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Immunity in human schistosomiasis

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Two considerations render important the study of immunity to schistosome infection in man. In the short term, chemotherapy is currently the preferred method of control: but the duration of the effectiveness of chemotherapy in limiting both transmission and morbidity, and the factors that determine this duration, are poorly understood. Reinfection occurs after treatment, but it is not apparent at the moment whether some individuals become reinfected because of a behavioural or genetic predisposition, or because they have failed to mount an appropriate immune response. It is important, therefore, to determine the extent and duration of immunity to reinfection after treatment among different individuals, and to understand the constraints that age, previous experience of infection or other factors may place upon its development: this may then help in the targetting of treatment or retreatment campaigns at appropriate sections of the community. In the longer term, however, the problem of drug-resistant strains of parasite is likely to emerge: and, although chemotherapy is useful at present, it would be short-sighted to stop looking for alternatives. The development of a vaccine is now a real possibility: but, in order to use such a vaccine most effectively, it is important to determine which human immune responses should be elicited or avoided during vaccination – that is, to understand the effector mechanisms of naturally-acquired human immunity, which may be different from those observed in the various experimental animal models.

The shape of age-specific prevalence and intensity curves of *Schistosoma mansoni* and *S. haematobium* infections in communities living in endemic areas, with a characteristic decline in infection in the older age groups, suggested to early workers that acquired immunity to superinfection might be acting in older individuals [1, 2]. This view was partially supported by anecdotal observations on groups of heavily-exposed adults [3], but was challenged in the early 1970s. It was argued that the decline in infection in older individuals could be attributable, not to acquired immunity, but rather to a spontaneous death of

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adult worms together with a reduced level of exposure to new infections [4, 5]. Two previous studies have suggested that this explanation might not be sufficient. In the first, Kloetzel and da Silva [6] had shown that the rise and decline of prevalence and intensity of infection showed a similar time course in adult immigrants to an endemic area and in individuals who had resided in an endemic area since childhood: that is, it was dependent on duration of exposure rather than on age as such. Since levels of water contact are strongly dependent on age [5], it seems unlikely that the age-independent changes observed in the adult immigrants were attributable to changes in exposure. In the second study, Bradley and McCullough carried out a longitudinal study on a cohort of children with *S. haematobium* infection, and concluded that the stability of ranking of egg excretion was attributable to the expression of immunity in some individuals [7, 8].

Neither of these studies was entirely conclusive, and the problems remain threefold.

1. In the continued presence of a primary infection, with adult worms dying at an unknown and possibly variable rate, it is difficult to estimate the levels of new infection that may be occurring, and hence the degree of immunity to these new infections.
2. It is difficult to determine the relative contributions of acquired immunity and reduced exposure to an observed reduction in new infections in older individuals.
3. Immunity in man – as in many of the experimental animal models – may well be incomplete, and yet still of considerable value in reducing both transmission and also disease, by reducing the numbers of individuals who bear heavy infections.

An approach to these problems adopted by Wilkins and colleagues [9, 10] was to compare age-specific intensities of *S. haematobium* infection in two communities, in one of which transmission was interrupted by application of molluscicides. In the molluscicide-treated area, the decline in intensity of infection over a three-year period indicated that adult worms were dying with a mean life-span of 3.4 years. This allowed the calculation, in the untreated area, of the numbers of eggs deposited by worms *newly* acquired over the same three year period. It was found that the acquisition of new infections by adults over 25 years old was one thousand fold less than that of 5 to 8 year old children, a difference that could not be attributed to a comparable one thousand fold reduction in water contact levels among the adults.

An alternative approach to all three problems is to measure intensities of reinfection after treatment of infected individuals, at the same time that levels of exposure to contaminated water are closely monitored. The obvious disadvantage of such a "treatment and reinfection" study – namely that treatment itself may modify the immune status of the patient – is outweighed by the fact that, since the starting worm burden is reduced to zero, it is possible to deter-

mine accurately low levels of new infections. In addition, since chemotherapy is a preferred form of control, it is clearly desirable to understand the extent and duration of any immunity to reinfection that may remain after treatment.

Parallel studies involving this approach have been carried out for *S. mansoni* in Kenya and for *S. haematobium* in The Gambia [11–16], while similar but less complete data have been reported by Katz for *S. mansoni* in Brazil [quoted in 23]. In each case, the conclusions have been identical, namely that there is evidence for an age-dependent acquired resistance to reinfection that can be clearly distinguished from changes in exposure.

In The Gambia, two studies have been carried out on intensities of reinfection following treatment for *S. haematobium* infections. In the first [15], a random sample of 40% of individuals within a community, comprising all age groups, were treated and were re-examined three months later to check the effects of treatment. Water contact observations were carried out during the subsequent transmission season, and intensities of reinfection were determined fourteen months after the initial treatment. Intensities of reinfection were tenfold lower among 10 to 14 year old individuals than among 5 to 9 year old children, and were 100 fold lower in females over the age of 15. In contrast, levels of exposure were only slightly less in the 10 to 14 year age group than in the 5 to 9 year group, and were only fivefold less in females over 15. In addition, when individuals were stratified according to levels of exposure, there was a clear and significant relationship between degree of exposure and intensity of reinfection within age groups: but, for each stratum of exposure, there was an additional and marked age-dependence of reinfection, with heavily-exposed 2 to 9 year old children showing intensities of infection 100 fold greater than comparably-exposed individuals of more than 15 years.

In the second study [16], treatment was administered to children of a limited age range of 8 to 13 years. Fifty children showed egg counts of less than one egg/10 ml urine three months later, and were included in the subsequent analysis. Blood samples were taken before the next transmission season, during which water contact observations were carried out, and egg counts were determined 15 months after treatment. As in the preceding study, incidence of reinfection was again related both to levels of exposure and to age. In addition, however, reinfection was also related to eosinophil levels, being significantly lower in individuals with high esosinophil counts, suggesting that eosinophils might be involved in resistance to reinfection. Antibodies mediating eosinophil-dependent killing of schistosomula were not detected in these children, although they were present in older individuals: the mechanism whereby eosinophils might be involved in the expression of resistance was therefore unclear.

Immunological studies by Colley and colleagues [25] on Egyptian patients treated for *S. mansoni* infections have also provided preliminary evidence for a role for the immune system in limiting reinfection after treatment. Following an extensive analysis of the regulation of lymphocyte proliferative responses to

schistosome antigens of individual patients from various areas before and after treatment [e.g. 26–30], Colley et al. have examined the incidence of reinfection following treatment of 18 patients over a 24 month period, in relation to lymphocyte responses to egg (SEA), adult (SWAP) and cercarial (CAP) antigen preparations [25]. Seven of the 18 patients became detectably reinfected over the 24 month period: these patients did not differ significantly from those who failed to become reinfected in pretreatment intensity of infection or location of their households in relation to water contact sites. However, they did differ in lymphocyte proliferative responses. Those patients who failed to become reinfected showed high responses to CAP, SWAP and SEA throughout the study period. At the time of the last blastogenesis assay *before* they became reinfected, the seven "susceptible" patients showed significantly lower responses to CAP and SEA, and somewhat lower responses to SWAP, than those who failed to become reinfected. The authors were careful not to claim a causal relationship between resistance to reinfection and lymphocyte proliferation: but, clearly, this is an exciting finding and requires further attention.

Similar studies on *S. mansoni* in Kenya have been in progress since 1980. The design of the first study [11] was to follow reinfection in a cohort of children, mainly aged 9 to 16 at the time of treatment, whose levels of contact with contaminated water bodies could be observed. This choice of a restricted age range was deliberate: since intensities of infection classically peak and start to decline during the second decade of life, it was considered that immunity might be present in the older children, but absent in the younger ones, while water contact levels would not vary markedly.

The community of Iietune, in an area of typical rural transmission of Machakos District in Kenya, was selected for this study. Following mapping of the area and registration of the individuals, observations were carried out over a one year period of prevalence and intensity of infection, snail numbers and infection rates in the waterbodies used by the community, and various aspects of water contact by all members of the community with such water [11]. Subsequently, 129 children from the community primary school were selected for treatment. Duplicate Kato preparations of each of three stool samples were examined before treatment, five weeks after treatment with oxamniquine, and thereafter at three-monthly intervals for 21 months. Venous blood samples were collected before treatment and at 5 weeks and 6, 12 and 18 months after treatment. Throughout the post-treatment period, water contact observations were made at monthly intervals at each site used by the community. Snails were collected from the same sites at fortnightly intervals and checked for infection.

During the six months following treatment in October 1981, there were high levels of transmission in the study area, and over this period most of the treated children did become reinfected. However, the geometric mean intensities of reinfection were tenfold lower than the pretreatment levels: and, thereafter, these levels stayed relatively constant over the remainder of the 21 month

follow-up. In addition, there was a marked heterogeneity in the levels of reinfection between different individuals. Twenty-two children showed high intensities of reinfection of over 100 eggs per gram by 6 months after treatment, and thereafter continued to show high egg counts. In another 70 children, in contrast, reinfection was either undetectable or present at only low levels (<32 epg) throughout the study period. Half of these lightly-reinfected children showed high observed levels of water contact, indicating that the relative lack of reinfection could not reasonably be attributed to a lack of exposure, and must instead reflect some form of resistance to reinfection [12]. Thus, in an initial analysis, it was possible to identify two extreme groups of children – a "susceptible" group, showing high intensities of reinfection, and a "resistant" group, showing low intensities of reinfection in spite of high levels of observed water contact – and to show that these two groups did not differ in mean pretreatment intensities of infection, but did differ in age, the resistant children being on average 2.0 years older, within a restricted starting age range. This indicated that the observed resistance was an acquired and age-dependent event, and the most reasonable interpretation was that the older children had developed a degree of immunity [12, 13].

This finding was subsequently confirmed by Kendall's rank correlation analyses performed on the study group as a whole (Bensted-Smith et al., unpublished results). Both pretreatment and post-treatment intensities of infection were correlated with duration of water contact at certain sites, these sites consistently containing infected snails. Contact at other sites showed either no correlation with infection, or a negative correlation, this reflecting a negative correlation between the levels of contact at the different sites. In addition, levels of water contact in this particular age-group of children were *not* strongly correlated with age. In contrast, pretreatment intensities of infection were positively correlated with age, while post-treatment intensities of reinfection showed a highly significant negative correlation with age. These findings confirm that observations of water contact do provide an indicator of exposure to infection, and that the strong age dependence of reinfection cannot be attributed to age-dependent changes in exposure. In a separate analysis of the relationship between pre- and post-treatment intensities of infection, Bensted-Smith et al. [14] have found evidence for an individual predisposition to reinfection (possibly attributable to either exposure or other factors, including genetic). However, this effect is much stronger among the *younger* children, being considerably reduced among older individuals as the age-dependent acquired resistance becomes more marked.

The contribution of various immune responses to the expression of the age-dependent resistance in this group of 129 children is described below. In the meantime, further evidence was obtained that indicated that the age-dependent effect observed in these children reflected a narrow window of a broader age-dependent acquisition of resistance to reinfection that occurred from childhood

throughout adult life. In October 1983, after two years of follow-up of the treated children, treatment was offered to all infected residents in the Iietune community. Treated individuals were re-examined five and nine weeks after treatment. During the subsequent nine months, transmission continued in the community because of contamination of the waterbodies communally used by people outside the area: and, by August 1984, a high incidence of infection was observed both among infected and treated individuals and among previously-uninfected individuals [13]. However, there was a marked difference in the age dependence of reinfection between the two groups. Among the younger children, aged 0 to 9 years, incidence of infection was higher among those who had previously been infected than among those who had not: and this was reflected by higher levels of water contact among the previously-infected children. After the age of 9, however, the pattern was reversed. Previously-uninfected individuals (or individuals who had spontaneously lost their infections) showed an even incidence of new infection throughout adult life. In contrast, previously-infected and treated individuals showed a progressive decline with age in the incidence of reinfection. This difference in incidence between the two groups could not be attributed to a difference in exposure, since water contact levels remained higher for each age group among the previously-infected individuals. Therefore, it could be concluded that the previously-infected and treated individuals were showing an age-dependent resistance to reinfection that was dependent on recent experience of infection, since it was not observed in those who were not detectably infected in August 1983, immediately before the treatment campaign.

In summary, then, the data both from the selected schoolchildren and from the whole community provided evidence for an age-dependent resistance to reinfection that could not be attributed to changes in exposure, and that was dependent on recent experience of infection.

In an attempt to identify the immunological basis for the observed age-dependent resistance to reinfection, various immune responses were measured in blood samples taken from the study group of 129 schoolchildren before treatment and over an 18 month period after treatment [11, 12, 17–19]. Variables that were initially assayed included, among others; peripheral blood eosinophil counts; the levels of heat-stable (IgG) antibodies mediating eosinophil-dependent killing of schistosomula: IgE anti-schistosomular antibodies: the levels of antibodies that recognise a major 38Kd schistosomulum surface antigen, as judged by the inhibition of binding of two rat monoclonal antibodies [20]: and the levels of anti-adult worm and anti-egg antibodies.

The levels of IgG antibodies mediating eosinophil-dependent killing of schistosomula were consistently correlated both with the levels of anti-adult and anti-egg antibodies, and also with the inhibition of binding of the rat monoclonal antibodies, whereas there were no such associations for the IgE response. In addition, the levels of antibodies inhibiting the binding of the two rat monoclonal antibodies (and therefore recognising the 38Kd schistosomulum surface

antigen) were correlated with the anti-egg responses, but *not* with the anti-adult responses. These findings suggested that antibodies elicited in response to egg antigens cross-react with certain schistosomulum surface antigens, those antigens also being recognised by antibodies mediating eosinophil-dependent killing of schistosomula. In support of this interpretation, it has been found that both rat monoclonal antibodies, which recognise carbohydrate epitopes on the 38Kd molecule [21], also recognise miracidial antigens (Dissous and Capron, personal communication). In addition, a murine IgM monoclonal antibody that recognises the same 38Kd molecule, and that markedly inhibits the eosinophil-dependent killing of schistosomula mediated by pooled human infection sera, also recognises egg antigens, and in particular a major egg polysaccharide, K3 [24]. The significance of these findings is discussed below.

When the various immune responses assayed in the early blood samples were analysed with respect to *subsequent* reinfection intensities, no significant negative correlations were detected: that is, it was not possible to say that a high value for a particular response was associated with a low subsequent intensity of reinfection and was therefore an indicator of resistance. This is in contrast to an earlier pilot study in Kenya [22], and to the results of Hagan et al. in The Gambia [16], in which high eosinophil counts were associated with low subsequent reinfection. However, it was noted that there was a strong *positive* correlation between anti-egg antibodies, including antibodies that recognise the epitope that is seen by one of the two rat monoclonal antibodies, and subsequent reinfection [19]. These correlations were not solely attributable to age-dependent changes, since they remained significant after standardisation of the data for age.

The hypothesis that is most consistent with these results is that antigens released from eggs during early infections, including the major egg polysaccharide(s), elicit the formation of antibodies that cross-react with schistosomulum surface glycoproteins, and thereby block the binding of antibodies, directed against the same or different epitopes on the same surface molecules, that mediate effector functions such as eosinophil-dependent killing of schistosomula. These "blocking" antibodies may therefore prevent the expression of immunity, a phenomenon clearly established in the rat model [20]. The blocking antibodies might include IgM and possibly ineffective IgG isotypes, such as IgG4. This hypothesis is supported experimentally by various further observations.

1. Following fractionation of individual human infection sera by *Staphylococcus aureus* protein A absorption, it is found that fractions containing IgM not only fail to mediate eosinophil killing of schistosomula, but also block the killing that is mediated by IgG fractions from the same sera [17, 18]. These IgM-enriched fractions recognise both schistosomulum surface and also egg antigens.

2. Further analysis of the sera from the 129 schoolchildren subsequently revealed a positive correlation between the intensities of reinfection after treatment and the levels of IgM antibodies with specificity for the major 38Kd schistosomulum surface antigen, as assayed in an IgM capture assay [18].
3. Further analysis of the anti-egg antibody responses confirm a positive correlation with intensity of reinfection for both IgM and IgG classes. In addition, preliminary assays for antibodies against the major K3 polysaccharide, as detected in an antigen capture assay with a murine anti-K3 monoclonal antibody, have revealed a positive correlation between intensities of reinfection and both IgM and IgG anti-K3 antibodies in the pretreatment blood sample (Dunne et al., unpublished observations).

These findings not only support the hypothesis that blocking antibodies may prevent the expression of immunity, but also suggest that such antibodies may be of certain IgG as well as IgM isotypes, and may be elicited in response to egg polysaccharide antigens, subsequently cross-reacting with carbohydrate epitopes on schistosomulum surface glycoproteins.

These results are consistent with, but do not prove, the following hypothesis. During early trickle infections of young children, the major immunogenic stimuli are antigens released from eggs, which are present in much greater mass than either young larvae or adult worms. These antigens, including polysaccharides or heavily glycosylated glycoproteins, elicit predominantly IgM responses, or possibly certain inappropriate isotypes of IgG; and the antibodies formed cross-react with major glycoproteins on the schistosomulum surface. At the same time, the child mounts potentially-protective IgG responses (such as IgG1) against the same glycoproteins: these may be directed either against the same epitopes as the blocking antibodies, or against different epitopes on the same molecule, their binding then being blocked sterically. During early infections, the balance of the response is towards the blocking antibodies: and, although potentially-protective antibodies can be detected, the child is not immune. Subsequently, as the child ages, the balance may switch from a predominantly blocking to a predominantly protective type of response, and the child may then express his capacity to resist reinfection.

More work is required to test further this hypothesis, and we are currently investigating reinfection after treatment, and associated immunological responses, in a further group of patients of wider age range, of 2 to 66 years. In principle, however, the hypothesis would explain a previously puzzling feature, namely the extremely *slow* development of immunity, over a period of some ten years, in the face of a range of potentially-protective responses. In addition, these findings may be considered to bode well for the eventual development of a useful vaccine, in that the main requirement would simply be to vaccinate *early,* with a recombinant or other antigen, before the child is exposed to natural infection, and therefore before he develops a heavy egg load and high levels of blocking antibodies elicited in response to egg antigens.

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