

*SCHISTOSOMA MANSONI*

Anti-Egg Monoclonal Antibodies Protect Against Cercarial Challenge  
In Vivo

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The rationale for developing a vaccine for schistosomiasis, a disease affecting 200–300 million people worldwide, is based on observations that infected laboratory animals can develop resistance to challenge infections. For instance, in the mouse model of schistosomiasis, immunity to challenge infection is normally acquired by animals harboring adult worms (1, 2). Although resistance can be seen in mice harboring either unisexual or bisexual adult worms (3), mice with worms of one sex usually exhibit low levels of resistance to challenge (4, 5) as compared with mice having patent infections with organisms of both sexes.

Because bisexually infected mice develop high levels of resistance to challenge 6–8 wk after the primary infection (6) and female worms begin laying eggs ~5 wk postinfection, egg production has been linked to high levels of resistance.

In investigations of the putative link between egg production and immunity, immunization with isolated eggs or egg extracts has, in general, failed to demonstrate any protective effect (5, 7–10) although one study by Moore et al. (11) did demonstrate low levels of resistance to challenge in mice that were immunized with eggs or egg extract. Moreover, in experiments where immunization with eggs resulted in significant resistance to challenge, the protective effect was ascribed to the granulomatous response to eggs in the tissues, which was thought to kill or damage migrating parasites nonspecifically (6, 9, 12).

Thus, the data available are ambiguous as to the role of the egg in naturally acquired resistance. For this reason, we began production of lymphocyte hybridomas secreting monoclonal antibodies to egg antigens to find out if eggs or egg antigens could induce protective antibodies.

We report here the production of anti-egg monoclonal antibodies that recognize antigenic determinants on the surface membranes of developing schistosomula. We also show that these anti-egg monoclonal antibodies are partially protective against challenge infection, demonstrated by the *in vivo* passive transfer of immunity.

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### Materials and Methods

*Mice.* CBA/J, BALB/c, and C57BL/6 mice were purchased from The Jackson Laboratory, Bar Harbor, ME.

*Parasites.* A Puerto Rican strain of *Schistosoma mansoni* was maintained in our laboratory using *Biomphalaria glabrata* snails and CBA/J mice. *Schistosoma japonicum*- and *S. haematobium*-infected snails were obtained from Dr. Liang at the University of Lowell, Lowell, MA.

Skin schistosomula were prepared by allowing cercariae to penetrate rat abdominal skin, as described by Clegg and Smithers (13). In addition, schistosomula were prepared mechanically (14) using a vortex mixer in the case of cercariae of *S. mansoni* and *S. haematobium* or by passage of cercariae through a 23-gauge needle for *S. japonicum*. Parasite bodies were separated from tails by centrifugation on a Percoll (Pharmacia Fine Chemical, Piscataway, NJ) gradient, as was described by Lazdins et al. (15).

Lung worms were prepared as follows: mice (C57BL/6) were infected with ~10,000 mechanical schistosomula by tail vein injection. 4 d later, the lungs were removed and the lung chop assay was performed as described by Sher et al. (16), except that the lungs were not perfused before removal from the mouse.

Eggs were collected from the livers of mice infected for 8 wk with *S. mansoni*. The infected livers in 0.225 M saline were homogenized in a Waring blender. The homogenate was partially cleared by sedimenting and decanting, as was described by Coker and von Lichtenberg (17). Eggs were separated from contaminating liver tissue by centrifugation through a mixture of 40% Percoll in 0.225 M saline. The purified eggs were washed free of Percoll and resuspended in assay medium (minimum essential medium containing 20 mM Hepes and 2 mM L-glutamine, pH 7.4). Soluble egg antigen (SEA)<sup>1</sup> was prepared by homogenizing purified eggs in phosphate-buffered saline (PBS), pH 7.4, at 4°C for 1 h. The egg homogenate was centrifuged at 15,000 g for 60 min at 4°C and the supernatant was collected as SEA and stored at -70°C. Microfilaria of *Brugia malayi* were prepared by Dr. Louis Lamontagne and Dr. W. Piessens of the Department of Tropical Public Health at the Harvard School of Public Health.

*Infected and Normal Mouse Sera.* Female C57BL/6 mice were infected percutaneously with ~50 cercariae. Infected mice were bled at weekly intervals, starting at 12 wk postinfection. Sera for passive transfer of immunity experiments were collected from mice infected between 12 and 16 wk and pooled. Normal mouse sera were obtained from age- and sex-matched C57BL/6 mice. Myeloma IgG2b (Litton Bionetics Inc., Kensington, MD) was used as a control in some experiments.

*Production of Monoclonal Antibodies.* BALB/c mice were primed by intravenous (tail vein) injection of ~1,000 purified schistosome eggs. For one fusion, a group of mice were boosted in a manner identical to the way they were primed. A second fusion was done in which mice were boosted intravenously with 100 µg of SEA. In both cases, mice were sacrificed and their spleens were removed 4 d after the boost. The fusion protocol was according to Kennett et al. (18), using NS-1 cells as the myeloma line and polyethylene glycol 1000 as the fusing agent. Hybridomas positive for surface binding were cloned by limiting dilution using irradiated spleen cells as a feeder layer. Cloned hybridomas were maintained in culture, and culture supernatant was the source of monoclonal antibody for all experiments. In experiments where concentrated culture supernatants were used, concentration was by ultrafiltration using an Amicon YM-10 membrane (Amicon Corp., Danvers, MA). Antibody concentration was determined by quantitative rocket immunoelectrophoresis (19).

Monoclonal antibody EG1C4B1 (E.1), an IgG2b, was purified from culture supernatants by protein A chromatography as described by Ey et al. (20). Purified E.1 was dialyzed against 400 vol of PBS, pH 7.4, overnight at 4°C. The pure monoclonal antibody was next concentrated to 2 mg protein/ml by Amicon ultrafiltration. Protein concentration was determined by Bradford assay (21). Fab fragments of E.1 were prepared by papain

<sup>1</sup> Abbreviations used in this paper: DMEM, Dulbecco's modified Eagle's medium; ELISA, enzyme-linked immunosorbent assay; FCS, fetal calf serum; PBS, phosphate-buffered saline; SEA, soluble egg antigen; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

digestion according to the method of Dresser (22). Fab fragments were purified from Fc and undigested antibody by protein A chromatography, and the purity of the Fab fraction was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (23).

*Isotype Analysis.* Culture supernatants from cloned hybrids were assayed for immunoglobulin isotype using the immunoglobulin subtype enzyme-linked immunosorbent assay (ELISA) assay (Boehringer Mannheim Biochemicals, Indianapolis, IN).

*Detection of Surface Binding Monoclonal Antibodies.* Living, skin-transformed schistosomula and purified cercarial tails were used in all of the indirect immunofluorescence assays. Schistosomula were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g glucose/ml and supplemented with 5% heat-inactivated fetal calf serum (FCS), 100  $\mu$ g/ml penicillin, and 100  $\mu$ g/ml streptomycin. Cultures of schistosomula at a concentration of 2,500 per milliliter were maintained in a humidified atmosphere in 7.0% CO<sub>2</sub> at 37°C.

For the fluorescence assay, schistosomula and cercarial tails were washed separately in assay medium (minimum essential medium containing 20 mM Hepes and penicillin/streptomycin with no FCS, pH 7.4), then resuspended in assay medium at a concentration of 2,000 per milliliter. Approximately 200 schistosomula plus 200 cercarial tails were delivered into wells of a 96-well V-bottomed microtiter plate (Dynatech Laboratories, Alexandria, VA). Then an equal volume of undiluted hybridoma culture supernatant or control (infected or normal mouse) serum diluted 1:40 in assay medium was added. The plates were incubated for 45 min at room temperature, then washed four times by aspiration and centrifugation using a Dynatech miniwash to aspirate and refill the wells with assay medium. Rabbit anti-mouse IgG (Cappel Laboratories, Cochranville, PA) was diluted 1:100 in assay medium and added to each well for 30 min at 4°C. After washing four times, fluorescein-conjugated goat anti-rabbit IgG (Boehringer Mannheim Biochemicals), diluted 1:100 in assay medium, was added to each well for 30 min at 4°C. Parasites were washed four times and then examined using a Leitz fluorescent microscope.

*In Vitro Complement-mediated Killing Assay.* Schistosomula obtained after skin penetration (3-h skin schistosomula) were used in these experiments. Before culture, the schistosomula were washed in assay medium and resuspended in DMEM containing 2.0% FCS, 10% NCTC 109, 2 mM L-glutamine, and penicillin/streptomycin as an antibiotic. Approximately 150 schistosomula per well were delivered into a Linbro 96-well flat-bottomed plate (Linbro Chemical Co., Hamden, CT). Then, each well received either 100  $\mu$ l of undiluted hybridoma culture supernatant or 100  $\mu$ l of infected or normal mouse sera diluted 1:10. Parasites were incubated for 30 min followed by the addition of fresh or heat-inactivated guinea pig sera. Cultures were kept in a humidified atmosphere at 37°C with 7.0% CO<sub>2</sub>. Damage to the tegument of the parasites was scored by examination with a microscope. Death of parasites was assessed microscopically by toluidine blue uptake and loss of motility.

*In Vivo Passive Transfer: Lung Assay.* In these experiments, hybridoma culture supernatants were used at 1 $\times$  and 5 $\times$  concentrations. Controls were normal mouse sera or infected mouse sera diluted 1:40 in DMEM plus 15% FCS. 10-wk-old female C57BL/6 mice were given 1.5 ml culture supernatant or control sera by intraperitoneal injection 24 h before challenge. Mice were challenged with ~500 skin schistosomula given by intravenous tail vein injection. 4 d later, the mice were sacrificed, lungs removed, and the lung chop assay was performed as described by Sher et al. (16). The percentage reduction in recoverable worms was calculated as  $[(A - B)/A] \times 100$ , where A is the mean of the control group and B is the mean of the experimental group.

*In Vivo Passive Transfer of Immunity: Adult Worm Assay.* As in the lung assay, culture supernatants were the source of monoclonal antibody in all experiments. In experiments where concentrated culture supernatants were used, they were concentrated as described above. Control sera are as described for the lung assay. A myeloma IgG2b (Litton Bionetics, Inc.) was used as an additional control in most experiments.

Monoclonal antibody E.1 and control sera were administered by intraperitoneal injection to 10-wk-old female C57BL/6 mice between 5 and 20 h before challenge. Mice were

challenged with cercariae (100–180 per mouse depending on the experiment) by belly penetration. Adult worms were collected 6–8 wk postchallenge by mesenteric vein perfusion (24, 25). Livers were also squashed and examined for worms. The percentage reduction of adult worms was calculated as described for the lung assay. The significance of the differences between control and experimental groups was calculated using the Student's *t* test.

*Western Blot Analysis of Antigens.* Molecular weights of egg and schistosomula antigens recognized by anti-egg monoclonal antibodies were determined by Western blot (26). For this analysis, we used soluble egg antigen and a detergent extract of whole mechanical schistosomula, which enriched for surface membrane components. Soluble egg antigen was prepared as described for egg purification; however, the following protease inhibitors were added before homogenization: chymostatin (4 mM), leupeptin (4 mM), aprotinin (0.03%), phenylmethylsulfonyl fluoride (2 mM), *N*- $\alpha$ -*p*-tosyl-L-lysine chloromethyl ketone HCl (TLCK) (0.2 mM), and L(tosylamide-2-phenyl)ethyl-chloromethyl-ketone (TPCK) (0.2 mM).

Surface extract of schistosomula was prepared by incubating mechanical schistosomula (cultured for 3 h) in 50 mM phosphate buffer, pH 8.0, containing 4 mM deoxycholate and the same protease inhibitors as were used in the preparation of SEA. Approximately 100,000 schistosomula per milliliter were kept in detergent solution on ice for 30 min. Soluble schistosomula surface preparation was collected by centrifugation of the detergent extract at 15,000 *g* for 60 min at 4°C. The supernatant was collected and stored at -70°C.

For Western blotting, antigen samples were electrophoresed according to Laemmli (23). At the completion of SDS-PAGE, the proteins were transferred from the gels to nitrocellulose paper as described by Towbin et al. (26). After transfer, the nitrocellulose paper was quenched by incubation at room temperature for 1 h in PBS containing 0.30% Tween 20. The nitrocellulose strips were then incubated with anti-egg monoclonal antibodies or control antibody (anti-*Leishmania* IgG2b provided by Dr. Diane McMahon Pratt, Harvard Medical School) overnight at 4°C. After this incubation, the strips were washed in PBS containing 0.05% Tween 20. Finally, the strips were incubated with <sup>125</sup>I-rabbit anti-mouse IgG, washed in PBS-Tween, dried, and exposed to x-ray film.

## Results

*Detection of Surface-Binding Antibodies.* Anti-egg hybridoma culture supernatants were screened by indirect immunofluorescence for surface-binding antibodies on living skin-stage schistosomula and isolated cercarial tails. Hybridomas yielding supernatants that were positive in the initial assay were cloned and then rescreened for surface binding antibody. Fig. 1 compares the surface-binding patterns seen on skin-stage schistosomula when monoclonal antibody E.1, infected mouse serum, or normal mouse serum were examined. It can be seen that both monoclonal antibody E.1 and infected mouse serum bound uniformly to the surface of the schistosomular body. Although not shown in the photographs, E.1 and chronic mouse serum also bound to cercarial tails. Infected mouse serum and the monoclonal antibody exhibited comparable levels of fluorescence intensity in all indirect immunofluorescence assays performed. All surface-binding monoclonal antibodies produced using lymphocytes from mice immunized with whole living eggs bound to both schistosomular bodies and cercarial tails. Monoclonal antibodies generated from the fusion in which SEA was used for the final boost (see Materials and Methods) demonstrated two different surface-binding patterns: those which bound to the surface of schistosomular bodies and cercarial tails, such as E.1, and those which only bound to the surface of schistosomula but not to cercarial tails.

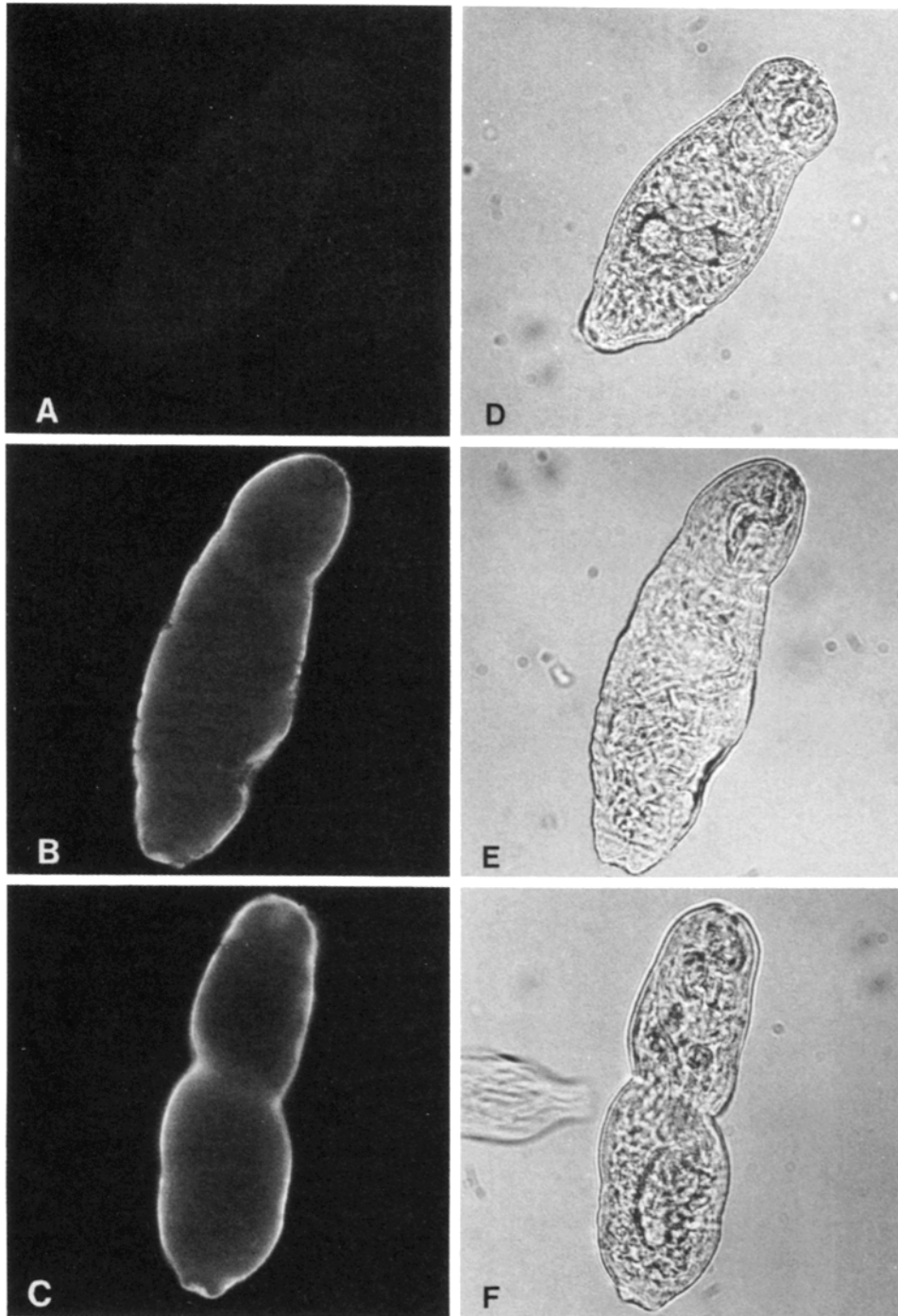


FIGURE 1. (A, B, C) fluorescence patterns, (D, E, F) light microscope (A + D) normal mouse serum (B + E) monoclonal antibody EG1C4B1, (C + F) infected mouse sera.

*Persistence of Antigens on Developing Schistosomula.* Studies were performed to assess how long after transformation of cercariae to schistosomula the monoclonal antibodies could still detect surface antigens. Skin-stage schistosomula were cultured as described for up to 96 h posttransformation, and, during this period, the parasites elongated and gut development occurred. 11 monoclonal antibodies were assayed for surface binding at 24, 48, and 96 h after transformation of the schistosomula. Table I lists the 11 monoclonal antibodies tested and the last time point at which uniform surface membrane binding was detectable. Monoclonal antibody E.1 was representative of the group of monoclonal antibodies that bound to 96-h schistosomula. However, E.1 did not bind to freshly harvested lung worms or to lung worms that were cultured for 24–48 h after collection.

*Species Specificity Assay with E.1.* To see if monoclonal antibody E.1 could detect antigen on other species of human schistosomes or on heterologous helminths, we assayed for surface binding on mechanical schistosomula of *S. haematobium* and *S. japonicum* and on microfilaria of *Brugia malayi* using indirect immunofluorescence. E.1 bound to the schistosomula bodies and to the cercarial tails of both species of schistosomes. No detectable fluorescence was observed on the microfilaria. The intensity of fluorescence was the same in *S. mansoni* and *S. japonicum* but lower on the *S. haematobium* organisms.

*In Vitro Complement-mediated Helminthotoxicity Using E.1.* Monoclonal antibody E.1 was used in these experiments because it detected antigen on the surface of developing schistosomula for at least 96 h posttransformation and was shown to be an IgG2b in the isotype ELISA. Infected and normal mouse sera were used as positive and negative controls. Experiments were done using fresh or heat-inactivated guinea pig serum as complement source and control. Monoclonal antibody E.1, when incubated with fresh guinea pig serum, caused tegumental blebbing and granulation of parasites with 24 h of incubation. Control schisto-

TABLE I  
*Persistence of Surface Membrane Antigens on Cultured Schistosomula*

Clone	Isotype	Hours post-transformation that antigen is still detectable*
EG1C4B1	G <sub>2</sub> b	96
EG564C7	G <sub>2</sub> b	96
EG2H5A9	G <sub>3</sub>	96
EG5H1	G <sub>3</sub>	96
SG1D12D8	M	96
SG1D12D10	M	96
SGZ1D4E5	M	96
SG5E12.D11	M	48
SG1D12.E12	A	48
SG43B11E8	M	48
SG5E12.C8	M	24

E denotes monoclonals generated by immunization with schistosome eggs. S denotes monoclonals generated from mice whose final boost was SEA.

\* Determined by indirect immunofluorescence on living parasites.

somula cultured for the same period of time in normal mouse serum and fresh guinea pig serum appeared normal. Fig. 2 demonstrates the contrast between parasites in normal mouse serum and complement and parasites in monoclonal antibody and complement. The maximal number of dead parasites, as deter-

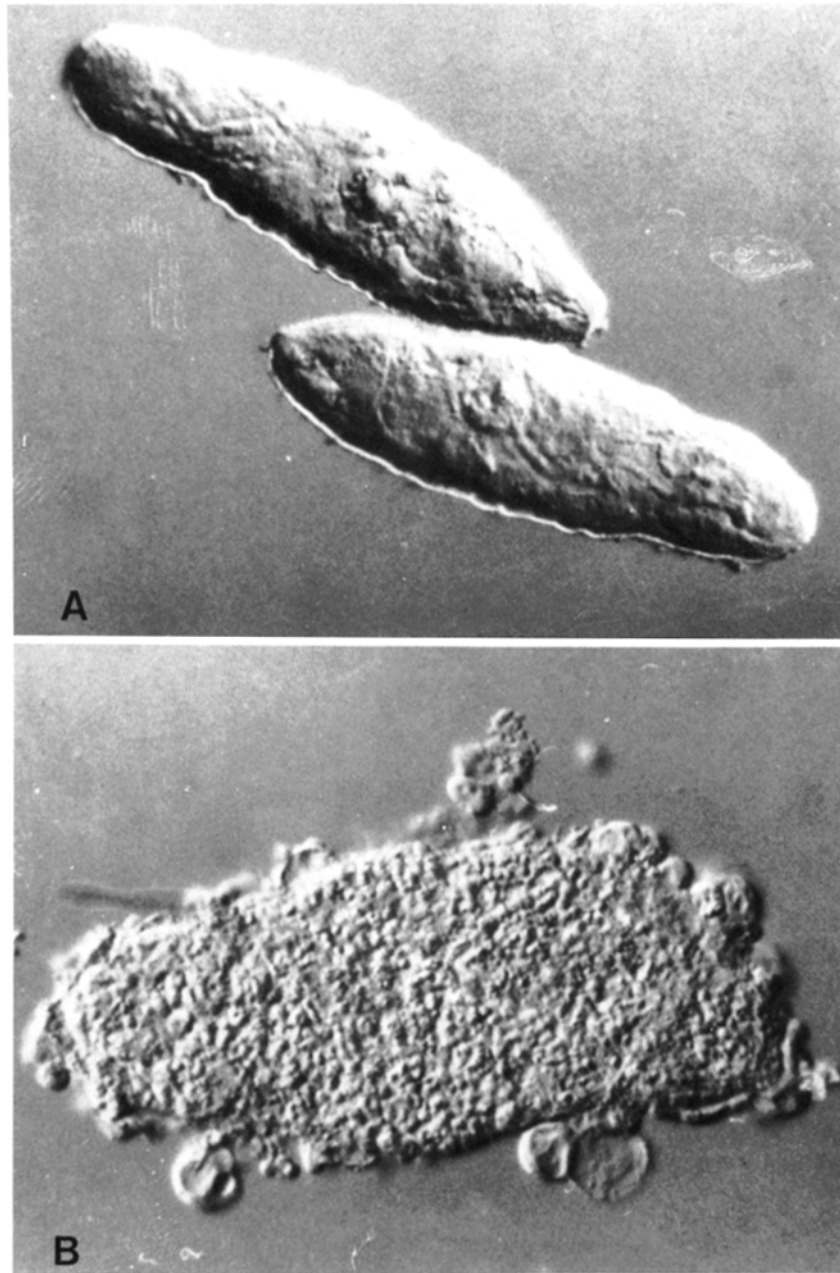


FIGURE 2. Complement-mediated damage to schistosomula. (A) normal mouse sera plus fresh guinea pig sera, (B) EG1C4B1 plus fresh guinea pig sera.

mined by dye uptake and loss of motility, was found on day 3. The percentage of parasites killed by monoclonal antibody or infected mouse serum plus fresh guinea pig serum was similar, 65 and 73%, respectively, while only 2.4% were killed when normal mouse serum was used (see Table II). In experiments where heat-inactivated guinea pig serum was substituted for fresh guinea pig serum, only 7.0 to 8.0% of the parasites were killed when coincubated with monoclonal antibody or infected mouse serum, indicating that parasite killing was complement dependent.

*In Vivo Passive Transfer of Immunity: Lung Assay.* These experiments were done to see if monoclonal antibody E.1 could alter patterns of invasion and/or migration of larvae in vivo. In all of these experiments, monoclonal antibody or control sera were administered to the mice at least 3 h before challenge. The delay of migration of parasites was measured by lung assay. Normal and infected mouse sera were used as controls. As is shown in Table III, monoclonal antibody E.1 was as effective or more effective than infected mouse serum in reducing the percentage of worms detected in the lungs on day 4 postchallenge. In the

TABLE II  
*Complement-mediated Damage/Killing of Skin Schistosomula Using Monoclonal Antibody EGIC4B1*

	Guinea pig sera	Percent of schistosomula damaged* at 24 h	Percent of schistosomula killed** at day 3
Normal mouse serum	Fresh	2.5	2.4
	Heated	2.0	1.7
Chronic sera (1:10)	Fresh	42.0	72.6
	Heated	9.9	7.8
EGIC4B1	Fresh	33.3	64.8
	Heated	15.4	7.0

\* Membrane blebbing and granularity.

‡ Determined by toluidine blue uptake/exclusion and nonmotility.

TABLE III  
*Passive Transfer of Protection to Schistosoma Mansoni Using Monoclonal Antibody EGIC4B1\**

Experi- ment		Mean number of worms	Percent protection
1	Normal sera	44 ± 15	—
	Chronic sera	16 ± 11	66
	Monoclonal antibody	19 ± 8	57
2	Normal sera	40 ± 20	—
	Chronic sera	12 ± 9	70
	Monoclonal antibody	5 ± 6	88
3	Normal sera	63 ± 16	—
	Chronic sera	19 ± 16	60
	Monoclonal antibody	16 ± 11	75

\* 500 mechanical somula injected intravenously. Monoclonal antibody (culture supernatant) given 3–12 h earlier. Lung worms harvested on day 4. Experiments 2 and 3 used 5× concentrated culture supernatant.



three experiments shown, the reduction in the number of lung worms recoverable on day 4 ranged from 57 to 88%. The reduction was maximum in experiments 2 and 3, where five times concentrated culture supernatants were used as the source of monoclonal antibody.

*In Vivo Passive Transfer of Immunity: Adult Worm Assay.* To test the effects of monoclonal antibody E.1 on natural infections in mice, we performed passive transfer of immunity experiments, assaying for surviving adult worms 6–8 wk postchallenge. The level of protection was based on the reduction in the number of surviving adult worms in mice given E.1 as compared with mice given normal mouse serum or myeloma G2b. In five of these experiments, mice were given varying amounts of monoclonal antibody to see if the protective effect of E.1 might be dose dependent. In one of these experiments, we also tested to see if antigen-binding Fab fragments could mediate an *in vivo* protective effect. Results from these five separate experiments demonstrate that significant levels of protection were achieved even when extremely low levels of monoclonal antibody were used. Fig. 3 summarizes five experiments and shows that the level of protection was apparently dose dependent within the 40–150  $\mu\text{g}$  range of monoclonal antibody concentrations. Levels of protective immunity ranged from 21% for 40  $\mu\text{g}$  of antibody to 41% for 300  $\mu\text{g}$  of antibody. Table IV shows the data for the experiment that gave a protective level of 41%. It is also shown in Table IV that antigen-binding Fab fragments were unable to mediate any protective immunity, indicating that the Fc portion is probably important in such immunity.

*Analysis of the Molecular Weights of Antigens Detected by Anti-Egg Monoclonal*

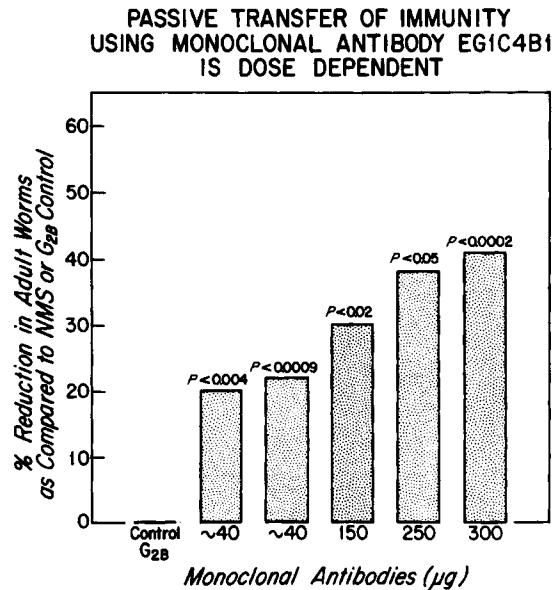


FIGURE 3. Passive transfer of immunity by EG1C4B1. Adult worm assay. Results of five different experiments using different amounts of monoclonal antibody are shown. The number of worms recovered from negative control mice for each experiment were as follows: 40  $\mu\text{g}$ ,  $67 \pm 9$ ; 150  $\mu\text{g}$ ,  $48 \pm 9$ ; 250  $\mu\text{g}$ ,  $81 \pm 7$ ; 300  $\mu\text{g}$ ,  $91 \pm 5$ .

TABLE IV  
*In Vivo Passive Transfer of Protection by Purified Monoclonal Antibody EG1C4B1, But Not by Fab Fragment of EG1C4B1: Adult Worm Assay\**

	Number of adult worms ± SEM	Percent protection
Myeloma G2b	91.2 ± 4.6 (n = 9)	—
Purified EG1C4B1‡	54.0 ± 6.5 (n = 9)	41 (P < 0.0002)
FAB of EG1C4B1§	97 ± 6.0 (n = 6)	0

Mice were challenged with 180 cercariae per mouse by belly penetration.

\* Recovered 6 wk postinfection.

‡ Protein A-Sepharose-purified; 150 µg was administered at 25 and 5 h before challenge (300 µg/mouse total).

§ Fab fragments of protein A-purified antibody administered as above.

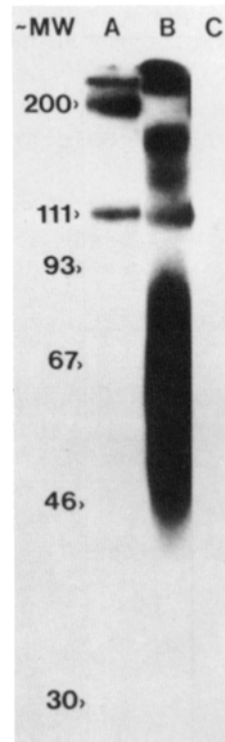


FIGURE 4. Western blot analysis of egg antigens detected by monoclonal antibodies. (A) monoclonal antibody EG1C4B1; (B) monoclonal antibody 5E12:D11, representative of the 48-h group of monoclonal antibodies; (C) anti-*Leishmania* monoclonal antibody (IgG2b).

*Antibodies.* Anti-egg monoclonal antibodies that bound to the surface of skin-stage schistosomula were used in Western blot analysis to determine molecular weights of the respective antigens. As is shown in Fig. 4, two different molecular weight patterns were exhibited by anti-egg monoclonal antibodies binding to egg antigens. Protective monoclonal E.1 (Fig. 4A) is representative of the group of monoclonals that bind to 96-h schistosomula. The antigens detected on eggs by these monoclonals are at approximate molecular weights of greater than 200,000,

200,000, and 111,000. Those monoclonal antibodies that bind to 48-h worms bind to the egg antigens shown in lane *B*. In both cases, the samples were not reduced. When comparing egg and schistosomular antigens recognized by E.1 (Fig. 5), we noticed that the two antigens in the schistosomular preparation were of 160,000 and 130,000 molecular weight, different from those found in soluble egg antigen.

### Discussion

The data presented herein show that monoclonal antibodies recognizing surface membrane antigens on schistosomula and/or cercariae can be produced from mice immunized with eggs. It is also shown that one anti-egg monoclonal antibody, E.1, partially protects naive mice from challenge with cercariae. Although other workers (5, 7-10) have tried previously to induce protective immunity by immunizing with eggs or egg extracts, such attempts have generally failed to generate resistance to challenge infection. On the other hand, it has been shown that there are many different egg antigens (27-29) and that egg antigens elicit several types of immune reactions (30-32). Significantly, eggs or egg antigens have been implicated in the suppression of the cell-mediated immune response (33-35).

Our data, taken together with the results of these earlier studies, suggest the

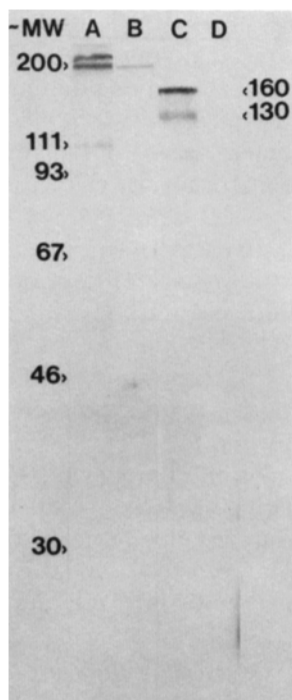


FIGURE 5. Western blot comparing egg and schistosomula antigens recognized by monoclonal antibody EG1C4B1. (A + B) SEA, (C + D) schistosomula surface extract, (A + C) monoclonal antibody EG1C4B1, (B + D) anti-*Leishmania* monoclonal antibody (IgG2b). The minor band (B) 200,000 mol wt, seen by anti-*Leishmania* monoclonal antibody, has only appeared this one time. Five other blots all appear in Fig. 4.

possibility that the inability to demonstrate protection in mice immunized with eggs or complex egg extracts results from suppression of the cell-mediated immune response, rather than the lack of protective antibody. We have demonstrated here, as have Lichtenberg et al. (8) and Bickle et al. (10) in earlier studies, that immunization with eggs gives rise to antibodies that recognize surface membrane epitopes on cercariae and schistosomula. Thus, if there is a suppressive response to avoid, it may be necessary to immunize with only one or a limited number of egg epitopes. Although several laboratories have reported the purification of different egg antigens (36–39), we know of no attempts to induce protective immunity with them.

However, we do know from results presented here that there are at least three egg antigens that cross-react with surface determinants on developing schistosomula. One of these antigens is putatively protective, and is present on the surface membranes of *S. japonicum* and *S. haematobium* as well as *S. mansoni*. The same antigen has also been shown to persist on developing larvae for up to 96 h posttransformation.

The observation that antigens persist on developing larvae is interesting in light of the work by Samuelson et al. (40) and Dessein et al. (41). Both of those studies showed that a majority of the antigenic components on the early schistosomulae were shed within the first 24 h of culture. In this study, we examined 11 different anti-egg monoclonal antibodies and detected three different time patterns of antigen persistence. However, both of the other studies used serum antibodies, either from immunized rats or infected patients, whereas we used monoclonal antibodies prepared from mice immunized with eggs or egg products. Since the serum antibodies probably recognized several different surface antigens, it is quite possible that a loss of one or more major surface antigens during development would dramatically lower the overall fluorescence intensity observed.

In our experiments on protective immunity, we concentrated on a monoclonal antibody (E.1) that bound to the surface of developing larvae for at least 96 h posttransformation. This monoclonal antibody, when injected intraperitoneally, markedly diminished the number of lung worms that were recoverable. The reduction in numbers of recoverable parasites was comparable to or better than that obtained when infected mouse serum was used. E.1 was also very effective in reducing the adult worm burden. The level of immunity in each experiment appeared to be related to the amount of monoclonal antibody administered. The upper levels of protection are comparable or higher than those achieved by Smith et al. (42) or Zodda and Phillips (43). In their studies, the source of monoclonal antibodies was ascites or serum. In either case, the level of antibody administered in their experiments was probably much higher than that used in this study.

In one passive transfer experiment, we found that purified Fab fragments were unable to reduce the adult worm burden when compared with purified intact E.1. Thus, the Fc portion of the monoclonal antibody was necessary for *in vivo* protection. Whether the intact antibody mediates protection *in vivo* via complement or by antibody-dependent cellular cytotoxicity is not known. In our experiments, E.1 was capable of killing parasites *in vitro* with complement; however,

Sher et al. (44) have demonstrated that complement-depleted mice do not show diminished levels of resistance to challenge *in vivo*.

Although protective monoclonal antibodies to *S. mansoni* have been produced by Smith et al. (42), Zodda and Phillips (43), and Grzych et al. (45), only the latter two groups have been able to use the monoclonal antibodies to characterize the relevant antigens by immunoprecipitation. The antigen detected by E.1 is different in molecular weight from the antigen described by Dissous et al. (46). The molecular weight of our antigen is similar to that of the antigen described by Zodda and Phillips (43), but in side by side Western blot experiments performed at the Schistosome Antigen Workshop (47), their monoclonal antibody did not bind to the same antigen preparations as did E.1.

Surface antigens recognized by monoclonal antibodies produced by others (48–50) are also probably different from the antigen detected by E.1. The surface antigen reported by Taylor and Butterworth (48) is different in molecular weight from that detected by E.1. Though the molecular weights of antigens reported by Norden et al. (49) and Aronstein et al. (50) are similar to that of the antigen detected by E.1, their monoclonal antibodies differ in that they do not bind to eggs (49) or to cercariae (50).

Although our results suggest that egg antigens may be important in inducing naturally acquired resistance, it is of note that effective protective immunity can also be induced by irradiated cercariae that do not produce eggs (51, 52).

The finding that an anti-egg monoclonal antibody is partially protective suggests the possible use of egg antigens that cross-react with schistosomula for vaccination studies. It must be considered, however, that egg antigens can sensitize for granuloma formation (26). However, studies by Warren and Domingo (53) have shown that immunization with cercariae, unisexual infections, or dead worms do not sensitize for granuloma formation. Because our protective antigen cross-reacts with epitopes on larval stages, it also is likely not to sensitize for granuloma formation, although this must be tested.

The data presented in this paper suggest that if naturally acquired immunity to schistosomes is dependent on the production of eggs, the function of the egg in protection may not be totally nonspecific. The results clearly show that eggs and egg antigens elicit the production of antibodies that cross-react with membrane antigens on invading larval stages and that can mediate protective immunity.

### Summary

Monoclonal antibodies that bind to surface membranes of developing schistosomula and/or cercarial tails were generated from mice immunized with living schistosome eggs or soluble egg antigen. These monoclonal antibodies detected at least three different surface epitopes. One surface antigen detected by anti-egg monoclonal antibody EG1C4B1 (E.1) persisted on the surface of developing schistosomula for 96 h posttransformation. The same or a cross-reactive antigen was also detected on the surfaces of *S. japonicum* and *S. haematobium* schistosomula and cercarial tails. Monoclonal antibody E.1 killed schistosomula *in vitro* as well or better than infected mouse sera and transferred immunity to naive mice when administered *in vivo*. The monoclonal antibody reduced the number of lung

worms recoverable on day 4 postchallenge by up to 85% and reduced the adult worm burden up to 41% as compared with controls. The data also show that the molecular weights of the egg antigens detected by monoclonal antibody E.1 were different from those detected on schistosomula.

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