INFECTION AND IMMUNITY, Apr. 1986, p. 230–235
0019-9567/86/040230-06$02.00/0

Vol. 52, No. 4

Surface Antigen Detected by a *Schistosoma mansoni* Monoclonal Antibody in Worm Extracts and Kidney Deposits of Infected Mice and Hamsters

CÉLIA REGINA WHITAKER CARNEIRO[1] AND JOSÉ DANIEL LOPES[2]*

Discipline of Parasitology, Escola Paulista de Medicina, 04023, São Paulo,[1] and Ludwig Institute for Cancer Research, São Paulo Branch, 01509, São Paulo,[2] Brazil

Received 21 August 1985/Accepted 13 December 1985

Four monoclonal antibodies (MAbs) derived from a *Schistosoma mansoni*-infected mouse reacted against the tegument or the cell layer of the digestive tract of the adult worm. They also showed similar patterns of immunofluorescence staining when schistosomula were used as antigens. Two of the MAbs (4A10 and 4D3) recognized immune complexes deposited in the kidneys of infected mice and hamsters as detected by indirect and direct immunofluorescence reactions. When adsorbed to polystyrene beads, both MAbs allowed the quantitative detection of antigen by an enzyme immunoassay. ^{125}I-labeled 4A10 binding to live schistosomula and corresponding inhibition assay ruled out the possibility that this binding could be through its Fc fragment. The same MAb detected an antigen migrating as an 80-kilodalton protein by Western blot analysis of soluble worm antigen preparation after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Use of MAb 4A10 may help to elucidate mechanisms of renal pathology and also be of value in the development of immunodiagnostic assays.

In experimental *Schistosoma mansoni* infections of the mouse, the development of immunity can be detected from week 7 onwards (27), with some immunoglobulins being important mediators of the protection mechanisms of the host (24). Specific immune complexes have been found to be associated with glomerular lesions observed in experimental (22) and human infections (13). Antigens located on the surface of the worm or at the cell layer of the digestive tract are directly exposed to the immune system of the host (23). They are important targets for antibody response and are presumably relevant to immunity mechanisms (5, 27). Furthermore, it has been demonstrated that antibodies eluted from kidney deposits react with the tegument or the digestive tract of adult worms (13, 19). Several circulating antigens of different chemical structures, polysaccharides, glycoproteins, and proteoglycans, have already been identified and found to be associated with the digestive tract cell layer of the adult worm (3, 6, 21). However, their complete characterization, relevance to resistance mechanisms, and morbidity have not yet been determined.

Several anti-*S. mansoni* monoclonal antibodies (MAbs) have been instrumental in the identification of either stage-specific or non-stage-specific individual antigens (2, 7, 28, 29). The isolation of such antigens may allow studies on mechanisms of protection and on immunopathology. MAbs have been used in passive immunization experiments (7, 28, 29) and also as reagents for serodiagnostic tests (1).

In the present work we have used MAbs to investigate antigens produced during an experimental infection by *S. mansoni* which might be of immunopathological relevance. We report an MAb derived from spleen cells of an infected mouse that recognizes a previously unknown antigenic protein at the surface of the adult worm and schistosomulum as well as in worm extracts. This antigen is also found in immune complexes deposited along the glomerular basement membrane in kidneys of infected rodents.

* Corresponding author.

MATERIALS AND METHODS

Animals. Inbred, 4-to-8-week old, BALB/c mice (kindly provided by T. A. Mota-Santos and G. Vieira) were bred at the Discipline of Parasitology, Escola Paulista de Medicina and used for hybridoma and ascites fluid production. Hamsters were obtained from the animal house of Escola Paulista de Medicina and were infected when 12 weeks old.

Infection of mice and hamsters. Cercariae of *S. mansoni* freshly shed by snails of the species *Biomphalaria glabrata* were injected subcutaneously into BALB/c mice (20 per mouse) and into hamsters (200 per hamster). Infection was ascertained by stool examination 6 weeks after the injection, and by indirect immunofluorescence testing of mouse sera with frozen cryostat sections of adult worms. Worms were obtained by liver perfusion of infected mice and hamsters. One mouse with the highest immunofluorescence titer (>1:320) at week 7 of infection was used as a spleen donor for cell fusion without any further immunization.

Hybridoma production and characterization. The production and characterization of MAbs against *S. mansoni* have been described elsewhere (16). Briefly, spleen cells from the infected mice were fused with the SP2/0.Ag14 murine myeloma cell line by using polyethylene glycol. After selection by growth restriction in hypoxanthin-aminopterin-thymidine medium, supernatants of hybrid colonies were tested for the presence of antibodies by indirect immunofluorescence with frozen, 4-μm thick sections of adult worms. The fluorescein conjugate used was either anti-mouse immunoglobulin G (IgG) and IgM (Miles Laboratories, Inc. Elkhart, Ind.) or protein A (Pharmacia, Uppsala, Sweden).

Antibody subclasses were determined by double diffusion (Ouchterlony) with concentrated supernatants and specific antisera (Miles Laboratories). Supernatants were concentrated by vacuum dialysis with Millipore membranes (immersible CX-10 ultrafiltration unit, Millipore Corp., Bedford, Mass.).

Production of ascitic fluids. BALB/c mice, previously primed (24 to 96 h before) by an intraperitoneal injection of

0.5 ml of pristane (2,4,10,14-tetramethylpentadecane; Aldrich Chemical Co., Milwaukee, Wis.), received 1×10^6 to 2×10^6 hybrid cells by the same route. Ascitic fluids could be tapped after 2 weeks.

Purification of antibodies. MAbs from either culture supernatants or ascitic fluids were precipitated by 50% (wt/vol) ammonium sulfate solution, buffered with 0.05 M Tris hydrochloride (pH 8.6), and applied to a 5-ml Sepharose-protein A (Pharmacia) column which had been previously equilibrated with 0.05 M Tris hydrochloride containing 0.15 M NaCl (pH 8.2) (15). Stepwise elution of antibodies was performed by lowering the buffer pH as described by Ey et al. (8).

Preparation of SWAP. Soluble worm antigen (SWAP) was prepared from worms obtained by liver perfusion of infected mice and hamsters which were washed and stored at −70°C. After thawing, 200 to 300 worms were suspended in 1.0 ml of either 0.2 M NaOH (alkaline extract) or 0.1 M NaCl (saline extract) and homogenized by grinding (14). These suspensions were incubated at 4°C overnight with continuous shaking. The alkaline extract (S1) was sonicated for 1 min and neutralized with 2.0 M HCl. Sodium thioglycolate and thimerosal (0.01%) were added to the saline extract (S2), and the suspension was incubated at 4°C for 24 h. Both S1 and S2 were centrifuged at 10,000 × *g* at 4°C for 30 min, the supernatants were dialyzed against phosphate-buffered saline (PBS), pH 7.4 at 4°C, filter sterilized (Millipore filter, 0.22-μm pore size), and stored in samples at −20°C. Protein and carbohydrate contents of samples were determined before use.

Immunofluorescence and solid-phase EIA. Determination of indirect and direct immunofluorescence reactions of MAbs was performed as previously described (16) against 4-μm-thick, frozen sections of either whole worms, kidneys, or livers of infected mice and hamsters. Normal mouse sera, kidneys, and livers were used as controls. Conjugation of purified MAbs with fluorescein isothiocyanate was performed by the dialysis procedure described by Clark and Sheppard (4).

Solid-phase enzyme immunoassay (EIA) was carried out with coated 6-mm polystyrene beads (Precision Plastic Ball Co., Chicago, Ill.). Coating was done by immersion in a solution of MAbs in double-distilled water for 3 days at 4°C, during which time the beads were also incubated at 56°C for 30 min every day. The second antibody was a polyclonal sheep anti-*S. mansoni* serum described elsewhere (13) which had been conjugated with peroxidase by the method of Nakane and Kawaoi (20). For the assay, coated beads were washed three times in PBS, pH 7.4, containing 0.05% Tween-20 (PBS-T), and immersed in a solution containing SWAP diluted in PBS-T for either 45 min at 37°C or overnight at room temperature. The beads were washed again as above and immersed in conjugate diluted 1:400 in PBS-T containing 47% normal human or fetal calf serum plus 3% normal sheep serum. After washing as before, the beads were transferred to clean tubes and bound enzyme was revealed with *o*-phenylenediamine and hydrogen peroxide. Catalysis was interrupted with 0.5 M H_2SO_4 by the method of Goding and Handman (10). Absorbances of supernatants were determined at 492 nm. All reactions were performed in duplicate.

Binding of ^{125}I-labeled MAb 4A10 to living schistosomula. Schistosomula were prepared from freshly shed cercariae by the method of Ramalho-Pinto et al. (25). Purified MAb 4A10 was labeled with 1 mCi of ^{125}I (4,000 Ci/mmol; New England Nuclear Corp., Boston, Mass.) by the Iodo-Gen method (9). A total of 500 viable schistosomula, as seen by phase microscopy were suspended in 100 μl of Earle balanced salt solution containing 1% lactalbumin hydrolysate, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), and 24 mM sodium bicarbonate. This suspension as mixed with 2×10^5 cpm of labeled 4A10, with or without unlabeled 4A10, normal human serum, normal mouse serum, or RF2, an MAb of the IgG1 subclass reactive with carcinoembryonic antigen (12). All reactions were performed in duplicate and incubated overnight at room temperature. After gentle washing three times by centrifugation in PBS, the pellet was counted in a counter (Biogamma TM; Beckman Instruments, Inc., Fullerton, Calif.) The viability of the schistosomula was always checked at the end of each experiment. Similar experiments were done with ^{125}I-labeled RF2.

Immunoblot analysis of MAbs 4A10 and 4D3. A duplicate sample of 50 μg of SWAP protein was subjected to electrophoresis in 10% polyacrylamide gel, as described previously (11). One half of the gel was stained by Coomassie blue for the visualization of total proteins, and the other half was electrophoretically transferred to a nitrocellulose filter (Millipore Corp.; 0.45-μm pore size) (31). Filters had been preincubated overnight with 3% bovine serum albumin in PBS-T and extensively washed with the same buffer containing 1% bovine serum albumin. Filters were then incubated overnight under continuous stirring with ascites of MAbs 4A10 and 4D3 previously diluted 1:10 in the same buffer. After being washed, reactions were detected by incubation with anti-mouse IgG coupled to peroxidase (Miles Laboratories), followed by diaminobenzidine as described previously (18). Anticarcinoembryonic antigen MAb RF2 and normal mouse serum were used as controls.

RESULTS

Production of MAbs. To obtain MAbs against *S. mansoni* circulating antigens in a natural infection, spleen cells from a chronically infected BALB/c mouse were fused with the SP2/0 mouse myeloma line. Four clones of 14 positive cultures could be maintained and expanded as stable hybrids. Table 1 summarizes the MAbs subclasses and specificities for different developmental worm stages. The indirect immunofluorescence reactions with adult worm sections were predominantly against the tegument or the cell layer of the digestive tract (Fig. 1a and b). All four MAbs reacted with the tegument and internal structures of mechanically prepared schistosomula (Fig. 1c), with little differences in reaction intensity. Only MAb 3C6 reacted simultaneously with parasite eggs present in infected rodent livers.

Reactions against kidney sections. Immunofluorescence reactions were performed to check whether the antigens detected by the MAbs were present as part of immune complexes deposited in the kidneys, thus having im-

TABLE 1. Antidevelopmental stage specificities and subclass characterization of MAbs against *S. mansoni*

MAb	Immunoglobulin subclass	Patterns and intensity of immunofluorescence reactions with:		
		Adult worms	Schistosomula	Eggs
4D3	IgG1	Digestive tract	+ +	−
4A10	IgG1	Tegument	+ + +	−
3C6	IgM	Digestive tract	+ + +	+ + + +
4B2	IgG1	Tegument	+ + + +	−

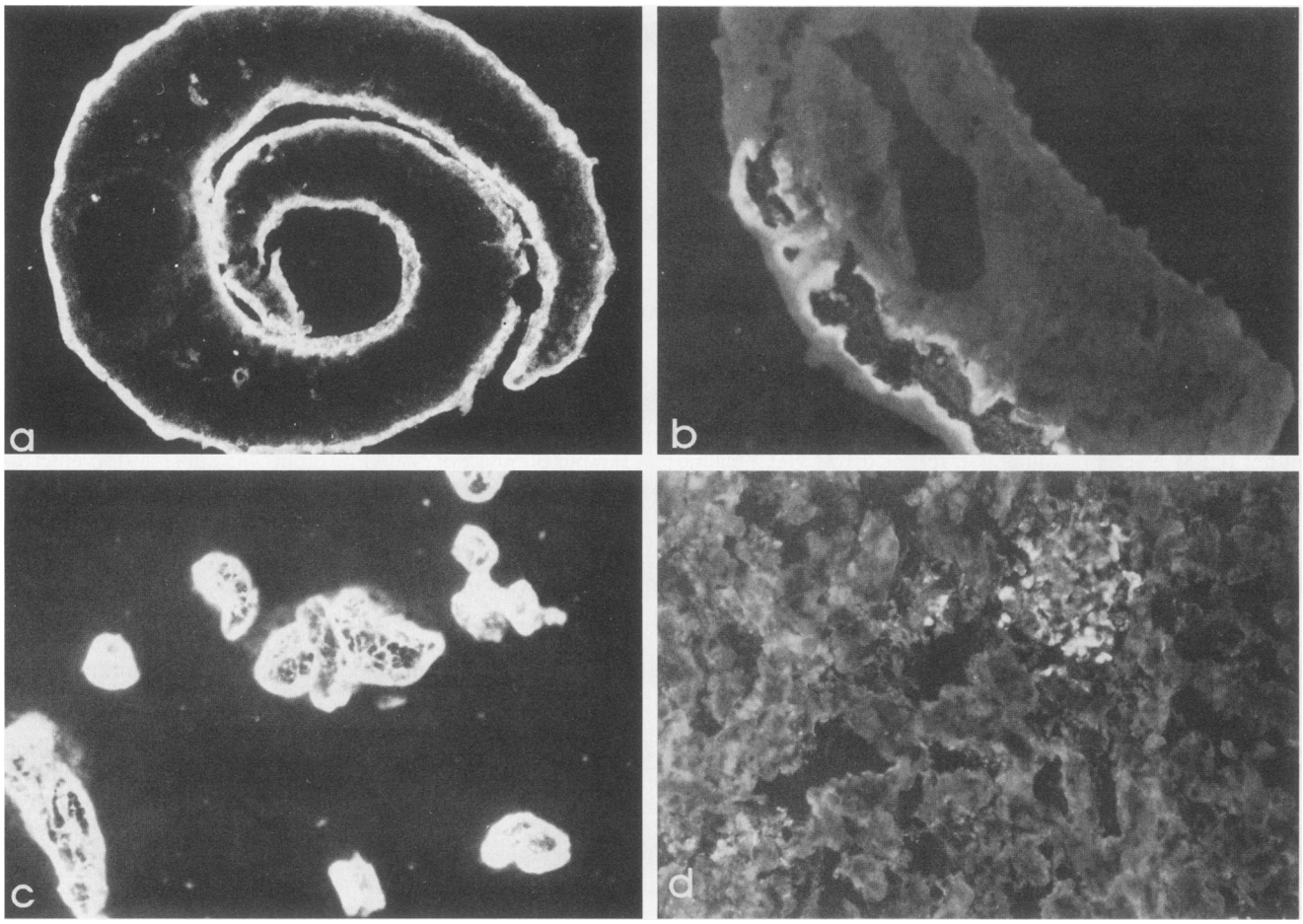

FIG. 1. *S. mansoni*, indirect immunofluorescence reactions with MAbs against sections of whole worms. Panels: a, Tegument stained with MAb 4A10; b, digestive tract stained with MAb 4D3; c, schistosomula stained with MAb 4A10; d, glomerular staining in a kidney section from an infected hamster with MAb 4A10 by direct immunofluorescence.

munopathological relevance. Two MAbs (4A10 and 4D3) reacted with the deposits seen along the glomerular basement membrane of kidney sections from chronically infected mice and hamsters. To rule out the possibility that the indirect reaction was detecting mouse immunoglobulins instead of *S. mansoni* antigens in the glomerular deposits, direct reactions with FITC-conjugated MAbs 4A10 and 4D3 were also performed, yielding similar results (Fig. 1d). Nonspecific fluorescence was greatly reduced by previous incubation of the tissue sections with 1% bovine serum albumin and by using fluorescein isothiocyanate-conjugated protein A instead of anti-mouse IgG. No glomerular staining was observed in normal kidney sections.

Solid-phase EIA with SWAP. Since quantification of the antigens recognized could be related to the intensity of infection, a solid-phase EIA was devised and used to determine levels of antigens in SWAP. Figure 2 shows typical dose-response curves obtained with polystyrene beads coated with MAbs 4A10 and 4D3 incubated with SWAP. The best results were obtained when preparations S2 and S1 were used, respectively, with 4A10 and 4D3. When 4A10 was used with S1 and 4D3 was used with S2, the sensitivity of both reactions was greatly reduced.

Binding of MAb 4A10 to living schistosomula. Since the specificities of MAbs had been determined only by indirect immunofluorescence in cryostat worm sections, a binding assay with living larvae was also performed. Figure 3 shows the curve obtained for the inhibition of binding of labeled 4A10 by unlabeled 4A10 to living schistosomula. Inhibition was not observed with normal human serum, normal mouse serum, or unlabeled RF2 (a murine MAb of the same IgG1 subclass). Also, when the same amount of labeled RF2 was used instead of labeled 4A10, no significant binding was obtained.

Immunoblot analysis of MAbs 4A10 and 4D3. To characterize the antigens detected in kidney deposits and quantified by solid-phase EIA, 50 μg of SWAP was fractionated in sodium dodecyl sulfate-polyacrylamide gels and electrophoretically transferred to nitrocellulose filters. A single band of ca. 80,000 daltons was revealed when MAb 4A10 was incubated with the antigen (Fig. 4). No bands could be visualized when MAbs 4D3 or RF2 were tested.

DISCUSSION

In the present report we describe the production of hybridomas from an *S. mansoni*-infected mouse that secrete MAbs specific for antigens located predominantly at the tegument of the worm or at the cell layer of the digestive tract. It has been suggested that those structures are the source of most antigens released by living worms into the bloodstream of the host during the course of a natural infection (23, 27). Thus, the specificities of the MAbs ob-

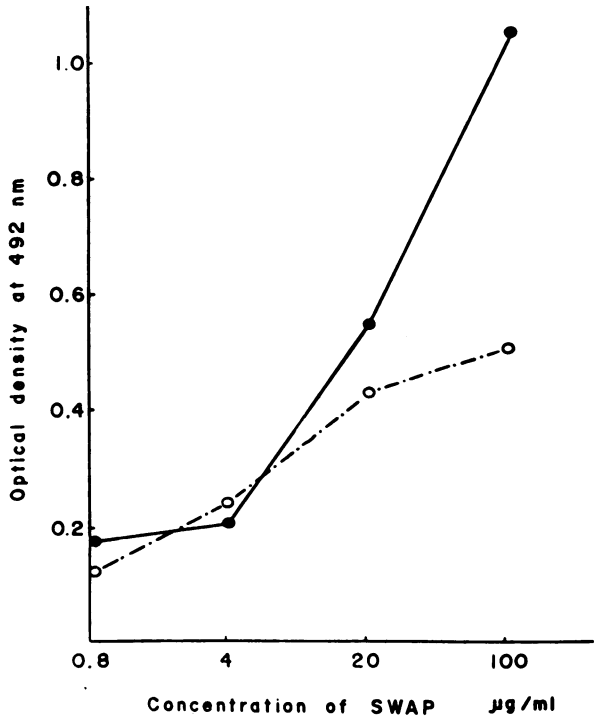

FIG. 2. *Schistosoma mansoni*, solid-phase EIA with polystyrene beads coated with MAb 4A10, 370 µg/ml, against SWAP-S2 (●) and MAb 4D3, 800 µg/ml, against SWAP-S1 (○). Reactions were performed as described in the text. Each point represents the average of duplicate reactions.

tained, using a chronically infected mouse as the spleen donor, are in accordance with these previous findings.

It has been demonstrated that some antigenic determinants are shared by different life-cycle stages of the parasite (5, 7, 27, 28). The four MAbs obtained probably recognize some of these epitopes, since all stained schistosomula and one of them also reacted with egg antigens by immunofluorescence (Table 1).

To further investigate the location of one of those antigens, a binding test with ^{125}I-labeled MAb 4A10 and living larvae was devised. The observation that living schistosomula had bound labeled antibody at the end of this experiment suggests that antibody binding was at the surface (Fig. 3). The inhibition of binding with unlabeled 4A10 and the failure to do so with either another murine monoclonal IgG or normal sera also argue against nonspecific binding through the Fc fragment of IgG. This possibility must be ruled out because a receptor on the schistosomular surface has been described (30).

The recognition of circulating antigens of a chronic infection by the MAbs was checked by immunofluorescence on kidney sections. Two MAbs (4A10 and 4D3) detected antigens in immune complexes in kidneys of infected mice and hamsters. The possibility that the binding was against immunoglobulins present in immune complexes, and not against *S. mansoni* antigens, could be ruled out by direct staining (Fig. 1d). These results are in agreement with previous demonstrations (17, 32) that the antigens in immune complexes deposited in renal glomeruli are released by the adult worm. Quantification of these antigens by EIA (Fig. 2), also present in SWAP, revealed a potential use of these MAbs in immunodiagnosis.

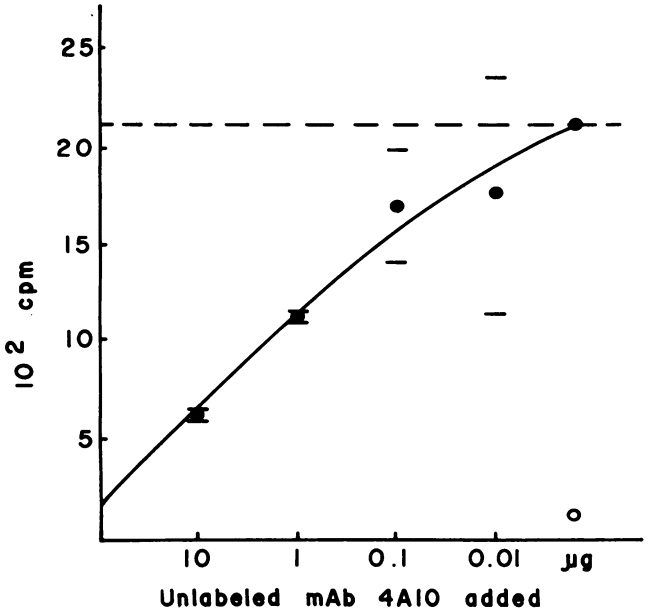

FIG. 3. *Schistosoma mansoni*, inhibition of binding of ^{125}I-labeled MAb 4A10 to living schistosomula by unlabeled 4A10. Reactions were performed as described in the text. Each point (●) represents the average of duplicate reactions and traces (−) are the absolute values obtained. The broken line represents total binding as measured without inhibitor.

Characterization of antigens is an important application of hybridoma technology. Surface proteins of low molecular weight (28, 29) and epitopes of higher-molecular-weight molecules shared by different developmental *S. mansoni* stages (2) can be recognized by MAbs. Our immunoblot experiments with electrophoretically fractionated SWAP and MAb 4A10 revealed a protein antigen of ca. 80,000 daltons (Fig. 4) not previously reported. No other bands

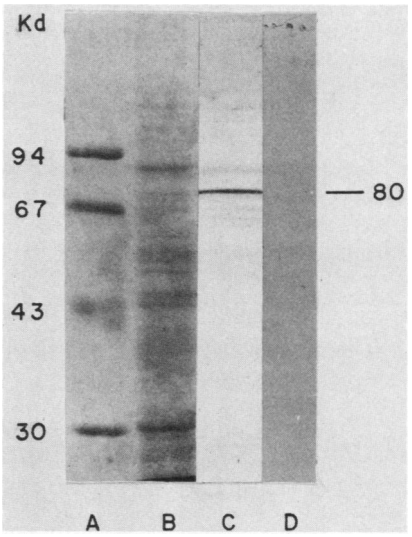

FIG. 4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot analysis of extracts of *S. mansoni* under reducing conditions. Lanes A and B, Coomassie blue staining of molecular weight markers (A) and *S. mansoni* whole-saline extract (B). Lanes C and D, immunoblot analysis of SWAP after treatment with MAb 4D10 (C) and RF2 (D) used as a negative control.

could be visualized when either the unrelated MAb RF2 or the anti-*S. mansoni* MAb 4D3 were tested.

The inability of MAb 4D3 to detect protein antigens by immunoblotting is in agreement with EIA results in which best reactions were seen with this MAb and the alkaline extract of SWAP (Fig. 2), which points to the possibility that the recognized antigen is not of protein nature. The staining by 4D3 of the digestive tract cell layer of the worm, where most of the already characterized antigens are polysaccharides (6, 21, 32), further corroborates this assumption. On the other hand, the possibility that 4D3 recognizes a protein epitope which is denatured in sodium dodecyl sulfate-polyacrylamide gel electrophoresis was not ruled out.

This is the first characterization of an antigen that is present on the surfaces of the worm and the schistosomulum and also in kidney immune complexes by using an MAb. Further studies with the antibodies described may lead to a better understanding of the mechanisms of glomerular damage by antigens and antibodies. Purification of the 80,000-dalton *S. mansoni* antigen by affinity chromatography may afford the development of immunodiagnostic tests.

ACKNOWLEDGMENTS

We are indebted to E. Plessmann Camargo for his critical comments and encouragement, to J. R. Magalhaes for the photographic documentation, to Leny Aiach for the technical assistance, and to C. Christo for the preparation of SWAP.

This work was funded in part by Conselho Nacional de Desenvolvimento Científico e Tecnológico, Fundação de Amparo à Pesquisa de Estado de São Paulo, Financiadora de Estudos e Projetos, and World Health Organization Special Programme for Research and Training in Tropical Diseases.

LITERATURE CITED

1. **Abdel-Hafez, S. K., S. M. Phillips, and D. M. Zodda.** 1983. *Schistosoma mansoni*: detection and characterization of antigens and antigenemia by inhibition enzyme-linked immunosorbent assay (IELISA). Exp. Parasitol. **55**:219–232.
2. **Aronstein, W. S., A. P. Norden, and M. Strand.** 1983. Tegumental expression in larval and adult stages of a major schistosome glycoprotein. Am. J. Trop. Med. Hyg. **32**:334–342.
3. **Carlier, Y., D. Bout, G. Strecker, H. Debray, and A. Capron.** 1980. Purification, immunochemical and biologic characterization of the *Schistosoma* circulating M antigen. J. Immunol. **124**:2442–2450.
4. **Clark, H. F., and C. C. Shepard.** 1963. A dialysis technique for preparing fluorescent antibody. Virology **20**:642–644.
5. **Damian, R. T.** 1984. Immunity in schistosomiasis: a holistic view. Contemp. Top. Immunobiol. **12**:359–420.
6. **Deelder, A. M., and D. Kornelis.** 1980. A comparison of IFA and the ELISA for the demonstration of antibodies against schistosome gut-associated polysaccharide antigen in schistosomiasis. Z. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **64**:65–75.
7. **Dissous, C., and A. Capron.** 1983. *Schistosoma mansoni*: antigenic community between schistosomula surface and adult worm incubation products as a support for concomitant immunity. Fed. Eur. Biochem. Soc. Lett. **162**:355–359.
8. **Ey, P. L., S. J. Prowse, and C. R. Jenkin.** 1978. Isolation of pure IgG1, IgG2a and IgG2b immunoglobulins from mouse serum using protein A-Sepharose. Immunochemistry **15**:429–436.
9. **Fraker, P. J., and J. C. Speck.** 1978. Protein and cell membrane iodinations with sparingly soluble chloroamide 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril. Biochem. Biophys. Res. Commun. **80**:849–857.
10. **Goding, J. W., and E. Handman.** 1984. Immunoassays for the detection of parasite antigens and monoclonal anti-parasite antibodies, p. 373–382. *In* C. M. Morel (ed.), Genes and antigens of parasites. UNDP/WORLD BANK/WHO/FINEP/CNPq/FIOCRUZ, Rio de Janeiro.
11. **Goding, J. W., and E. Handman.** 1984. Electrophoretic analysis of protein antigens, p. 383–415. *In* C. M. Morel (ed.), Genes and antigens of parasites. UNDP/WORLD BANK/WHO/FINEP/CNPq/FIOCRUZ, Rio de Janeiro.
12. **Hartfiel, R., J. D. Lopes, T. P. Lopes, and M. M. Brentani.** 1985. Tissue carcinoembryonic antigen and estrogen receptor in human breast cancer. Int. J. Cancer **35**:165–167.
13. **Hoshino-Shimizu, S., T. de Brito, H. Y. Kanamura, A. L. Canto, A. O. Silva, A. R. Campos, D. O. Penna, and L. C. da Silva.** 1976. Human schistosomiasis: *Schistosoma mansoni* antigen detection in renal glomeruli. Trans. R. Soc. Trop. Med. Hyg. **70**:492–496.
14. **Kanamura, H. Y., S. Hoshino-Shimizu, and L. C. da Silva.** 1981. Solubilization of antigens of *S. mansoni* adult worms for the passive hemagglutination test. Preliminary report. Rev. Inst. Med. Trop. (Sao Paulo.) **23**:92–95.
15. **Kohler, G.** 1981. The technique of hybridoma production, p. 285–298. *In* I. Lefkovitz and B. Pernis (ed.), Immunological methods, v.II. Academic Press, Inc., New York.
16. **Lopes, J. D., C. R. W. Carneiro, A. A. Vilela, and E. P. Camargo.** 1982. Production of monoclonal antibodies against *Schistosoma mansoni* and a trypanosomatid: a methodological report. Rev. Inst. Med. Trop. (Sao Paulo) **24**:327–336.
17. **Lopes, J. D., A. A. B. Moreira, R. Campos, H. Y. Kanamura, S. Hoshino-Shimizu, L. C. C. Gavotto, and L. C. da Silva.** 1981. Circulating antigens, antibodies and glomerular immune complexes in mice with unisexual *Schistosoma mansoni* infection. Rev. Inst. Med. Trop. (Sao Paulo) **23**:155–160.
18. **Moriearty, P. L.** 1984. Characterization of antigens by Western blotting, p. 365–372. *In* C. M. Morel (ed.), Genes and antigens of parasites. UNDP/WORLD BANK/WHO/FINEP/CNPq/FIOCRUZ, Rio de Janeiro.
19. **Moriearty, P. L., and E. Brito.** 1977. Elution of renal antischistosome antibodies in human schistosomiasis mansoni. Am. J. Trop. Med. Hyg. **26**:717–722.
20. **Nakane, P. K., and A. Kawaoi.** 1974. Peroxidase-labeled antibody. A new method of conjugation. J. Histochem. Cytochem. **22**:1084–1091.
21. **Nash, T. E., Nasir-ud-Din, and R. W. Jeanloz.** 1977. Further purification and characterization of a circulating antigen in schistosomiasis. J. Immunol. **119**:1627–1633.
22. **Natali, P. G., and D. Cioli.** 1976. Immune-complex nephritis in *Schistosoma mansoni* infected mice. Eur. J. Immunol. **6**:359–364.
23. **Quian, Z. L., and A. M. Deelder.** 1982. Circulating antigens in schistosoma infections. Acta Leiden. **49**:71–80.
24. **Ramalho-Pinto, F. J., R. de Rossi, and S. R. Smithers.** 1979. Murine schistosomiasis mansoni: anti-schistosomula antibodies and the IgG subclasses involved in the complement and eosinophil-mediated killing of schistosomula in vitro. Parasite Immunol. **1**:295–308.
25. **Ramalho-Pinto, F. J., G. Gazzinelli, R. E. Howells, T. A. Mota-Santos, E. A. Figueiredo, and J. Pellegrino.** 1974. *Schistosoma mansoni*: defined system for stepwise transformation of cercaria to schistosomule in vitro. Exp. Parasitol. **36**:360–372.
26. **Santoro, F., M. Capron, M. Joseph, R. Rousseaux-Prevost, and A. Capron.** 1978. Circulating antigens and immune complexes in *Schistosoma mansoni*-infected rats. Characterization by radioimmunoprecipitation PEG assay (RIPEGA). Clin. Exp. Immunol. **32**:435–442.
27. **Smithers, R. S., and R. J. Terry.** 1976. The immunology of schistosomiasis. Adv. Parasitol. **14**:399–422.
28. **Tavares, C. A. P., R. de Rossi, G. Pavares, A. J. G. Simpson, D. J. McLaren, and S. R. Smithers.** 1984. A monoclonal antibody raised against adult *Schistosoma mansoni* which recognizes a surface antigen on schistosomula. Z. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **70**: 189–197.
29. **Taylor, D. W., and A. E. Butterworth.** 1982. Monoclonal antibodies against surface antigens of schistosomula of *Schistosoma*

mansoni. Parasitology **84**:65–82.
30. **Torpier, G., A. Capron, and M. A. Ouassi.** 1979. Receptor for IgG (Fc) and human β-microglobulin on *S. mansoni* schistosomula. Nature (London) **278**:447–449.
31. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
32. **Van Mark, E. A. E., A. M. Deelder, and P. L. J. Gigase.** 1981. *Schistosoma mansoni*: anodic polysaccharide antigen in glomerular immune deposits of mice with unisexual infections. Exp. Parasitol. **52**:62–68.