Rapid Competitive Enzyme-Linked Immunosorbent Assay Using a Monoclonal Antibody Reacting with a 15-Kilodalton Tegumental Antigen of Schistosoma mansoni for Serodiagnosis of Schistosomiasis

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A competitive enzyme-linked immunosorbent assay (CELISA) for antibody detection was developed by using a monoclonal antibody which reacts with a 15-kDa tegumental antigen of the adult worm of Schistosoma mansoni. This monoclonal antibody was not able to react with antigens of Schistosoma japonicum or Schistosoma haematobium in enzyme-linked immunoelectrotransfer blot (EITB) and indirect immunofluorescence tests. The assay was performed in a period of 1 h using an adult worm crude extract antigen. To evaluate the CELISA, a total of 73 serum samples was analyzed: 35 were from S. mansoni-infected patients, 23 were from individuals with parasitic infections other than schistosomiasis, and 14 were from healthy individuals. All serum samples from healthy individuals and from patients infected with other parasites were negative, as were two (6%) samples from patients infected with S. mansoni. EITB analysis showed that 32 of 33 CELISA-positive samples were positive in the EITB but with different patterns of reactivity. A 15-kDa protein reacted with 60% of serum samples, and a 60-kDa protein showed the highest level of reactivity (85%). The two samples from patients infected with S. mansoni that were negative in the CELISA reacted with 70-, 60-, 50-, 47-, and 38-kDa proteins. One sample, positive in CELISA, did not react with proteins of the antigenic extract.

Parasitological methods have been the choice for the diagnosis of schistosomiasis, although many reports have indicated the possibility of using immunological methods for this aim (21). The enzyme-linked immunosorbent assay (ELISA) has been employed for the detection of antigens (9, 12, 15) and antibodies (11, 14, 28) in schistosomiasis as well as in other helminthic diseases (1, 10, 13). Despite the reported cross-reactivity (17), specificity and sensitivity of ELISAs can be improved by using purified antigens (25) or monoclonal antibodies (MAbs) (8). This latter reagent can be used to develop competitive ELISAs (CELISAs), which allow the detection of specific antibody (2), since the reactivity pattern of each MAb is addressed to a specific molecule epitope. In CELISAs, less-purified antigens can be used, thus eliminating difficult purification steps.

As reported by Lewis and Strand (20), adult worm tegumental proteins can be immunoprecipitated by sera from Schistosoma mansoni-infected humans. This indicates a possible source of immunogens suitable for use in immunodiagnosis. In addition, the 15-kDa tegumental antigen was found to react with antibodies in mice, monkeys, and humans infected with S. mansoni (24). In this report we describe a CELISA for antibody detection applying MAb A 61, which reacts with an epitope of a 15-kDa molecule present in S. mansoni adult worm tegument. Samples used in this study were also analyzed by enzyme-linked immunotransfer blot (EITB) in order to evaluate antibody reactivity against the antigen employed in the assay.

MATERIALS AND METHODS

Antigen. S. mansoni adult worm crude extract antigen was obtained from worms recovered from infected mice 8 weeks after infection by liver perfusion. The worms were homogenized in a glass homogenizer in a 0.05 M Tris-HCl buffer, pH 8.0. The homogenate was initially centrifuged at 100 × g for 15 min at 4°C, and the supernatant obtained was centrifuged at 30,000 × g for 30 min at 4°C. The pellet was sonicated with 100% power, 20% pulse duty cycles (three cycles of 30 s) with a model 375 sonicator (Heat system, Plainview, N.Y.), and the conditions of the last centrifugation were repeated. Protein concentration was assessed by a Bio-Rad protein assay with bovine serum albumin as the standard. This antigen was used in all immunoenzyme assays.

Purification of MAb and conjugation with horseradish peroxidase. MAb A 61 was previously obtained and characterized by Peralta et al. (24). The secreting cell clones were inoculated in the peritoneal cavity of BALB/c mice. MAb A 61 was purified from ascitic fluid by ammonium sulfate precipitation and ion-exchange chromatography with a DE-52 cellulose column. Purified MAb A 61 was then conjugated to horseradish peroxidase (Sigma type IV RZ, 3.2) by the periodate method of Wilson and Nakane (27). The horseradish peroxidase-conjugated MAb A 61 (A 61-POD) was titrated against different concentrations of S. mansoni adult worm crude extract antigen by ELISA.

Sera. All serum samples were obtained from the Evandro Chagas Hospital, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil. A total of 73 serum samples was used in the CELISA evaluation. Thirty-five samples were from individuals who demonstrated S. mansoni eggs in stools (age, 11 to 67 years;
were washed where median, 13.4 years), evaluated by the Kato-Katz (18) and sedimentation methods. Egg counts ranged from 23 to 1,679/g of feces, with a median of 198.85/g. Of these 35 patients, 6 were evaluated by the sedimentation method only. Fifteen samples were from individuals with no apparent infection and who did not demonstrate schistosome eggs in the stools. None of these individuals had been in an area where schistosomiasis is endemic. The 23 samples from individuals infected with parasites other than S. mansoni included 10 from malarial patients, 7 from patients with leishmaniosis, and 6 from patients with Chagas’ disease.

CELISA. To perform the CELISA, Immunolon I plates (Dynatech Laboratories, Alexandria, Va.) were coated with, per well, 100 μl of 5.0-μg/ml crude adult worm extract antigen in 0.5 M Tris-HCl buffer, pH 8.0, and incubated for 1 h at 37°C. After storage overnight at 4°C, the test plates were washed three times with phosphate-buffered saline containing 0.3% Tween 20 (PBS-T), after which 50 μl of serum specimen diluted at 1:2 was added to each well. This was immediately followed by the addition to each well of A 61-POD, diluted at 1:600 in PBS-T containing 5% dried skim milk. Plates were gently tapped to ensure mixing and incubated for 30 min at 37°C. After three wash steps with PBS-T, 100 μl of substrate solution containing 0.04% ortho phenyl diamine, 0.04% hydrogen peroxide (Sigma), and 0.05 M acetate buffer at pH 5.0, was added to each well. After incubation of the plates at room temperature in the dark for 30 min, the reaction was stopped by adding 100 μl of 8 N sulfuric acid per well. Optical density was measured with an automated ELISA reader (Multiskan; Titertek) at 492 nm. The optical density values were expressed by inhibition percentage, with the values of pooled positive control sera as 100% and those of the pooled negative control sera as 0%. The value established for the cutoff was 50% inhibition. Above this value sera were considered positive.

EITB. Analysis by EITB was performed to determine the reactivity patterns of 35 serum samples from schistosomiasis patients. The technique was performed as described by Tsang et al. (26). Briefly, 100 μg of crude antigen was electrophoretically resolved in sodium dodecyl sulfate-polyacrylamide gels (12% polyacrylamide running gels) and electrophoretically transferred onto a nitrocellulose membrane. The protein-containing membrane was cut into 3-mm strips and assayed immunoenzymatically with serum samples from 35 S. mansoni-infected patients. One sample with a high percentage of inhibition in the CELISA was chosen as a positive control. The pooled negative serum control used in CELISAs was also used in EITB tests. Serum was considered positive when it reacted with three or more of the following proteins: 90-, 71-, 60-, 47-, 38-, 30-, 21-, and 13-kDa protein. When no reactivity was found, the serum was considered to be negative, and when it did not fit this criterion, the result was designated nonconclusive.

RESULTS

Standardization of CELISA. The optimal concentration of antigen and A 61-POD to be used in the test was determined by ELISA. Titration curves resulting from different concentrations of reagents are shown in Fig. 1. The optimal concentration of the antigen was established to be 5.0 μg/ml. Although there was little difference between the antigen concentrations used, this was the lower concentration with optical density values present in the sharper part of the curve, which was the best zone of competition. For the A 61-POD the optimal dilution was found to be 1:600. The same criterion as that used above was used.

Detection of antibodies by CELISA. Antibodies against S. mansoni were detected in 33 (94%) of 35 serum samples from parasitologically proved cases when we considered 50% inhibition as the cutoff value of the test (Fig. 2). The two CELISA-negative samples (65 and C) presented 36 and 21% inhibition, respectively. None of the samples from no-schistosomiasis patients (healthy or with other parasitologic infections) was positive in CELISA.

Reactivity pattern of A 61-POD and schistosomiasis samples in EITB. The A 61-POD reacted with three different proteins of the S. mansoni adult worm antigens: 21-, 20-, and 15-kDa

![FIG. 1. Titration curves with different concentrations of S. mansoni adult worm antigen (AG) and various dilutions of A 61-POD. O.D., optical density.](http://jcm.asm.org/DownloadedFrom)
proteins (Fig. 3, lane A). However, the most intense reaction was observed with the 15-kDa band. The serum sample analysis of the 35 parasitologically proved cases by EITB showed that 34 had positive patterns recognizing three or more bands with different molecular weights. Included among the positive patterns were the two CELISA-negative samples (65 and C), which were shown to have reacted with the 70-, 60-, 47-, and 38-kDa bands (sample 65) and the 51-, 47-, and 38-kDa bands (sample C) (Fig. 3, lanes 1 and 6). Neither of these two serum samples reacted with the 15-kDa protein. Sample 75 (Fig. 3, lane 3), which showed 65% inhibition in CELISA, did not react with any protein. The pattern of EITB reactivity among the positive samples was different, and the highest reactivity was found with the 60-kDa protein (85%); however, the 15-kDa protein reacted only with 60% of serum samples.

**DISCUSSION**

As described by Mitchell (22), the complexity of immune response and parasite biology involved in helminth diseases has been an impairment for the development of immunological tests appropriate for use in epidemiological studies. In schistosomiasis, various strategies have been designed for tests to be applied in a particular situation. Detection of circulating antigen by ELISA (5), for example, can detect active schistosome infections (7) and can be useful to assess treatment efficacy (6). On the other hand, antibody detection could have a wide application in determination of the prevalence of infection (21), but because of antigen sharing between schistosomes and other parasites (4, 19), it is necessary to use specific reagents such as purified or cloned antigens (3, 16, 25) or MAbs (22).

In the present study, a MAb was used to develop a CELISA method. This MAb was characterized by Peralta et al. (24) as an immunoglobulin G1 isotype which reacts with a 15-kDa adult worm tegumental antigen. The CELISA was able to detect specific antibodies in 33 (94%) of 35 serum samples from control groups (healthy or with other parasitic infections).

The analysis of the A 61-POD against a crude *S. mansoni* adult worm antigen by EITB showed results different from
those described before when we used indirect assay (24). In this study, we found that A 61-POD reacted with 20- and 21-kDa proteins in addition to the 15-kDa protein. On the basis of this result it is possible to postulate that the recognized epitope could be present in a protein of higher molecular weight which could be degraded by the EITB sample preparation. Another possibility is that this epitope is present in different proteins of S. mansoni adult worm tegument.

For serum samples analyzed by EITB, it was found that only 60% of them reacted with the 15-kDa protein, contrasting with the positive results of 94% in the CELISA. This could indicate a lower sensitivity of EITB to detect antibodies against this protein. However, when the criterion in which the presence of three or more reactive bands was used, 34 serum specimens had positive reactions in EITB, including the 2 CELISA-negative samples. On the other hand, one CELISA-positive sample was negative in EITB. Nevertheless, when this sample was evaluated by the sedimentation method, no egg was found, but by the Kato-Katz technique, no viable eggs were detected. This result suggested a lack of specificity of CELISA, but it could mean also a past infection because of which the patient was still producing small amounts of antibodies against the 15-kDa molecule, assessed only by CELISA and not by EITB. The parasitological methods used to diagnosis were not sensitive enough to clear these results. The EITB was not as sensitive as the CELISA in detecting antibodies against the 15-kDa protein.

Despite a few conflicting results in relation to EITB and parasitological examination, the CELISA described here showed good sensitivity (94%) and specificity (97%). It takes only 1 h to perform, allowing many samples to be processed in a relatively short period of time. Besides that, new MAbs could be evaluated to have higher spectrum of reactivity to increase both sensitivity and specificity. A further evaluation with a large number of serum samples in areas where schistosomiasis is endemic also has to be performed to confirm whether this test can be used as a new tool for epidemiological studies.

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REFERENCES


