

ANALYSIS OF ANTIBODY RESPONSES OF *SCHISTOSOMA MANSONI* INFECTED PATIENTS AGAINST SCHISTOSOMAL ANTIGENS

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Identification of major schistosome antigens recognized by patients from endemic areas have long been a goal in the studies of the immune response to the schistosome infection. The objectives are several, including identification of major antigens to be used in immunodiagnosis (Mott & Dixon, 1982; Mott, 1984; Tsang et al., 1984; Ruppel et al., 1985; Ruppel et al., 1987) and vaccines (Horowitz et al., 1982; Smit & Clegg, 1985; James, 1984; James et al., 1985). Another area of major interest in which identification of specific schistosome antigens may be important is the understanding of the etiology of development of severe form of schistosomiasis. In this area our laboratory has been investigating the antibody responses of *Schistosoma mansoni* infected patients to schistosome derived antigens. An initial survey of the responses of infected patients to soluble adult worm antigens (SWAP) by "western" immunoblot showed that the response developed by these patients increased with the number of eggs in the stool indicating a relationship between egg counts (intensity of infection) and recognition of schistosome antigens. Furthermore, analysis of antibody responses of patients classified by the clinical form presented at the time of stool examination, showed that there was a marked difference in reactivity to SWAP between patients of the intestinal (I) form of and those with the hepatosplenic (HS) form of schistosomiasis. When patients from *S. mansoni* endemic areas carrying other infections such as hookworm, *Ascaris lumbricoides*, and others were tested for their response to SWAP, we observed that, as previously described (Mott & Dixon, 1982; Mott, 1984), patients carrying either hookworm or *A. lumbricoides* showed a high level of cross-reactivity to schistosome antigens. However, in our hands this cross-reactivity was dependent on the source of the antigen used. While sera from patients carrying hookworm infections showed cross reactivity to

SWAP, whole schistosomula, schistosomula derived vesicles (Kusel et al., 1984) as well as lethal antibody activity to living schistosomula, antibodies from patients infected with *A. lumbricoides* showed cross-reactivity only to SWAP. These results are important since besides showing cross-reactivity between parasites commonly found infecting man concomitantly with *S. mansoni*, they also show a possible biological activity of these cross-reactivity, mainly due to the observation that sera from patients infected with hookworm can mediated complement killing of schistosomula.

Due to the possible influence of these cross-reactive antibodies in the analysis of the anti-schistosome specific antibody responses, all patients in the studies described below had only *S. mansoni* infection at the time of blood collection. Comparison of anti-SWAP antibody responses of patients developing the less severe form of schistosomiasis (intestinal -I-) and the most severe form (hepatosplenic-HS-) showed that in both groups there was an extremely high level of heterogeneity in their response but in the first group there in a major antigen of approximately 31kDa that is recognized by 82% of intestinal patients and by only 13% of HS individuals. On the other hand, sera from all HS patients recognize antigens of MW of 14 kDa and 66 kDa. The former antigen is also present in 25% of I patients and the latter is completely absent when sera from I patients are analysed. Although differences were observed between both groups of patients, it is important to say that in these studies all individuals of both groups (I and HS) were of ages of 16 and up. The reason for this choice came from our experiments where the antibody responses to SWAP of *S. mansoni* infected children were analysed. In the I group of children (ages 0-10) we observed an extremely low level of reactivity to SWAP as determined by "Western" im-

munoblot, that increased with age. It is important to emphasize that the response of these I children when analysed together as a group of ages from 0 to 10, did not show any clear difference from the adult I group. However, when these children were grouped by the age, i. e. only 6 or only 7 year old children in the same group, major differences in reactivity were observed when compared to the adult group. These differences due to the age were obvious. There was an increase in reactivity as age increased and only those children of ages over 10 years started to show a similar pattern presented by the adult group of I patients. Coincidentally with the adult I group, children of this clinical form also reacted strongly to the 31 band of adult worm. The reactivity to this antigens was present in all age group tested. In contrast to the differential reactivity presented by I children and adults to SWAP, no major differences due to age were observed between the HS groups of children and adults.

To further investigate the differences in reactivity to SWAP antigens due to the age of the patient and clinical form, and to investigate whether the lack of reactivity also occurred in children from non-endemic areas that were not of the HS group, we studied the responses of children during the acute phase of schistosomiasis. In contrast with the results obtained when intestinal children were tested for their antibody response to SWAP, children with the acute form of schistosomiasis were able to recognize a wide range of antigens that did not show any major increase in number of antigens recognized with age. These results together with those presented above, clearly show that one can distinguish patients with the I and HS forms of the disease by analysing their response to SWAP using "western" immunoblot. Furthermore they show a relationship between the development of hepatosplenic and the detection of an antibody response to the 66 kDa antigen. Although all HS patients recognize the 14 kDa antigen the presence of this band alone does not suggest hepatosplenism. An important observation is that children from endemic areas with the intestinal form of the disease show a low level of reactivity to SWAP and that this reactivity increases with age. The lack of reactivity observed with sera of children from endemic areas may explain the high levels of infection presented by these patients, since their immune response to schistosome antigens is low, it is possible that

these patients can not reject a subsequent infection, increasing the parasitism and consequently the number of eggs eliminated in the stool. The explanation of why these children do not seem to develop immunity to secondary infection is important and deserves further investigation. Another factor that may be influencing the immune response of these children is their mother's immune response, since these patients are often born from infected mothers, it is possible that the antibodies from the mother that cross the placenta are able to interfere with the development of an effective anti-schistosome antibody response by the child. In addition, the lack of reactivity of some infected intestinal children impose a great problem for the development of an efficient immunodiagnostic procedure since in most instances these patients will fail to react to schistosome derived antigens.

The identification of antigens for immunodiagnosis of schistosomiasis are frequently directed only to the diagnosis of disease. However, there is also an important area of diagnosis that has been neglected by most investigators that is the identification of antigens to be used in the diagnosis of drug cure. In order to approach this problem, we have been studying the post-treatment antibody responses of drug treated patients to SWAP and soluble egg antigens (SEA). In these studies we used two assays, ELISA and "Western" immunoblot. The sera used in this study was obtained from patients that had been treated and the treatment efficacy assayed by stool examination up to one year after treatment. In the ELISA assays we could not detect any differences in pre and post-treatment responses to either SWAP or SEA. However, when "Western" immunoblot analysis of the post treatment response was used, we were able to detect a decrease in reactivity to SWAP but not SEA antigens as the patients were parasitologically considered cured by stool examination. These results are important since they show one of the first evidences of an assay that can detect drug cure serologically and correlates the serological findings with the stool examinations. Further studies in this area will allow the definition of antigens that disappear soon after treatment and that correlate with drug cure of schistosomiasis. In order to identify these antigens a larger number of serum samples from patients considered parasitologically cured

will be analysed together with those in which the drug treatment was not effective.

A detailed analysis of the antibody responses of *S. mansoni* infected patients although extremely complex and labor intensive has given us valuable informations concerning major questions often asked about development of immunity as well as the different form of schistosomiasis and the immune responses to defined antigens. We believe that by further investigating a larger number of serum samples from schistosome infected patients for their antibody response to the various schistosome antigens will help us in the identification of major schistosome antigens involved in the development of severe schistosomiasis as well as identification of antigens to be used in both pre and post-treatment diagnosis.

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