SCHISTOSOMIASIS MANSONI: IMMUNOBLOT ANALYSIS TO DIAGNOSE AND DIFFERENTIATE RECENT AND CHRONIC INFECTION

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Abstract. One hundred seven patients classified into three different groups (11 with acute schistosomiasis, 58 with chronic schistosomiasis, and 38 children with high IgM-specific antibody titers against schistosome gut-associated antigens living in an endemic schistosomiasis area) were studied by immunoblotting for the presence of IgG, IgM, and IgA antibodies against Schistosoma mansoni soluble adult worm antigen preparation. We used sera from 15 individuals infected with various intestinal parasites, as well as sera from 19 uninfected individuals, as controls. An immunogenic fraction with a molecular weight of 31–32 kD (Sm31/32) was the most frequently recognized by the different antibody isotypes. In the group with acute disease, this fraction was recognized by IgG and IgM antibodies of all patients, and by 10 (90.9%) of 11 samples for IgA antibodies. Approximately 98% of the patients with chronic infections had IgG antibodies against Sm31/32, but only about 10% had IgM and IgA antibodies against this fraction. The IgG immunoblot profiles of the children from the endemic area were similar to those obtained for the group with acute schistosomiasis. This observation suggests recent infection of these children. Our data show that the Sm31/32 protein fraction is highly immunogenic and may be a useful serologic marker for diagnosing and differentiating between acute and chronic schistosomiasis infection.

The diagnosis of acute schistosomiasis has been based largely on clinical and epidemiologic data associated with laboratory findings. However, clinical symptoms observed in the early stages of infection with Schistosoma mansoni are not pathognomonic of acute schistosomiasis. Other diseases such as malaria, typhoid fever, and influenza produce symptoms similar to those observed in acute schistosomiasis and may delay diagnosis.

A simple serologic test capable of distinguishing acute from chronic schistosome infections would be valuable as a diagnostic tool. The use of such a tool could contribute to epidemiologic studies by defining geographic regions with active transmission. Several serologic tests for the differentiation between acute and chronic forms of schistosomiasis have been evaluated for clinical and epidemiologic purposes. IgA antibodies specific to S. mansoni gut antigens can be detected mainly during the acute phase of schistosomiasis. Therefore, detection of specific IgA antibodies can provide the basis for the development of serologic tests for the differential diagnosis of acute schistosomiasis.

We present the results of Western blot profile analysis of different serum samples that were previously studied by ELISA against an S. mansoni soluble worm antigen preparation (SWAP). The correlation between the Western blot patterns, clinical forms, and epidemiologic characteristics of the population was studied. Our data indicated that a 31–32 kD fraction of SWAP antigen (Sm31/32) can be valuable in the diagnosis of schistosomiasis mansoni, as well as in differentiating acute from chronic disease through the analysis of the anti-Sm31/32-specific antibody isotypes.

MATERIALS AND METHODS

Sera. A total of 141 serum samples were collected from individuals in five different groups.

Group A. This group was composed of 11 individuals living in nonendemic areas in the State of São Paulo, Brazil who acquired schistosomiasis while traveling to disease-endemic areas within Brazil. All of these patients were excreting S. mansoni eggs and presented clinical symptoms compatible with acute schistosomiasis 30–65 days after the exposure to infective cercaria. All had high levels of IgM and IgA antibodies against gut-associated antigens by an immunofluorescence test (IFT).

Group B. This group was composed of 58 patients with chronic schistosomiasis. All of them were excreting S. mansoni eggs at the time of the diagnosis, and all lived at one time in an area endemic for schistosomiasis. Patients in this group had never been treated for S. mansoni, and the IFT revealed IgM antibodies specific for adult worm gut antigens in 93.1% of these patients.

Group C. This group was composed of 38 school children from a schistosomiasis-endemic area in the State of São Paulo, Brazil. They were selected because of their high IgM reactivity against S. mansoni gut-antigens by the IFT. IgA antibodies specific to S. mansoni gut antigens were also present in 66% (25 of 38) of these children. Parasitologic confirmation through the detection of S. mansoni eggs in stool samples was possible in only 18 children. In the remaining 20 children, S. mansoni eggs were not detected despite the examination of 3 or more stool samples by two different parasitologic methods. These children had never received specific treatment for schistosomiasis and had no specific symptoms related to schistosomiasis on clinical examination.

Group D. This group was composed of 15 school children living in a nonendemic area for schistosomiasis in the State of São Paulo, Brazil. All individuals in this control group were infected with one or more intestinal parasites other than S. mansoni. Trichuris trichiura was detected in 87%, Ascaris lumbricoides in 80%, Strongyloides stercoralis in 13.3%, Endolimax nana and hookworm in 27%, and Entamoeba histolytica and Giardia lambia in 6.7%. At least 3 stool samples from each child were examined and no S. mansoni eggs...
Figure 1. Western blot patterns for specific IgG antibodies to *Schistosoma mansoni* worm antigen. Group A (patients with acute schistosomiasis), lanes 1–11; group B (patients with chronic schistosomiasis), lanes 12–69; group C (school children from an area endemic for schistosomiasis), lanes 70–107; group D (school children without schistosomiasis but with other intestinal parasitic infections), lanes 108–122; group E (healthy individuals), lanes 123–141. kDa = kilodaltons.
FIGURE 2. Western blot patterns for specific IgM antibodies to *Schistosoma mansoni* worm antigen. Group A, lanes 1–11; group B, lanes 12–69; group C, lanes 70–107; group D, lanes 108–122; group E, lanes 123–141. kDa = kilodaltons. For definitions of groups, see Figure 1.
were found. Children were also negative by IFT for IgM antibodies specific for *S. mansoni* gut-associated antigens.

**Group E.** This control group was composed of 19 healthy individuals without evidence of parasitic or other infectious disease, as indicated by both parasitologic and serologic evaluation.

This study was reviewed and approved by the Institutional Review Board at the Federal University of Espírito Santo. Informed consent for participation in the study was obtained from all patients or their guardians.

**Soluble worm antigen preparation.** Adult *S. mansoni* worms were recovered from hamsters after 6–7 weeks of

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**Table 1**

Frequency (%) of IgG, IgM, and IgA antibody responses against 12-, 24-, 31/32-, 52-, and 62-kD protein fractions of worm antigen of *Schistosoma mansoni* by western blot in patients sera of all groups*

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<thead>
<tr>
<th>Group</th>
<th>Ig isotype</th>
<th>Protein fractions</th>
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<td>12</td>
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<td>A</td>
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* Ig = Immunoglobulin; – = not detected.
and homogenized in 0.01 M phosphate-buffered saline (PBS), pH 7.2, containing a mixture of proteases inhibitors: 20 μg/ml of phenylmethylsulfonyl fluoride, N-D-p-tosyl-L-lysine chloromethyl ketone, antipain, aprotinin, and leupeptin (Sigma, St. Louis, MO). The worm suspension (1,500 worms in 1 ml of PBS) was sonicated repeatedly (8 times) at 40 kHz for 45 sec on ice, with a 30-sec interval between each step. The homogenate was then centrifuged at 10,000 × g for 45 min and the supernatant was collected and stored at −20°C. The protein concentration of the SWAP was determined according to the method of Lowry and others.6

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blotting. An aliquot of 100 μg of SWAP diluted in 30 μl of 0.5 M Tris buffer solution was applied to a 12.5% sodium dodecyl sulfate–polyacrylamide gel. After the electrophoresis, the separated protein fractions were transferred onto a nitrocellulose membrane as described. The membrane with the antigenic fractions was cut into 3-mm strips and blocked in a 5% skim milk PBS solution. Serum samples were diluted to 1/100 for the detection of IgG and IgM antibodies, and to 1/20 for detection of IgA. Strips were incubated overnight at room temperature, then washed thoroughly with PBS. Peroxidase-labeled goat anti-human antibodies specific to either IgG, or IgM, or IgA were added and incubated for 2 hr at room temperature, and the strips were washed as described earlier. Reactive bands were revealed after the incubation of the strips with a substrate solution containing 0.02% diaminobenzidine and 0.04% H2O2.

Immunofluorescence test. The IFT was performed as described elsewhere4,8,9 to detect IgA- and IgM-specific antibodies to the gut-associated antigens, respectively, in the 1/10 and 1/40 dilutions of the sera. Since a fluorescent reaction against worm tegument was observed in some patients without schistosomiasis, only serum samples with gut-specific IgM antibodies were considered positive for schistosomiasis.

RESULTS

As shown in Figures 1, 2, and 3, all patients with acute infections demonstrated an intense antibody reaction (IgG, IgM, and IgA, respectively) against proteins bands ranging from 30-kD to 32 kD, referred to here as Sm31/32. This protein fraction (Sm31/32) was recognized by 97% of the patients with schistosomiasis (A, B, and C groups). In group A, 100%, 100%, and 90.9% of the patients with acute disease had IgG, IgM, and IgA antibodies specific for Sm31/32, respectively. In contrast, the frequencies of patients with chronic diseases (group B) had circulating IgG, IgM, and IgA antibodies specific for Sm31/32 were 98.2%, 10.5%, and 10.0%, respectively (Figure 4 and Table 1). The protein fractions recognized by IgM antibodies in group B patients differed both in number and molecular weight, and recognition by these antibodies produced a weak reaction. IgA antibodies in group B patients recognized several protein bands but in contrast sera from patients with acute disease, only 10% reacted with Sm31/32.

No antibodies to Sm31/32 were detected in the group E. In Group D, IgG and IgA antibodies were detected in 12.3% and 6.7% of the patients, respectively.

DISCUSSION

Antibodies against two immunodominant antigens in S. mansoni worms characterized as two digestive proteolytic enzymes, cathepsin B10 and asparaginyl endoproteinase,11 have been described. Both cathepsin B (31 kD) and asparaginyl endoproteinase (32 kD) are capable of inducing an early and intense immune response detectable approximately 4 weeks after infection. Antibody levels against these two antigens may decrease after treatment with praziquantel.12,13 Klinkert and others14 described the usefulness of recombinant proteins related to the Sm31/32 fraction as antigens for immunodiagnostic purposes. According to these investigators, the recombinant proteins were recognized by 82.2% and 88.6% of the patients depending of the portion and length of the fragment used.

Another protein fraction of the 36-kD protein was described by Noya and others15 as a protein that reacts with different classes and subclasses of antibodies from schistosome-infected individuals. Based on its relative molecular weight and immunoblot pattern, these investigators suggested that this fraction may correspond to the Sm31 and Sm32 protein bands previously described by Ruppel and others.12 In our work, we also detected a fraction with a molecular weight ranging from 30 to 32 kD (Sm31/32). This fraction is highly immunogenic and useful as a serologic marker in the diagnosis of schistosomiasis by detection of IgG antibodies. The IgM and IgA antibodies against Sm31/32 were detected in 100% and 90.9%, respectively, of the patients with acute schistosomiasis, and in only about 10% of the patients with chronic infection. The children in group C demonstrated an intense IgG antibody reactivity against the Sm31/32 fraction, with weak reactivity to bands of higher molecular weight (Figure 1). This profile was similar to that of the group with acute disease (group A). Some of the patients in group C also had IgM and IgA antibodies against Sm31/32 fraction, suggesting a recent infection.

Our results indicate that the detection of antibodies against the Sm31/32 fraction is a sensitive serologic tool that might prove to be valuable in the diagnosis of schistosomiasis. Furthermore, the identification of the antibody isotypes responsible for the positive reaction would help to differentiate between acute and chronic Schistosoma mansoni infection and also would serve as an epidemiologic marker of transmission in disease-endemic areas.

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