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HUMAN IgE RESPONSES TO *SCHISTOSOMA MANSONI* AND RESISTANCE TO REINFECTION

DAVID W. DUNNE; ANTHONY E. BUTTERWORTH; ANTHONY J. C. FULFORD;
JOHN H. OUMA* & ROBERT F. STURROCK**

Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, UK
*Division of Vector Borne Diseases, Ministry of Health, Nairobi, Kenya
**Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, London, UK

Schistosoma mansoni *infected Kenyan patients were treated and the intensities of their reinfections were followed over the next two years. In addition, their pre- and six month post-treatment serum levels of IgG1-4, IgM, and IgE, specific for schistosomula, egg and adult worm, were measured in ELISA. No reinfection took place before six months post-treatment. Reinfection intensities varied with age; the younger children becoming reinfected at significantly higher intensities than older individuals. When antibody and reinfection levels were compared, only the six month post-treatment IgE reponse against adult worm correlated negatively with intensities of reinfection and, therefore, was predictive of resistance or immunity to reinfection. IgE and IgG specific Western Blots were carried out. The adult worm antigens recognized by IgE were restricted compared with the IgG responses of the same patients, although no individual antigen was uniquely recognized by the IgE isotype. A dominant 22 kDa antigen was recognized by most but not all high IgE responders. Patients with IgE responses against this antigen suffered significantly lower subsequent levels of reinfection, compared with non-responders. A monospecific rabbit antiserum against the 22 kDa adult worm antigen showed that this antigen is specifically located in the tegument of the adult worm and of 'lung' and 'liver' stage schistosomula, but is absent from the early 'skin' schistosomula. It is possible that this antigen is a target for human IgE mediated immune effector mechanisms active against the post skin stage schistosomula and that this is boosted by the death of adult worms.*

Key words: Schistosoma mansoni – resistance – human IgE

RESISTANCE TO REINFECTION WITH SCHISTOSOMES IN ENDEMIC POPULATIONS

It has been repeatedly reported that, in areas which are endemic for human schistosomiasis, children have higher intensities and prevalences of infection than adults; for example, the observations of Kloetzel & Da Silva (1967). Similarly, after chemotherapeutic cure of existing infections of either *Schistosoma mansoni* or *S. haematobium*, younger individuals in an endemic population become reinfected at much higher intensities (as judged by the numbers of parasite eggs excreted) than adults. For many years it was not clear whether these differences in reinfection rates were caused by age related differences in exposure to infection (Warren, 1973) or by the development of a protective immunity. More recently, careful field studies have measured individual intensities of reinfection after treatment of *S. mansoni* (Butterworth et al., 1985; 1988b) and *S. haematobium* (Wilkins et al., 1987), whilst also monitoring the amount of contact the same individuals had with infective water. These studies have shown that, although intensities of reinfection are related to exposure to infection, the differences in intensities between children and older groups are too great to be accounted for merely by differences in exposure. This age related resistance to reinfection is also dependent on previous exposure to infection, since the incidence of infection in individuals within an endemic population, who have no evidence of previous infection, shows no decline with age; whereas the incidence of reinfection in previously infected and treated individuals, within the same population with

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comparable patterns of contact with infectious water, shows a dramatic decline with age (Sturrock et al., 1987; Butterworth et al., unpublished results). Peak levels of prevalence occur at an earlier age in endemic populations which have higher mean intensities of infection with *S. haematobium* (Woolhouse et al., 1991). Based on the predictions of mathematical models of immunity, these observations have been interpreted as proving the existence of protective immunity in human schistosome infections (Woolhouse et al., 1991; discussed by Hagan elsewhere in this volume). Similar age shifts in peak intensities of infection have been observed in *S. mansoni* infections by Fulford et al. (submitted for publication), although these authors question the ability of such models to predict the existence of immunity.

Thus, it seems likely that age-related differences in susceptibility to reinfection are due, at least in part, to the development of a protective immunity rather than to non-specific, age-related changes in human behaviour or physiology. If this is true then, in contrast to immunity to many virus and bacterial infections, protective immunity to schistosomes takes many years to manifest itself after initial infection. One of the main goals in studies of schistosomiasis in human populations is to identify the mechanisms involved in this remarkably slow development of immunity.

HUMAN ANTIBODY RESPONSES AGAINST SCHISTOSOMES

Despite the continuing susceptibility to reinfection of young children, a wide range of anti-schistosome immunological responses can be demonstrated throughout the whole infected population, irrespective of age. However, there are age-related qualitative and quantitative differences, both in the isotype composition of human antibody responses and the epitopes to which these antibodies are directed (Jassim et al., 1987; Iskander et al., 1981; Dunne et al., 1988; Hagan et al., 1991). We have previously demonstrated that, in a population of 129 *S. mansoni*-infected Kenyan schoolchildren between the ages of 6 and 16 years, the youngest (most susceptible) children tend to respond particularly strongly against carbohydrate epitopes present both on polysaccharide antigens in the parasite egg and on glycoproteins and other glycoconjugates associated with the outer tegument of the early 'skin-stage' schistosomulum (Dunne & Bickle, 1987; Dunne et al., 1987). The composition of the antibody responses against these epitopes is relatively rich in IgG2 and IgM. The levels of anti-carbohydrate responses generally, and of IgG2 and IgM responses in particular, decline with age (Dunne et al., 1988). When the levels of individual antibody responses were compared with the intensities of reinfection found in these children after chemotherapeutic cure, it was found that high levels of anti-egg and anti-carbohydrate antibodies and high levels of IgG2 and IgM at the time of treatment, correlated positively with high intensities of reinfections in the same individuals over the subsequent 18 months period (Butterworth et al., 1987; 1988a). These findings suggested that anti-carbohydrate IgG2 and IgM antibody responses, rather than being host protective in nature, were at best unhelpful to the patient or at worst actually acting as 'blocking' responses. Thus, it is possible to postulate that all individuals in the population have potentially protective immunological responses directed against the surface of the 'skin-stage' schistosomula, but younger children also produce large amounts of ineffective anti-carbohydrate epitope IgG2 and IgM. These isotypes are selectively induced by the T independent type II nature of the polysaccharide egg antigens which share epitopes with various glycoconjugates associated with the schistosomula tegument (Mazza et al., 1990). IgM antibodies directed against the schistosomulum surface have been shown to be ineffective in mediating antibody-dependent eosinophil killing of schistosomula *in vitro* (Khalife et al., 1986), and IgG2 mediated schistosomula killing is more dependent on the activation state of the eosinophil effector cell than is the killing mediated by IgG1 (Khalife et al., 1989).

CORRELATIONS BETWEEN IgE RESPONSES AND REINFECTION

In the course of these studies on the interaction between IgG subclasses responses and reinfection with *S. mansoni,* no response was identified which negatively correlated with intensities of reinfection after treatment (that is, no antibody response was predictive of subsequent low intensities of reinfection) and, therefore, had the characteristics of a potentially host protective response. However, in a recent study of a cohort of individuals of wider age range with *S. haematobium* infections, Hagan and his colleagues have demonstrated a

significant relationship between IgE antibodies against adult worm or egg antigens and resistance to reinfection (Hagan et al., 1991). After allowing, in logistic regression analysis, for age, sex and exposure, Hagan and his colleagues found that those individuals in the highest quintile of IgE responses were tenfold less likely to become reinfected than those in the lowest quintile, thus suggesting the involvement of IgE in protective immunity. The involvement of IgE in immunity to infection with schistosomes had previously been demonstrated in the *S. mansoni*-rat model (Capron et al., 1981), and in *in vitro* studies of various antibody-mediated anti-schistosome effector mechanisms (Capron et al., 1984). Here we report some of our recent work which concentrated on the relationship between reinfection with *S. mansoni* and human IgE responses in a group of 151 Kenyan patients, between 6 and 66 years of age.

These patients were all examined to assess the number of eggs they were excreting before treatment and bled to assess their pretreatment serum antibody levels. They were then treated with praziquantel. A subsequent reinfection score for each patient was calculated from the results of six stool surveys between 12 and 36 months post-treatment, each survey consisting of duplicate Kato thick smear preparations from three separate stool samples. Little or no reinfection took place in the first 12 months post-infection. When IgE levels, before and 6 months after treatment, were measured against egg, schistosomulum and adult stages of the parasite life-cycle in ELISA, and then compared statistically with levels of pretreatment infection and levels of reinfection, it was found that the 6 month post-treatment anti-adult worm response correlated negatively with the observed levels of reinfection (Pearson's correlation coefficient: -0.307; $p < 0.01$). The pretreatment anti-adult worm responses did not correlate with either pretreatment intensities of infection or post-treatment reinfection. No negative correlation was found between reinfection and IgE anti-egg or schistosomular antigens: instead, the anti-egg IgE responses correlated positively with intensities of reinfection (+ 0.261; $p < 0.01$). Thus, the negative relationship with subsequent reinfection was peculiar to IgE responses to the adult worm, and then only when measured after chemotherapeutic cure (but before exposure to reinfection). The restriction of this association between IgE and lack of reinfection to the adult stage of the parasie life-cycle, suggests that it is not merely due to a generalized increase in the level of IgE with age. This, to some extent, is in contrast to the situation described for human *S. haematobium* infections, where negative correlations with reinfection were found with IgE responses against both egg and adult worm developmental stages (Hagan et al., 1991).

In order to ascertain if the association between anti-worm responses and lack of reinfection reflected a general increase in anti-adult worm antibodies or was restricted to the IgE isotype, individual IgG subclass responses, and IgA, IgM and total IgG responses to adult worm were also compared with intensities of reinfection in these patients. No other isotype response correlated negatively and significantly with intensities of reinfection. However, IgE responses against a number of different adult worm antigen preparations, including a purified outer tegument preparation and a sodium periodate-treated (to oxidize carbohydrate epitopes) adult worm preparation, also negatively correlated with reinfection. Again these negative relationships were only found if antibody levels were measured after treatment of the primary infection, suggesting the possibility that production of these particular IgE antibodies is enhanced by the death of the adult worms.

A consistent confounding factor in attempts to correlate particular antibody responses with resistance to reinfection with *S. mansoni* infections in man is the strong association between intensities of reinfection and age. As anti-worm IgE and reinfection are both strongly correlated to age, it is possible that the association of high IgE anti-adult worm antibodies with lack of reinfection after treatment was fortuitous rather than causal. Initial attempts to control for the effects of age in multiple regression or logistic regression analysis were unsuccessful, since the age effects were too strong for residual effects to be detected (the Pearson correlation coefficient between age and 'reinfection' was -0.718; the correlation for IgE with age was +0.261). However, when the correlations between intensities of reinfection and the IgE responses against these worm antigen preparations were re-analysed separately for the 6 to 16 year old group and for the greater than 16 year olds, it was found that the IgE responses of the younger group were negatively correlated with reinfection, but no

significant correlation was found in the older group. In fact, the negative correlations between IgE anti-worm and anti-periodate treated worm and reinfection were stronger in the under 16 year old group than the population as a whole. Furthermore, the correlation between anti-worm IgE responses and age is lost if the individuals under the age of 16 years are considered separately. Thus, it appears that the older members of the population are contributing more strongly to the associations with age than to the associations with reinfection and IgE. This is probably because older individuals in the population form a more heterogeneous group than do the school-age children in terms of their water contact behaviour, with some of the adults being unexposed and neither showing strong IgE responses nor becoming reinfected. The under 16 year old children were reanalysed by multiple regression analysis and this showed a significant effect of anti-worm IgE ($F = 4.96$ with 1, 56 df; $p < 0.05$) or anti-periodate treated worm IgE ($F = 15.01$ with 1,56 df; $p < 0.001$) even after allowing for the effects of age. Therefore, in the younger age groups, the development of post-treatment IgE antibodies against worm antigens is associated with the development of an age-related resistance to reinfection, and the development of these antibodies can be dissociated from the effect of age itself.

ADULT WORM ANTIGENS RECOGNIZED BY HUMAN IgE

The association between the IgE response against adult worm and resistance to reinfection suggests that the antigens recognized by IgE may be target antigens for protective immunity. In order to identify these antigens, we subjected the *S. mansoni* adult worm preparation which was used in the ELISA assays to SDS polyacrylamide electrophoresis followed by Western blot and then compared the individual adult worm antigens which were recognized by human IgG or IgE. None of the antigens were recognized exclusively by IgE. However, the pattern of antigens recognized by human IgE in this system was very restricted compared with that recognized by IgG. In particular, a 22 kDa antigen was strongly recognized by many, but not all, of the patients who were high IgE anti-worm responders in ELISA. When the whole study population was scored, as being either positive or negative for IgE recognition of the 22 kDa worm antigen in Western blots, it was found that intensities of reinfection were significantly lower among those who had detectable IgE antibodies against the 22 kDa antigen than among those who did not. A monospecific serum was raised by immunizing a rabbit with 22 kDa antigen which had been electro-eluted from SDS-polyacrylamide gels. When this serum was used to immunoprecipitate the 22 kDa antigen from homogenates of metabolically labelled, 'skin-stage' schistosomula, 'lung-stage' schistosomula, 12, 18, 21 day old immature worms and adult worms; this antigen was not found in the 'skin-stage' schistosomula, but it was found in lung-stage larvae and all subsequent developmental stages up to adult worm. Immunogold electron-microscopy revealed that the 22 kDa antigen was only expressed in the tegument of the parasite.

The absence of this antigen in the early 'skin' schistosomulum may account for the lack of a negative relationship between IgE responses against this developmental stage and resistance to reinfection in this study population. There is no strong evidence to suggest that immunological effector mechanisms directed against the adult worm play an important role in protective immunity against *S. mansoni* in man. Indeed, the weight of evidence suggests that under normal circumstances the adult worm is impervious to immunity attack. However, the occurrence of antigens in 'post-skin' stage, pre-adult worms allows the possibility that protective immunological responses are effective against migrating 'post-skin' stage larvae. Preliminary experiments using rabbit anti-22 kDa rabbit serum in indirect immunofluorescence assays against the surface of live schistosomula and pre-adult worms failed to demonstrate the presence of this antigen on the surface of the parasite. Therefore, this antigen may not be available to immune effector mechanisms, such as antibody mediated cellular cytotoxicity, which are directed against the outer tegument of the parasite. However, metabolically labelled 'lung-stage' schistosomula, cultured *in vitro,* were found to secret the 22 kDa antigen into the culture medium. Thus, it is possible that IgE responses directed against antigen released by the migrating larva may trigger a series of inflammatory reactions in tissues adjacent to the parasite, and these reactions are detrimental to the the parasite's successful migration and development.

We have used the monospecific rabbit anti-22 kDa tegumental antigen to screen an adult

worm cDNA library. A large number of cDNA clones have been identified with these antibodies and these clones are now being characterized (Havercroft et al., personal communication). As not all high IgE responding, apparently immune, individuals have antibodies which recognise this 22 kDa antigen, other antigens which have the same pattern of developmental expression and ability to induce IgE antibodies may also be involved in the induction of protective IgE responses. We are screening adult worm cDNA libraries for clones which are recognised by IgE from selected patients.

REFERENCES

BUTTERWORTH, A. E.; BENSTED-SMITH, R.; CAPRON, A.; CAPRON, M.; DALTON, P. R.; DUNNE, D. W.; GRZYCH, J-M.; KARIUKI, T. K.; KHALIFE, J.; KOECH, D.; MUGAMBI, M.; OUMA, J. H.; ARAP SIONGOK, T. K. & STURROCK, R. F., 1987. Immunity in human schistosomiasis mansoni. Prevention by blocking antibodies of the expression of immunity in young children. *Parasitol., 94:* 281-300.

BUTTERWORTH, A. E.; CAPRON, M.; CORDINGLEY, J. S.; DALTON, P. R.; DUNNE, D. W.; KARIUKI, H. C.; KIMANI, G.; KOECH, D.; MUGAMBI, M.; OUMA, J. H.; PRENTICE, M. A.; RICHARDSON, B. A.; SIONGOK, T. K.; STURROCK, R. F. & TAYLOR, D. W., 1985. Immunity after treatment of human schistosomiasis mansoni. II. Identification of resistant individuals, and analysis of their immune responses. *Trans. R. Soc. Trop. Med. Hyg., 79:* 393-408.

BUTTERWORTH, A. E.; DUNNE, D. W.; FULFORD, A.; CAPRON, A.; KOECH, D.; OUMA, J. H. & STURROCK, R. F., 1988a. Immunity in human schistosomiasis mansoni: crossreactive IgM and IgG2 anti-carbohydrate antibodies block the expression of immunity. *Biochimie, 70:* 1053-1063.

BUTTERWORTH, A. E.; FULFORD, A. J. C.; DUNNE, D. W.; OUMA, J. H. & STURROCK, R. F., 1988b. Longitudinal studies on human schistosomiasis. *Proc. Roy. Soc. London B, 321:* 495-511.

CAPRON, M.; BAZIN, H.; JOSEPH, M. & CAPRON, A., 1981. Evidence for IgE-dependent cytotoxicity by rat eosinophils. *J. Immunol., 126:* 1764-1768.

CAPRON, M.; SPEIGELBERG, H. L.; PRIN, L.; BENNICH, H.; BUTTERWORTH, A. E.; PIERCE, R. J.; OUAISSI, M. A. & CAPRON, A., 1984. Role of IgE receptors in effector function of human eosinophils. *J. Immunol., 132:* 462-468.

DUNNE, D. W. & BICKLE, Q. D., 1987. Identification and characterization of a polysaccharide-containing antigen from *Schistosoma mansoni* eggs which crossreacts with the surface of schistosomula. *Parasitol., 94:* 269-280.

DUNNE, D. W.; BICKLE, Q. D.; BUTTERWORTH, A. E. & RICHARDSON, B. A., 1987. The blocking of human antibody-dependent, eosinophil-mediated killing of *Schistosoma mansoni* schistosomula by monoclonal antibodies which crossreact with a polysaccharide-containing egg antigen. *Parasitol., 94:* 269-280.

DUNNE, D. W.; GRABOWSKA, A. M.; FULFORD, A. J. C.; BUTTERWORTH, A. E.; STURROCK, R. F.; KOECH, D. & OUMA, J. H., 1988. Human antibody responses to *Schistosoma mansoni;* the influence of different stages of the parasite life-cycle on the response to the schistosomulum. *Eur. J. Immunol., 18:* 123-131.

HAGAN, P.; BLUMENTHAL, U. J.; DUNN, D.; SIMPSON, A. J. G. & WILKINS, H. A., 1991. Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium. Nature (London), 349:* 243-245.

ISKANDER, R.; DAS, P. K. & AALBERSE, R. C., 1981. IgG4 antibodies in Egyptian patients with schistosomiasis. *Int. Arch. Allergy Appl. Immunol., 66:* 200-207.

JASSIM, A.; HASSAN, K. & CATTY, D., 1987. Antibody isotypes in human schistosomiasis mansoni. *Parasit. Immunol., 9:* 627-650.

KHALIFE, J.; CAPRON, M.; CAPRON, A.; GRZYCH, J-M.; BUTTERWORTH, A. E.; DUNNE, D. W. & OUMA, J., 1986. Immunity in human schistosomiasis. Regulation of protective immune mechanisms by IgM blocking antibodies. *J. Exp. Med., 164:* 1626-1640.

KHALIFE, J.; DUNNE, D. W.; RICHARDSON, B. A.; MAZZA, G.; THORNE, K. J. I.; CAPRON, A. & BUTTERWORTH, A. E., 1989. Functional role of human IgG subclasses in eosinophil-mediated killing of schistosomula of *Schistosoma mansoni. J. Immunol., 142:* 4422-4427.

KLOETZEL, K. S. & DA SILVA, J. R., 1967. Schistosomiasis mansoni acquired in adulthood: behaviour of egg counts and the intradermal test. *Am. J. Trop. Med. Hyg., 16:* 167-169.

MAZZA, G.; DUNNE, D. W. & BUTTERWORTH, A. E., 1990. Antibody isotype responses to the *Schistosoma mansoni* schistosomulum induced by different stages of the parasite life-cycle. *Parasite Immunol., 12:* 529-543.

STURROCK, R. F.; BENSTED-SMITH, R.; BUTTERWORTH, A. E.; DALTON, P. R.; KARIUKI, H. C.; KOECH, D.; MUGAMBI, M.; OUMA, J. H. & ARAP SIONGOK, T. K., 1987. Immunity after treatment of human schistosomiasis mansoni. III. Long-term effects of treatment and retreatment. *Trans. R. Soc. Trop. Med. Hyg., 81:* 303-314.

WARREN, K. S., 1973. Regulation of the prevalence of schistosomiasis in man: immunity or ecology? *J. Infect. Dis., 127:* 595-609.

WILKINS, H. A.; BLUMENTHAL, U. J.; HAGAN, P.; HAYNES, R. J. & TULLOCH, S., 1987. Resistance to reinfection after treatment of urinary schistosomiasis. *Trans. R. Soc. Trop. Med. Hyg., 81:* 29-35.

WOOLHOUSE, M. E. J.; TAYLOR, P.; MATANHIRE, D. & CHANDIWANA, S. K., 1991. Acquired immunity and epidemiology of *Schistosoma haematobium. Nature, 351:* 757-759.