RESEARCH NOTE

Antigenic Recognition Pattern of Schistosomiasis Patients Bearing Different Parasite Burdens

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Direct parasitological methods detect schistosome eggs in stool or rectal mucosa. Immunological methods quantify the immune response to certain *Schistosoma mansoni* antigens or the concentration of parasite-derived antigens in serum. Whereas the specificity of parasitological methods usually comes close to 100%, the specificity of the immunological methods varies considerably.

In the chronic phase of disease, when there are few eggs in the feces, the infection is usually detected by immunological methods. However cross-reaction with other parasitic disease have been observed (NR Bergquist 1990 *Mem Inst Oswaldo Cruz 87*: 29-38, SML Montenegro 1992 *Mem Inst Oswaldo Cruz 87*: 333-335, H Feldemeir & G Poggensee 1993 *Acta Tropica 52*: 205-220). Thus, the use of chemically defined antigens and the molecular and immunological characterization of the parasite antigenic components are of utmost importance.

In the present communication we analyzed the antigenic recognition pattern of subjects bearing different parasite burdens, aiming to identify diagnostic antigens or markers of resistance.

S. mansoni adult worms (Belo Horizonte-BH and São Lourenço da Mata-SLM strains) were collected by perfusion of mice seven weeks after infection with 200 cercaria. The worms were separated according to sex and stored in liquid nitrogen. The life cycle stages of the parasites (egg, miracidium, cercariae, and schistosomulum) were obtained and stored as above. Male and female adult worm soluble antigen was obtained and the protein concentration determined by the method of OH Lowry et al. (1951 *J Biol Chem 193*: 263-275).

Patients infected with *S. mansoni* were from São Joaquim and Itapinassu-Tracunhaém, State of Pernambuco, Brazil. The patients were identified by two stool examinations (N Katz et al. 1972 *Rev Inst Med Trop São Paulo 14*: 397-400) and separated according to intensity of infections established by WHO (1993 Tech Report Series p. 830). Eight patients were selected: 4 with heavy infection (HI) and 4 with light infection (LI). Blood was obtained by venipuncture and serum was obtained and stored at -20ºC until use. These sera were analyzed by Western blot. For this, whole parasite homogenates (eggs, miracidia≅1,500, cercaria≅1,000, schistosomula≅1,400, male=1 and female=1 adult worms) as well as soluble adult worm antigens (male and female) of both strains (50 μg) were solubilized in sample buffer and run in polyacrylamide gel (10%) according to UK Laemmli (1970 *Nature 227*: 680-685). Proteins were transferred (H Towbin et al. 1979 *Proc Nath Acad Sci 76:* 4350-4354) from the gel to a nitrocellulose sheet (NTS) (pore size = 0.45 μm) overnight at 4ºC. The NTS was treated with individual sera from patients harbouring different parasite burdens. As control, individual sera of normal endemic and non-endemic subjects as well as subjects with other parasitic disease (amebiasis, toxoplasmosis, African and American trypanosomiasis, visceral and tegumental leishmaniasis and filariasis) were used. Immunerecognition was performed by using 1:100 serum dilution. Human immunoglobulins bound to parasite proteins on the NTS were detected with a 1:1000 diluted horseradish peroxidase-conjugated goat antibodies to human IgG (γ chain specific). Visualization of immune complexes was carried out with 0.04% diaminobenzidine (DAB) and H_2O_2 (2 μl/ml) in phosphate buffered saline (PBS).

Patients with HI or LI produced antibodies against several antigenic components. In this work we only analyzed the more reactives polypeptides. The antigenic pattern recognized by the patients with HI was similar for both BH ans SLM strains (data not shown). However we observed differences in the pattern related to different stages of the parasite. A polypeptide of 45 kDa was identified in egg, miracidium, cercariae and schistosomulum whole homogenate but was absent in male and female adult worms. The reaction was more evident with a 32 kDa antigen. A 29 kDa polipeptide was only recognized in male adult

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worms. Respecting the soluble worm antigens, polypeptides of 45, 40, 32, and 29 kDa were recognized in male worms while in female worms only a polypeptide of 32 kDa (Fig. 1). Fig. 1 is representative of the experiments with HI sera.

Patient sera with LI identified a polypeptide 105 kDa present in all evolutive stages studied of parasite. However, a 37 kDa polypeptide was recognized in the whole and soluble antigens of male and female. This polypeptide was identified by all patients while the 105 kDa polypeptide was identified by only one patients (Fig. 2). No reaction with the 32 and 37 kDa polypeptides was observed with other parasitic disease. We are currently evaluating these patterns as markers for resistance. Fig. 2 is representative of the experiments with LI sera.

Several groups have tryed to use biochemical defined antigens in the diagnosis of schistosomiasis mansoni. A cloned 37 kDa *S. mansoni* larval antigen has recently atracted interest due to its proposed use as marker for resistance. Subjects with apparent high resistance to schistosome infection exhibit significantly increased levels of antibodies against this antigen compared to susceptible ones (A Dessein et al. 1988 *J Immunol 140*: 2727-2736, V Goudot-Crozel et al. 1989 *J Exp Med 170*: 2065-2080). A 50 kDa glycoprotein (GP50), present in eggs and secretions of several evolutive stages of the parasite shows cross-reaction with others species of *S. mansoni* and *T. spiralis* (E Linder et al. 1992 *J Parasitol 78*: 488-507). A field trial using an assay based on the 31/32 kDa gut-associated antigen presented excellent agreement with positive stool examinations but as many as 75% of egg-negative subjects also tested positively (A Ruppel et al. 1990 *Trop Med Parasitol 41*: 127-130), indicating that further study is required.

In the present work the 32 kDa polypeptide recognized by HI patients may be associated to susceptibility. On the other hand the 37 kDa polypeptide is recognized by sera of patients harbouring LI, supporting the findings of Dessein et al. (*loc. cit.*), and is potentially a resistance marker. These are preliminary results, and more samples are necessary in order to withdraw consistent conclusions.

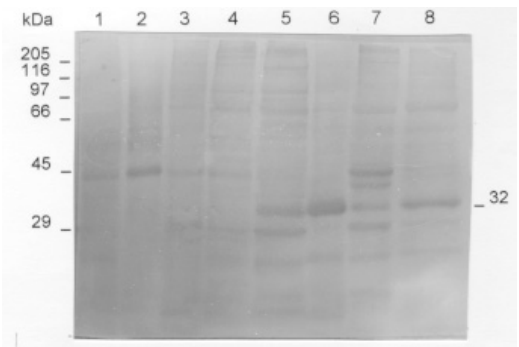

Fig. 1: antigenic pattern of life cycle stages of *Schistosoma mansoni* recognized by sera of patients with heavy infection. Whole homogenate: 1 → 6: 1 - egg; 2 - miracidium; 3 - cercariae; 4 - schistosomulum; 5 - male adult worm; 6 - female adult worm. Soluble antigen: 7 → 8: 7 - male adul worm; 8 - female adult worm.

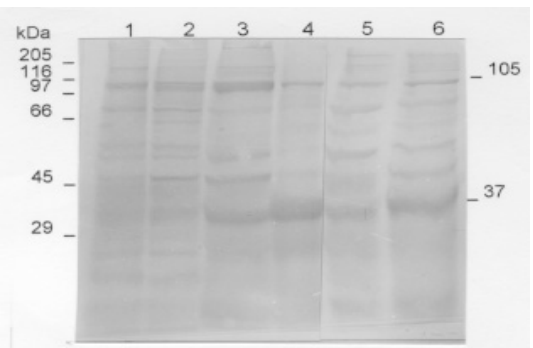

Fig. 2: antigenic pattern of life cycle stages of *Schistosoma mansoni* recognized by sera of patients with light infection. Whole homogenate: 1 → 4: 1 - cercariae; 2 - schistosomulum; 3 - male adult worm; 4 - female adult worm. Soluble antigen: 5 → 6: 5 - male adult worm; 6 - female adult worm.