

## THE SUSCEPTIBILITY OF ADULT SCHISTOSOMES TO IMMUNE ATTRITION

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*Mouse infection models are described that demonstrate reduction of egg production in Schistosoma haematobium infections and both worm loss and reduced fecundity in S. bovis infections. Neither phenomenon could be shown in S. mansoni infected mice. The immunological basis for these anti-adult responses was inferred by comparison with infections in T-cell deprived mice and by serum transfer of the ability to reduce a S. bovis worm burden into immunocompromised hosts. Vaccination with irradiation attenuated parasites was also shown to have consequences for the adults of a challenge infection of S. haematobium and S. bovis specifically. Prior vaccination resulted in an abrogation of the anti-fecundity and adult worm elimination that occurred in non-vaccinated similarly infected mice. These models are being used to define the targets and mechanisms involved in anti-adult attrition.*

*A serological assay, quantitation of a circulating antigen (CAA) has been assessed for its ability to measure worm burdens of different species of schistosome in mice. This assay will be used to question whether anti-adult immunity contributes to the pattern of infection with S. mansoni and S. haematobium in man.*

**Key words:** *Schistosoma haematobium* – *Schistosoma bovis* – *Schistosoma mansoni* – immunity – mouse – adult – larva – GST28 – circulating antigen – worm fecundity

Schistosomes do not replicate within their definitive hosts and intensity of infection in man is estimated by the number of parasite eggs excreted. As measured by egg excretion, endemic populations show an accumulation of parasites during childhood followed by a slow decline in intensity of infection with age. This pattern has been attributed to the induction of 'concomitant immunity' (Smithers & Terry, 1969). This describes a situation in which the established adult worms elicit an immune response which prevents continuous accumulation of worms while themselves remaining invulnerable to immunological attack. This hypothesis has been broadly confirmed by many investigations showing both relative adult worm refractoriness and larval stage susceptibility to immune attack.

However, this description of schistosome immunity is clearly an over-simplification since

considerable loss of worms occurs in man even in the period of net acquisition during the first decade of life (Wilkins & Scott, 1978). Wilkins argued that worm burdens are in a dynamic state and that 'concomitant immunity' – expressed as the decline in prevalence and intensity in later life, represents a balance between acquisition and loss of worms that is mediated by the immune response.

Despite general acceptance of this concept, adult schistosomes are not generally considered to be targets of an effective immune response. To what extent adult schistosomes are invulnerable, is a question that has not been asked, perhaps because the dominance of *Schistosoma mansoni* research has masked evidence that other species of schistosome exhibit considerable adult vulnerability to immune attack.

The natural host parasite relationships of *S. bovis* and *S. mattheei* present strong evidence that some schistosomes may suffer more effective immune regulation of the adult para-

site. Rapid reduction in established worm burden and progressive suppression of worm fecundity has been recorded during bovine *S. bovis* and *S. mattheei* infections (Bushara et al., 1980; Saad et al., 1980; Lawrence, 1973). Specific immunity has been implicated by serum transfer of reduced worm fecundity into recently infected cattle and by the recovery of egg production by worms transferred into naive animals (Bushara et al., 1983).

The patterns of infection in man may also reflect a species difference in this respect. *S. haematobium* infections reach a maximum at an earlier age and decrease in prevalence and intensity much more rapidly than *S. mansoni* and *S. japonicum* infections. This has been observed consistently between different geographical regions and cultures (*S. mansoni*: Arap Siongok et al., 1979; Smith et al., 1979; Abdel Wahab et al., 1980; *S. japonicum*: Pesignan et al., 1958; *S. haematobium*: Wilkins et al., 1984a, b; Chuks Ejezie & Ade Serrano, 1981; King et al., 1982; Audibert et al., 1983).

This difference cannot be attributed to sociological factors alone, since in areas where both *S. mansoni* and *S. haematobium* are transmitted in the same community, the two species still present distinct age prevalence and intensity patterns (Hairston, 1965; White, et al., 1982; Dennis, et al., 1983). The observation may be explained if increased resistance to superinfection, decreased lifespan of adult worms, reduced egg production or some combination thereof are a consequence of the immune status of the host and are stimulated more effectively by infection with *S. haematobium* than with *S. mansoni*.

Published experiments in permissive hosts support a difference between *S. mansoni* and *haematobium* group schistosomes in the susceptibility of their adult stages to immune attrition. Thus, in the baboon, adult *S. haematobium* suffer reduced fecundity in long term infections to a greater extent than do *S. mansoni* worms (Damian et al., 1976; Webbe et al., 1976). Moreover, unlike *S. mansoni* (Damian et al., 1986), this reduced egg production can be reversed by transfer into naive hosts (Webbe et al., 1976).

Comparison of *S. bovis* and *S. haematobium* with *S. mansoni* in the mouse has produced further evidence that *haematobium* group schistosomes are more susceptible to immune attri-

tion of their adult stages. We have previously shown that in 15 week *S. haematobium*-infected mice, egg numbers are reduced by as much as 60% in normal mice relative to T-cell deprived mice (Agnew et al., 1989a, b). *S. bovis* infected normal mice have reduced worm burdens after 10 weeks of infection relative to T-cell deprived mice (Murare & Doenhoff, 1987). Neither phenomena have been recorded in similar experiments infected with *S. mansoni* (Doenhoff et al., 1978).

In this study we present further evidence for an important role for anti-adult immunity in the host-parasite relationships of *S. haematobium* and *S. bovis* in mice. We also describe a study that is attempting to assess the contribution of anti-adult immunity to the pattern of infection in human schistosomiasis *mansoni* and *haematobium*.

#### IMMUNOLOGICALLY INTACT MICE CAN MOUNT EFFECTIVE ANTI-ADULT RESPONSES BUT IMMUNE-DEPRIVED MICE CANNOT

CBA mice were T-cell depleted by adult thymectomy and treatment with anti-thymocyte antibody. Comparison of different schistosome infections showed reduced tissue egg loads in *S. haematobium* and *S. bovis* and reduced worm burdens in *S. bovis* infections in normal relative to T-cell deprived mice. No significant differences were observed in either worm or egg burden in *S. mansoni* infections.

In a time course of infection with *S. haematobium* in normal compared to T-cell deprived mice, worm numbers remained constant in both groups for 20 weeks after infection. The mean number of eggs accumulating in the tissues in the two groups remained similar until 16 weeks post infection but by 20 weeks was 44% lower in immunologically intact mice.

Similar experiments with *S. bovis* infections in mice showed that over 14 weeks of infection the reduction in worm burden that occurred in intact mice was completely alleviated by T-cell deprivation. Worm numbers remained constant in both groups between 7 and 9 weeks after infection in two experiments with different intensities of infection. Both experiments showed a significant reduction in worm burden by 10 weeks in normal mice. At 14 weeks normal mice infected with high worm burdens showed a 75% loss of worms and the



less infected mice a 62% loss relative to comparably infected T-cell deprived mice. Worm loss occurred at a rapid rate shortly after the onset of oviposition but within 2 weeks this rate decreased and in other experiments a residual worm burden has been shown to persist for many months. This pattern suggests that the effective immune mechanism expressed at 9.5 weeks is down regulated by 14 weeks, allowing the survival of the remaining worm population.

#### SERUM TRANSFER OF ANTI-ADULT IMMUNITY

This interpretation is supported by the results of a serum transfer experiment. 71% of the worm loss induced in *S. bovis* infected normal mice can be reconstituted by acute infection serum (AIS) transfer into T-deprived mice. AIS was donated by *S. bovis* infected mice at a time when they would have been eliminating worms (9-12 weeks p.i.). Transfer of *S. bovis* chronic infection serum (CIS) did not induce worm loss in T-cell deprived mice. CIS was donated by mice that had undergone substantial worm loss in the previous 6 weeks of infection but were then carrying residual worm burdens (16 weeks p.i.). This result implies relatively rapid changes in the expression of anti-adult immunity and the following experiments also indicate that this response can be effectively switched or down regulated.

#### VACCINATION ABROGATES ANTI-ADULT IMMUNITY IN CHALLENGE INFECTIONS

The demonstration of specific anti-larval cross protection between *S. bovis* and *S. haematobium* using the irradiated vaccine model (Agnew et al., 1989a, b) raised the question of antigenic homology in the targets of the anti-adult response and the relationship between anti-larval and anti-adult immunity. In the first experiment, vaccination with 20 krad irradiated *S. haematobium* produced a reduction of 53% ( $P < 0.001$ ) relative to non-vaccinated *S. haematobium* challenged mice. A low level of protection (15%  $P < 0.05$ ) was also seen in vaccinated mice challenged with *S. bovis*. In agreement with previous experiments, *S. haematobium* did not protect against *S. mansoni*.

The number of challenging cercariae and the interval between challenge and perfusion was altered for each species to produce similar

worm and egg burdens at the termination of the experiment. Surprisingly, the effect of vaccination on the adults of the challenge infection was to abrogate the immune-mediated control of egg production seen in *S. haematobium* and *S. bovis* infections in naive mice. Thus 48% ( $p < 0.05$ ) more eggs per worm pair were deposited in the tissues of vaccinated and *S. haematobium* challenged mice compared to challenge control mice. Significantly more eggs (23%  $P < 0.02$ ) were also present in *S. bovis* challenged mice after vaccination compared to the control group but vaccination with *S. haematobium* had no effect on the fecundity of adult *S. mansoni* parasites.

A second experiment was designed to repeat the latter and extend it to examine the relationship between fecundity and worm survival in *S. bovis* infections. *S. haematobium* was again used as the vaccinating species because it does not normally induce anti-larval immunity against *S. bovis* challenge so that we could examine the effect on the adult population without consideration of density dependent effects. However, the *S. haematobium* vaccinated mice showed unusually high levels of anti-larval immunity to homologous challenge (80%  $P < 0.0001$ ) and the first clear demonstration of reciprocal cross immunity to *S. bovis* (55%  $P < 0.006$ ). Vaccination with *S. haematobium* again did not protect against challenge infection with *S. mansoni*.

The effect of vaccination on the egg production of the mature challenge infection confirmed the first experiment. *S. haematobium* vaccination resulted in 44% ( $P < 0.05$ ) more eggs per worm pair in the tissues of *S. haematobium* challenged mice and again did not affect the fecundity of *S. mansoni* adults. The numbers of eggs per worm pair in *S. bovis* challenge control mice was very variable due perhaps to individual differences in the rate of worm elimination. Thus total eggs per worm pair did not differ significantly between vaccinated and control groups but the rates of egg accumulation in the two groups showed the same trend. Thus between weeks 7 and 9 and weeks 9 and 11 the rates were similar but between weeks 11 and 12 the challenge control group showed an increase in egg burden per worm pair that was only 22% of that in the vaccinated group.

The effect of vaccination on *S. bovis* adult worm survival was much clearer. The chal-

challenge control mice had lost 34% (n.s.) of their worm burden between weeks 9 and 11 and 54% ( $P < 0.01$ ) after 12 weeks of infection. In contrast, the vaccinated group showed a 12% reduction that was not statistically significant between weeks 11 and 12. Thus the loss of worms that occurred in primary infections with *S. bovis* was abrogated or delayed by prior vaccination with *S. haematobium*.

#### NON-IMMUNOLOGICAL FACTORS

We considered the possibility that non-immunological factors, such as worm load or sex ratio, were contributing to the regulation of fecundity and worm burden. Although the size of the primary worm burden does affect the rate of worm loss, the abrogation of worm loss by vaccination cannot be attributed to differences in worm load alone because substantial worm death occurred by 10 weeks p.i. in naive mice infected with equivalent worm burdens. A review of other experiments in which *S. bovis* infected normal mice were compared with T-cell deprived mice confirmed that significant worm loss always occurred by 11 weeks post infection in normal mice irrespective of worm load.

Similarly, in previous mouse infections with *S. haematobium*, differences in worm burden of the order seen in the vaccination experiments did not affect worm fecundity. Differences in adult worm sex ratio are known to affect egg production in schistosomes (Harrison et al., 1982) but the sex ratios of the compared groups were not significantly different in any of these experiments. Thus these studies present clear evidence that *S. haematobium* and *S. bovis* but not *S. mansoni* adults are susceptible to immune mediated attrition of the established adult infection in mice.

#### ANTI-LARVAL AND ANTI-ADULT RESPONSES CONSTITUTE DISCRETE PHASES OF IMMUNITY

Vaccination with irradiated cercariae induced partial resistance to infection with challenge larval stages of *S. bovis*. The worms that escaped this attrition became established and were not affected by the immune response that develops after the onset of oviposition in naive mice given primary infections. This abrogation of anti-adult immunity, as a consequence of stimulating effective anti-larval immunity, suggests that these constitute two distinct phases of immunity mediated by discrete mechanisms and/or targets.

#### THE EFFECT OF IMMUNISATION WITH GLUTATHIONE -S -TRANSFERASE 28 ON THE EXPRESSION OF ANTI-ADULT IMMUNITY

The evidence that immunisation with GST28 could affect the egg production of a challenge infection (Boulanger et al., 1991) suggested that immune responses to this molecule could be responsible for adult worm loss in *S. bovis* infections.

Immunisation with purified *S. bovis* GST28 induced 40% ( $P < 0.03$ ) protection against challenge with *S. bovis* but no further worm loss occurred between weeks 9 and 11 after infection. Treatment of mice with cyclophosphamide, three days after immunisation with GST28 substantially reduced their ability to respond to GST28 after challenge infection and prevented the expression of anti-larval immunity. However, anti-adult immunity was unaffected (52% worm loss  $P < 0.02$ ) suggesting that responsiveness to GST28 is not wholly responsible for worm loss in this model.

This abrogation of adult worm loss associated with successful anti-larval vaccination with GST28 supports the idea that these are discrete phases of immunity mediated by different mechanisms and/or targets. A similar result has been reported recently by Boulanger. Two groups of baboons immunised with recombinant Sm28GST using different immunisation protocols showed that when anti-larval immunity was induced, no anti-fecundity was observed and *vice versa* (Boulanger et al., 1991).

#### IS ANTI-ADULT IMMUNITY AN IMPORTANT COMPONENT OF IMMUNITY IN MAN?

*Schistosoma haematobium* infections in man reach a maximum at an earlier age and decrease in prevalence and intensity much more rapidly than *S. mansoni* and *S. japonicum* infections. Measurement of worm burden independently of egg excretion would allow estimation of the contributions of reduced worm burden and reduced egg production to the distinct age prevalence and intensity patterns of these species.

A diagnostic tool was required that estimated worm numbers non-invasively. We chose quantitation of a proteoglycan circulating anodic antigen (CAA) which correlated well with worm numbers in *S. mansoni* infections in mice



(Deelder et al., 1980), with *S. mansoni* egg excretion levels in man (De Jonge et al., 1988), and is probably released only by living worms (De Jonge et al., 1989).

#### ASSESSMENT OF THE DIAGNOSTIC ASSAY

CAA was determined by a sandwich enzyme-linked immunosorbant assay (ELISA) using a mouse monoclonal antibody 120-1B10-A (IgG1) (Deelder et al., 1989). This monoclonal recognises a repeating epitope of CAA that was known to be genus common but model infections in mice were required to establish that the correlation between this circulating antigen and worm numbers was constant for other schistosome species.

CAA production by both *S. mansoni* and *S. haematobium* worms increases during maturation of the worms and reaches a plateau after the worms have become egg producing adults. CAA measurement correlated well with worm burden in mature infections of both *S. mansoni* and *S. haematobium* but the amount of CAA produced by *S. haematobium* worms is much less than that produced by *S. mansoni*.

Current studies are examining the relationship between CAA, worm burden and egg output in *S. haematobium* and *S. mansoni* infections in groups of mice with differing immune status. Normal and T-cell deprived mice infected with the same number of *S. mansoni* worms had similar levels of CAA. No differences in worm or egg burden have been observed in this type of experiment and this has excluded the possibility of a difference in the production and persistence of CAA due to T-cell deprivation alone. Further experiments that are not yet complete will establish whether CAA measurement will also correlate with worm burdens in *S. haematobium*-infected mice even when these worms are suffering a level of attrition that results in reduced egg production.

If the correlation remains constant under these conditions, quantitation of CAA will be used to examine the relationship between worm burden and egg output in schistosome infections in man. A comparative study of CAA measurements across an age profile of *S. haematobium* and *S. mansoni* is being used to assess the relative contributions of worm loss and reduced fecundity in the natural host-parasite relationship of these parasites. Differences

between these infections, in the relationship between CAA levels and eggs excreted in different age groups, will help to evaluate the evidence from animal models that *S. haematobium* is more susceptible to immune mediated anti-fecundity effects. For this purpose, a single time point collection of serum and good egg excretion data has been collected for 300 patients from discrete *S. haematobium* and *S. mansoni* endemic areas in Kenya.

#### CONCLUSIONS

These studies are addressing a basic question about the nature of the host-parasite relationship in different species of schistosome. While it is evident that immunological control of adult worm numbers and fecundity occurs in the natural hosts of *S. bovis* and *S. mattheei*, the role of anti-adult immunity to schistosomes that infect man has not been assessed. This clear demonstration of adult vulnerability should help to stimulate interest in the adult parasite as a potential target of vaccine induced immunity.

An understanding of the mechanisms of this anti-adult response will be important in the development of such vaccines. Protective immunity in man may also be a product of the two discrete phases of immunity seen in the mouse model. The importance of interaction between different immune effectors has been shown clearly by the role of blocking antibody in preventing the expression of protective immunity in man (Butterworth et al., 1987). The suggestion that the effectors of anti-larval and anti-adult immunity may be co-regulatory has major implications for the design of vaccines, especially when these will be deployed in endemic populations. Understanding these interactions could be critical to the design and deployment of vaccines in both human and veterinary schistosomiasis.

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