IgG fraction.

c) Murine IgM monoclonal antibodies which recognise epitopes present on both a major polysaccharide egg antigen, K_3, (D. W. Dunne & Q. D. Bickle, 1987, *Parasitol., 94:* 255-268) and on glycoprotein antigens on the surface of the skin stage schistosomulum, have been shown to inhibit the level of killing induced by human infection sera in, *in vitro,* eosinophil-mediated, schistosomula killing assays (D. W. Dunne et al., 1987 *Parasitol., 94:* 269-280).

d) Evidence of blocking antibodies was also found in preliminary analysis of the immune responses of 129 *S. mansoni*-infected, Kenyan schoolchildren, who were treated and then monitored for reinfection over the subsequent 21 months. A positive correlation was found between pretreatment levels of IgM antibodies with specificity for a major Mr38K schistosomulum antigen, and levels of reinfection as indicated by the number of parasite eggs excreted by individuals 9 months later. This suggested that high levels of these IgM antibodies are predictive of susceptibility to reinfection. The Mr38K antigen shares epitopes with developing miracidium. Pretreatment levels of IgM antibodies against a saline soluble extract of *S. mansoni* eggs (soluble egg antigen-SEA) were also found to correlate positively with subsequent rates of reinfection (A. E. Butterworth et al., 1985).

The influence of cross-reacting antigens on the response to the schistosomulum

The positive correlation between IgM responses to the parasite egg and susceptibility to reinfection; and the inhibition of infection serum mediated killing of schistosomula by anti-egg polysaccharide monoclonal antibodies, suggest that cross-reactive polysaccharide egg antigens might have a significant role, albeit a negative one, in immunity to schistosomes.

The early schistosomulum has been shown to be susceptible to a variety of immune effector mechanisms, both *in vitro* and *in vivo.* However, it appears that most of the antigens which can

be demonstrated on the schistosomulum surface shortly after transformation from the cercarial stage, are expressed *in vivo* for only two or three days. This is in contrast to the persistent antigen stimulation which results from even a single pair of mature worms. Indeed, the daily production of several hundred eggs from each female worm, over a life-span perhaps measured in years, might be expected to be a major influence on the immune response to the schistosomulum via cross-reacting antigens.

In order to examine the influence of cross-reacting egg antigens on the antibody responses to the schistosomulum, and to relate this to rates of reinfection, we have recently assayed the antibody responses of the same group of Kenyan schoolchildren described above, to a variety of schistosomulum and egg antigens.

The schistosomular antigens used in these antibody assays were dirived from mechanical transformed schistosomula. They consisted of:

a) a detergent solubilized membrane preparation, made using a method which extracted the outer membrane antigens, but left the schistosomulum intact;

b) a trichloroacetic acid soluble extract of the detergent solublized membrane (this contained mainly polysaccharide antigens, including antigens which cross-reacted with egg antigen K_3);

c) a periodate-insensitive fraction of the detergent solublished membrane (this had a much reduced carbohydrate content and did not cross-react with K_3);

d) antigenic material shed from schistosomula which had been cultured for 18 hours after transformation (this also cross-reacted with K_3 and appeared to contain relatively little protein).

The antibody responses against these schistosomular antigens, the egg (SEA), and the polysaccharide egg antigen K_3 were measured by ELISA and compared using Spearman rank correlations (D. W. Dunne et al., *Eur. J. Immunol.*, in press).

When the IgG responses to the four schistosomular membrane preparations were compared with each other, it was found that the response to the whole detergent solublized preparation was strongly, positively, correlated with the responses to the other preparations. However, although the responses to the carbohydrate-rich membrane and shed antigens also correlated positively with each other, neither of these correlated with the IgG responses against the membrane proteins. That is, the IgG responses to the protein components of schistosomulum membrane are independent of the responses to the polysaccharide antigens.

Positive correlations were observed between the total IgG responses to the SEA and the IgG responses to all the schistosomulum antigens. However, when individual IgG subclass responses to SEA were compared to the anti-schistosomular responses, a differential pattern of correlations emerged. Anti-SEA IgG_1, IgG_2 and IgG_3 responses, but not IgG_4, correlated positively with the IgG responses to the carbohydrate-rich membrane preparations and shed antigens. While, when the same anti-SEA IgG-subclass responses were compared with the IgG responses to the membrane proteins, positive correlations were observed with IgG_1, IgG_3 and IgG_4, but not IgG_2.

These results indicate that the egg and the schistosomulum outer membrane have both carbohydrate and peptide epitopes in common. However, the subclass composition of the IgG responses directed against these two types of epitope are different. No IgG_2 seems to be involved in the response against cross-reactive peptide epitopes, and no IgG_4 is directed against cross-reactive carbohydrates. This interpretation is supported by the comparison between the IgG subclass responses to whole SEA and the total IgG responses against the polysaccharide antigen K_3, which was purified from SEA. In this case positive correlations are found with the IgG_1, IgG_2 and IgG_3 responses, but not with the IgG_4 responses. Again this indicated that IgG_4 does not form part of the IgG response to polysaccharide egg antigens.

These correlation patterns appear to be the result of antigen-directed, subclass restriction in which the physico-chemical nature of the antigens dictate the subclass composition of the antibody responses directed against themselves. In this context, the lack of an IgG_2 response against protein epitopes might be predicted, since IgG_2 has been shown to be associated

with responses against other, unrelated, polysaccharide antigens (W. J. Yount et al., 1968, *J. Exp. Med., 127:* 633-646; W. F. Riesen et al., 1976, *Scand. J. Immunol., 5:* 383-390; A. Freijd et al., 1984, *Clin. Exp. Immunol., 56:* 233-241).

The associations between anti-egg responses and susceptibility or resistance to reinfection

As the schistosomulum has been shown to be the target for immune effector mechanisms, we compared the pretreatment anti-schistosomulum responses of Kenyan schoolchildren, with the rate at which individual children became reinfected after treatment. This was done in the hope of identifying protective immune responses. A protective response would be expected to manifest itself by correlating negatively with rates of reinfection as judged by the output of parasite eggs 9 months after treatment. No such negative correlations were observed between egg output and antibody responses against any of the schistosomular antigens.

However, when the antibody responses to SEA were compared with rates of reinfection, positive correlations were found with both the IgM and IgG_2 responses. Thus, both these responses to SEA were associated with susceptibility to reinfection and might therefore be acting as blocking antibodies.

It was also observed that the IgG_2 anti-SEA antibodies were present in large amounts in only a small proportion of the children, therefore it was possible to repeat the correlation analysis for the relationships between anti-schistosomulum responses and reinfection rates excluding those children who showed high levels of IgG_2 anti-SEA antibodies. Following the exclusion of the high IgG_2 responses, a negative correlation was found between the pretreatment IgG response to the antigens shed from cultured schistosomula and rates of reinfection. This suggests the possibility that IgG_2, and possibly IgM, anti-SEA antibodies are cross-reacting with epitopes on, and shed from, the surface of the schistosomulum, and that these isotypes may block protective mechanisms mediated by other isotypes (A. E. Butterworth et al., *Biochemie*, in press).

The production of these IgM and IgG_2 isotypes is probably determined by the polysaccharide nature of the cross-reacting egg antigens. At least some of the schistosomulum antigens, which have epitopes in common with these polysaccharides, are glycoproteins, and as such would not be expected to induce predominently IgG_2 responses.

We have some evidence that IgG responses to polysaccharide antigens actually decline with age, whereas IgG responses, particularly IgG_4, to protein and glycoprotein antigens, increase with age, and also with intensity and possibly duration of infection. These observations are consistent with the hypothesis that during early nature infection, polysaccharide egg antigens elicit a variety of antibody responses, including IgG_2 and IgM. Some of these antibodies cross-react with antigens on, or released from, the surface of the early schistosomulum. These antibodies block the expression of protective responses with specificity for the same or other antigens. Anti-polysaccharide responses decline with age, thus allowing the expression of protective immunity in older individuals.

It is also possible to speculate on the advantages to the parasite of this age-related blocking mechanism. Early in infection it would facilitate a relatively rapid build up of an initial population of sexually mature worms. However, the gradual increase in the expression of protective immunity would prevent the continued accumulation of worms to the point when the weight of the infection threatened the life of the host and, therefore, the future of existing population of parasites.