

Cloned *Schistosoma mansoni* Proteinase (Hemoglobinase) as a Putative Serodiagnostic Reagent

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Expressed cDNA encoding a proteolytic enzyme from *Schistosoma mansoni* has been cloned recently. Circulating antibodies reacting with the recombinant protein have been detected in the blood of mice and humans infected with *S. mansoni*, *S. japonicum*, or *S. haematobium*. *S. mansoni* and *S. haematobium* infection can be distinguished by antibody titer.

Schistosomiasis, a major parasitic disease, is associated with significant morbidity and mortality in endemic populations and in travelers. Diagnosis is usually made by visualization of parasite eggs in stools or urine. The chronic nature of this infection, which can persist for decades, results in cumulative damage to the liver, spleen, and colon. Early intervention with effective chemotherapeutic agents is most efficacious if sensitive and specific methods for detecting infection are available.

The antibody response to a variety of schistosome preparations and extracts has been studied in several laboratories (5). A 31,000- to 32,000-dalton cysteine proteinase (2) which degrades host globin (1; Zerda et al., in press) is among the first parasite antigens to be immunologically recognized in experimental infections in mice (4, 9) and baboons (11) and natural infections in humans (3, 8, 10).

Recently, Davis et al. (6) expressed λ gt11 cDNA clones encoding the hemoglobinase and purified the β -galactosidase fusion protein. The fusion protein retained proteolytic activity (6) and was recognized by a monoclonal antibody selected with the native cysteine proteinase (C. L. Chappell, unpublished data). In another study, Klinkert et al. (7) expressed a portion of a cloned 31,000- to 32,000-dalton immunogenic molecule from the adult schistosome. The product was recognized by antibodies present in the sera of acutely infected individuals, but less prominently in the sera of patients from endemic areas.

Antiproteinase antibody to the native enzyme can be detected earlier than fecal egg excretion (standard diagnostic technique). We were interested to learn whether the antigenic sites on the native proteinase were maintained on the recombinant proteinase, thus making this cloned protein a useful diagnostic reagent. To this end, we utilized the recombinant protein and native enzyme in immunoblots to screen the sera of mice experimentally infected with *Schistosoma mansoni*, *S. japonicum*, or *S. haematobium*. In addition, sera pooled from humans harboring *S. mansoni* were analyzed by dot blots, which may be assessed visually, a distinct advantage in field situations.

Immunoblot analyses were done essentially as described previously (4). The hemoglobinase recombinant protein and β -galactosidase were purified by a modification of techniques previously described (6). In these experiments, 1 μ g of purified recombinant protein was electrophoresed on a

sodium dodecyl sulfate-15% polyacrylamide gel and transferred to nitrocellulose. Primary sera from uninfected or schistosome-infected mice were diluted 1:100. For immunoglobulin E (IgE) detection, strips were incubated with rabbit anti-mouse IgE antibody (gift from Rabia Hussain) prior to incubation with conjugated antibody. For dot blots, the antigens, purified β -galactosidase (1 μ g), the recombinant proteinase (1 μ g), and the native proteinase, SMw32 (10 μ g), were spotted separately onto nitrocellulose sheets. The procedure followed was the same as in the immunoblot analysis.

Antibodies (IgG, IgE, and IgM) from *S. mansoni*-infected mouse serum which react with the native proteinase also recognized the fusion protein (Fig. 1A). The sensitivity of the fusion protein in detecting infection was tested with sera drawn at weekly intervals from infected mice. High levels of IgG antibody were seen in sera collected 4 weeks postinfection and thereafter (Fig. 1B). This is at least 2 weeks prior to the microscopic detection of parasite eggs in the feces. In this experiment, minimal reactivity was seen in the preinfection and 1-week sera. Band intensity at weeks 2 and 3 was increased slightly over preinfection level. Earlier studies with the native proteinase (enzyme-linked immunosorbent assay) showed IgG reactivity in 30% of the mice at week 2 and all the mice at week 3 of infection (4). It is not clear whether the increased sensitivity with the native enzyme may have been due to the assay method used or to antigenic differences between the native versus the cloned proteinase.

Antibody cross-reactivity among the schistosome species is an important consideration in the development of a diagnostic reagent. To examine this possibility, we collected sera from groups of mice infected with either *S. mansoni* or *S. japonicum* or hamsters infected with *S. haematobium*. (In earlier experiments, we demonstrated that hamsters infected with *S. mansoni* produced high-titer antiproteinase antibody.) The sera from animals separately infected with the three schistosome species all recognized the *S. mansoni* fusion protein (Fig. 1C). However, the titers of antibodies reacting with the fusion protein were highest in *S. mansoni*-infected animals, intermediate in *S. japonicum* infection, and lowest in *S. haematobium* infection. Further, *S. haematobium* serum, positive on blots at 1:10 and weakly positive at 1:100, could readily be distinguished from *S. mansoni* serum, which was routinely positive at 1:1,000. Thus, while serological screening with more concentrated sera detected all three schistosome species, use of more dilute sera distin-

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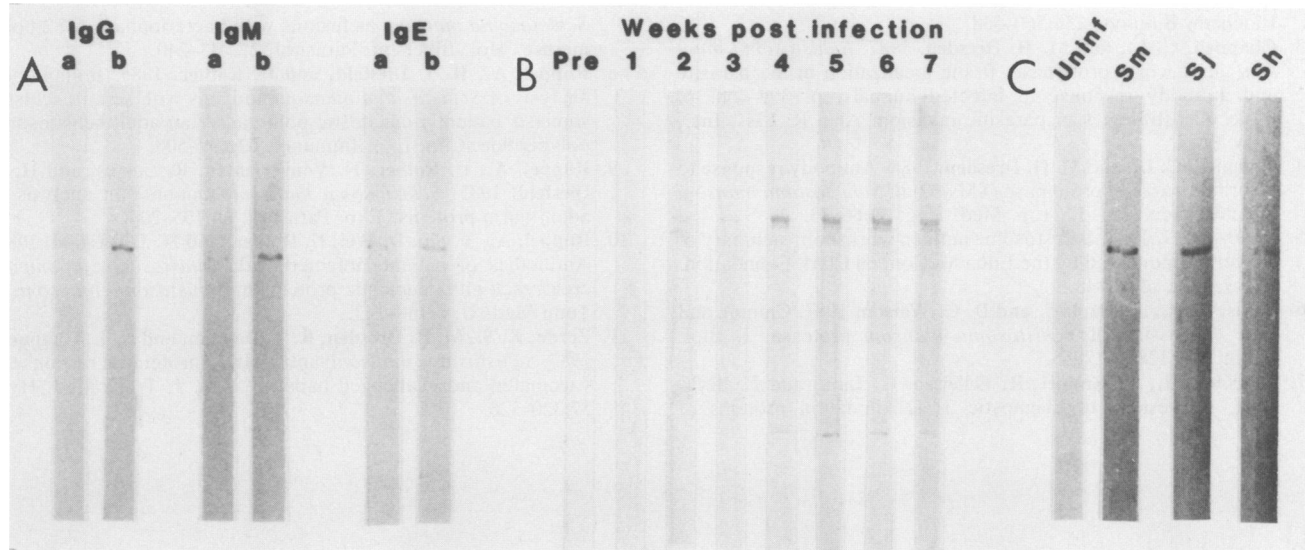


FIG. 1. Immunoblot analysis of the recombinant *S. mansoni* proteinase with the sera from schistosome-infected mice. (A) Strips containing the recombinant proteinase were incubated with sera from uninfected (lanes a) or *S. mansoni*-infected (lanes b) mice 7 to 8 weeks following exposure to 250 cercariae. Pencil marks at the edges of lanes a in IgM and IgE strips were added to aid in alignment. (B) Strips were incubated with sera collected from mice preinfection and at weekly intervals after infection with *S. mansoni*. Minor bands seen below the fusion protein may represent degradation products. (C) Strips were incubated with sera from uninfected mice (Uninf), mice infected with 250 *S. mansoni* cercariae (week 7) (Sm) or 200 *S. japonicum* cercariae (week 6) (Sj), or hamsters infected with 200 *S. haematobium* cercariae (week 6) (Sh).

guished between the two species which exhibit overlapping endemicity (*S. mansoni* and *S. haematobium*). This observation may be useful for epidemiological studies.

Adaptation of a serological assay to a simplified test system, such as the dot blot, is crucial for utilization in developing countries. As a first step, purified recombinant and native proteinases were directly immobilized on nitrocellulose. In addition, β -galactosidase purified from *Escherichia coli* (6) served as a control for the recombinant proteinase. Sera from patients excreting parasite eggs and from experimentally infected mice were incubated with the immobilized proteins. A strong reaction to both the native and recombinant proteinase was seen with each serum (Fig. 2). Essentially no reactivity to β -galactosidase was detected, suggesting that antibody to the *E. coli* protein did not interfere with the assay.

The immunoblot and dot-blot assays done as described above were each repeated a minimum of three times in separate experiments. No qualitative differences were noted among the trials. However, the intensities of the reactions

varied slightly from day to day, but did not vary so much as to alter the ability to distinguish a positive from a negative reaction. The stability of immobilized recombinant proteinase has not been carefully studied. However, in these experiments, strips containing proteinase were kept moist at 4°C for as long as 3 to 4 weeks. Under these conditions, no decrease in immunoreactivity was noted. Both positive and negative control sera were included in each trial to maintain a quality control on the assay system. Further studies of the antiproteinase response in a large number of infected individuals and as a function of worm burden are in progress.

The findings presented here are an important step toward a serodiagnostic test for schistosomiasis. The schistosome proteinase is a major antigen in all three human schistosome infections and is detected prior to egg excretion. These data suggest that antibody titers can be used to distinguish between *S. mansoni* and *S. haematobium* infections. The efficacy of this test in a system adaptable to field conditions is promising, and the abundance of the cloned enzyme makes its use as a serodiagnostic reagent economically feasible.

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LITERATURE CITED

1. Chappell, C. L., and M. H. Dresden. 1986. *Schistosoma mansoni*: proteinase activity of "hemoglobinase" from the digestive tract of adult worms. *Exp. Parasitol.* 61:160-167.
2. Chappell, C. L., and M. H. Dresden. 1987. Purification of cysteine proteinases from adult *Schistosoma mansoni*. *Arch.*

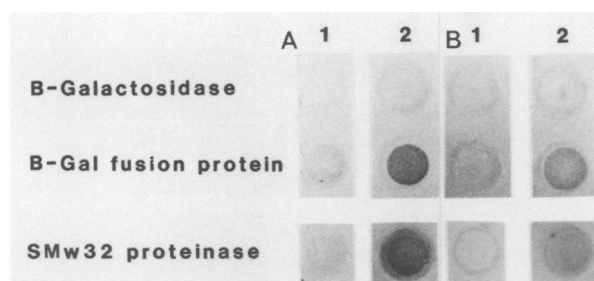


FIG. 2. Dot-blot analysis of recombinant and native proteinase with sera from *S. mansoni*-infected mice (A) and humans (B). In both panels, the blot in lane 1 was incubated with normal sera, while the blot in lane 2 was incubated with sera from *S. mansoni*-infected mice or humans. B-Gal, β -Galactosidase.

- Biochem. Biophys. **256**:560–568.
3. **Chappell, C. L., and M. H. Dresden.** 1987. *Schistosoma mansoni* adult worm proteinase: tissue localization in the parasite and antibody response in infected animals, p. 399–408. In *Molecular strategies of parasitic invasion*. Alan R. Liss, Inc., New York.
 4. **Chappell, C. L., and M. H. Dresden.** 1988. Antibody response to a purified parasite proteinase (SMw32) in *Schistosoma mansoni* infected mice. *Am. J. Trop. Med. Hyg.* **39**:66–73.
 5. **Colley, D. G.** 1983. Schistosome antigen workshop: summary of a meeting sponsored by the Edna McConnell Clark Foundation. *J. Parasitol.* **69**:45–48.
 6. **Davis, A. H., J. Nanduri, and D. C. Watson.** 1987. Cloning and gene expression of *Schistosoma mansoni* protease. *J. Biol. Chem.* **262**:12851–12855.
 7. **Klinkert, M., A. Ruppel, R. Felleisen, G. Link, and E. Beck.** 1988. Expression of diagnostic 31/32 kilodalton proteins of *Schistosoma mansoni* as fusions with bacteriophage MS2 polymerase. *Mol. Biochem. Parasitol.* **27**:233–240.
 8. **Ruppel, A., H. J. Diesfeld, and U. Rother.** 1985. Immunoblot analysis of *Schistosoma mansoni* antigens with sera of schistosomiasis patients: diagnostic potential of an adult schistosome polypeptide. *Clin. Exp. Immunol.* **62**:499–506.
 9. **Ruppel, A., U. Rother, H. Vongerichten, R. Lucius, and H. J. Diesfeld.** 1985. *Schistosoma mansoni*: immunoblot analysis of adult worm proteins. *Exp. Parasitol.* **60**:195–206.
 10. **Ruppel, A., Y. Shi, D. Wei, U. Rother, and H. J. Diesfeld.** 1986. Antibodies of patients infected with *Schistosoma japonicum* crossreact with diagnostic proteins of *Schistosoma mansoni*. *J. Tonji Med. U.* **6**:138–143.
 11. **Zerda, K. S., M. H. Dresden, R. T. Damian, and C. L. Chappell.** 1987. *Schistosoma mansoni*: anti-SMw32 proteinase response in vaccinated and challenged baboons. *Am. J. Trop. Med. Hyg.* **37**:320–326.