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Cloning of a cDNA encoding a surface antigen of *Schistosoma mansoni* schistosomula recognized by sera of vaccinated mice

(monoclonal antibody/antigen purification/cDNA expression library/fusion protein/schistosome)

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ABSTRACT **Spleen cells of mice vaccinated with radiation-attenuated *Schistosoma mansoni* cercariae were used to produce monoclonal antibodies directed against newly transformed schistosomular surface antigens. One of these monoclonal antibodies recognized a polypeptide of 18 kDa. This glycoprotein was purified by monoclonal antibody immunoaffinity chromatography and a polyclonal antiserum was prepared against it. Immunofluorescence assays showed that the polyclonal antiserum bound to the surface of newly transformed schistosomula and lung-stage organisms but not to the surface of liver-stage and adult worms. Using this polyclonal antiserum we isolated recombinant clones from an adult worm cDNA expression library constructed in λgt11. Clone 654.2 contained an insert of 0.52 kilobase and hybridized to a 1.2-kilobase mRNA species from adult worms. Most importantly, clone 654.2 produced a fusion protein of 125 kDa that was reactive with sera of vaccinated mice that are capable of transferring resistance. This result encourages future vaccination trials with the fusion protein.**

High levels of resistance to *Schistosoma mansoni* challenge infection can be induced in mice, rats, and baboons by vaccination with radiation-attenuated cercariae. At least in mice and rats, this resistance can be passively transferred to naive recipients using serum or purified immunoglobulins, indicating the importance of humoral immune responses in this resistance. The most consistent transfer of resistance is achieved when immune sera are administered while the schistosomula are in residence in the lungs. All such sera, including those of vaccinated mice, rats, and rabbits and of infected rats, contain antibodies that bind to the surface of lung-stage and newly transformed schistosomula. In contrast, sera from mice chronically infected with *S. mansoni* do not consistently transfer resistance, and antibodies in these sera do not bind to the surface of lung-stage schistosomula (reviewed in ref. 1). It is therefore likely that resistance following vaccination with radiation-attenuated cercariae is mediated by antibody binding to epitopes exposed on the surface of newly transformed and lung-stage schistosomula.

Several *S. mansoni* proteins have recently been molecularly cloned and expressed in *Escherichia coli*; however, none of these appears to be localized on the surface of early developmental stages (2–5).

Our approach to identifying potential immunoprophylactic antigens was to prepare monoclonal antibodies (mAb) from spleens of vaccinated mice and to screen such antibodies for their ability to immunoprecipitate surface-labeled schistosomular polypeptides. By this method we obtained a mAb, designated 654B2/1, that reacted with a single 18-kDa surface-labeled polypeptide. We purified this antigen and prepared a polyclonal antiserum (anti-654) against it. Immunofluorescence studies using anti-654 showed that some epitopes on the antigen are exposed not only on the surface of newly transformed schistosomula but also on the surface of lung-stage schistosomula.

To obtain sufficient antigen to carry out active vaccination experiments we have used polyclonal anti-654 to screen an adult worm cDNA expression library constructed in λgt11. We have isolated a recombinant clone containing an insert that encodes a protein reactive not only with mAb 654B2/1 and polyclonal anti-654 but, more importantly, also with sera of mice vaccinated with attenuated cercariae.

MATERIALS AND METHODS

Parasites. *S. mansoni* cercariae, newly transformed schistosomula, and worm pairs were obtained as described (6). Lung-stage schistosomula were recovered from the lungs of Syrian Golden hamsters (Charles River Breeding Laboratories) infected i.p. 7 days earlier with 5000 cercariae. Minced lungs were incubated at 37°C in Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal calf serum and 10 units of heparin per ml. After 1 hr, lung-stage schistosomula were recovered by sequential filtration through 0.2-mm and 5.0-μm nylon mesh. Liver-stage and adult worms were perfused from hamsters infected with 100 cercariae for 4 and 8 weeks, respectively.

Preparation of mAb. mAb 654B2/1 was prepared from spleen cells of mice vaccinated twice with γ-irradiated (50 krad; 1 rad = 0.01 gray) cercariae (7). Spleens were removed 3 weeks after the second vaccination. Antibody 654B2/1, IgG2a, was purified from ascites fluid by affinity chromatography (8). Immunoglobulin UPC-10 (Litton Bionetics), also IgG2a, was used as a control.

Immunoaffinity Purification. Ten milligrams of *S. mansoni* adult worm concanavalin A-binding glycoproteins, isolated as described (7), was applied and recycled six times over a 654B2/1-Sepharose 4B immunoaffinity column (5-ml packed volume; 1 mg of IgG per ml). The column was washed extensively with Tris·HCl (pH 8.0) containing 0.001 M EDTA, 0.1 M NaCl, 2.5 M KCl, and 0.1% *n*-octyl β-D-glucopyranoside, and bound proteins were eluted with 5.0 ml of 0.05 M diethylamine (pH 11.5). The eluted fractions were neutralized with 1.0 M $NaHCO_3$ (pH 8.0), dialyzed, and concentrated against 20 mM NaH_2PO_4 (pH 7.0) containing 0.1% *n*-octyl β-D-glucopyranoside. The concentrated sample (0.5 ml) was purified by high-pressure liquid chromatography using a TSK G2000SW column (Bodman Chemicals, Media, PA) equilibrated in the same buffer. Binding of mAb 654B2/1 to each fraction was measured by solid-phase radioimmunoassay (9). Immunoreactive fractions were pooled and dialyzed against phosphate-buffered saline (pH 7.3) containing 0.01% *n*-octyl β-D-glucopyranoside.

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Abbreviation: mAb, monoclonal antibody(ies).

Immunization. A New Zealand White rabbit was immunized four times at 3-week intervals with 500 ng of protein per immunization at two subcutaneous sites and bled 3 weeks after the last booster injection. Muramyl dipeptide-threonine (provided by A. Allison, Syntex, Palo Alto, CA) was used as an adjuvant.

Immunofluorescence. Live newly transformed schistosomula, lung-stage schistosomula, liver-stage worms, or adult worms were incubated on ice in mAb 654B2/1 ascites fluid or rabbit antiserum for 30 min. The organisms were washed and bound antibodies were detected by fluorescein-conjugated goat anti-mouse or goat anti-rabbit IgG as described (10). Immunofluorescent staining of frozen sections (6 μm) of adult worms and livers of hamsters infected for 7 weeks was performed as discussed (10).

Radiolabeling and Solubilization of Proteins. Newly transformed schistosomula and adult male worm proteins were metabolically labeled with [^{35}S]methionine (6). Newly transformed schistosomula were surface-labeled with ^{125}I using the Iodo-Gen method (11). Schistosome proteins were extracted as described (7, 12).

Antisera. Pooled sera of patently infected mice were obtained 13 weeks after infection of ten 8-week-old female C57BL/6 mice (The Jackson Laboratory) with 25 *S. mansoni* cercariae. Sera of vaccinated mice were obtained as described (6).

Immunoprecipitation and Gel Electrophoresis. These procedures have been described (13).

Isolation of Adult Worm RNA. Total RNA was isolated from worm pairs by hot NaDodSO$_4$/phenol extraction (14). Poly(A)$^+$ mRNA was purified as described (15).

Construction of Adult Worm cDNA Library. cDNA was synthesized using a commercially available kit (Amersham). Blunt-ended double-stranded cDNA was ligated to phosphorylated *Eco*RI linkers (New England Biolabs), followed by digestion with *Eco*RI. cDNAs of >500 base pairs were ligated to *Eco*RI-digested, alkaline phosphatase-treated λgt11 vector DNA (Promega Biotec, Madison, WI), packaged *in vitro*, and plated on *E. coli* host strain Y1090 (r$^-$, m$^+$). Approximately 2.3 × 10^5 independent recombinants were obtained with a nonrecombinant background of about 5%, as determined by growth on L plates supplemented with 2 mM isopropyl β-D-thiogalactopyranoside and 5-bromo-4-chloro-3-indolyl β-D-galactoside.

Screening of Adult Worm cDNA Library. Phages were screened essentially as described by Young and Davis (16), using a 1:75 dilution of the rabbit anti-654 serum and biotinylated anti-rabbit IgG followed by avidin-conjugated horseradish peroxidase (Vector Laboratories, Burlingame, CA).

Subcloning of cDNA and Generation of RNA Transcripts. Restriction mapping studies of plaque-purified 654 recombinant phage DNA revealed an insert of 0.52 kilobase (kb) containing a damaged *Eco*RI site at the left arm (data not shown). Insert cDNA and 2 kb of flanking phage DNA were excised using *Sac* I and *Kpn* I and purified as described (17). Digestion with *Eco*RI yielded a 1.54-kb *Eco*RI/*Kpn* I fragment containing the cDNA insert and 1.02 kb of vector DNA, which was ligated into an Sp6/T7 dual promoter transcription vector (Promega Biotec). Radiolabeled probes were transcribed from 250 ng of linearized recombinant plasmid using SP6 or T7 polymerase and [α-^{32}P]CTP (4 mCi/ml; 1 Ci = 37 GBq). Buffers and instructions were provided by Promega Biotec.

RNA Transfer and Southern Blot Analyses. For RNA transfer blots, duplicate samples of 4 μg of poly(A)$^+$ *S. mansoni* adult worm RNA were denatured and electrophoresed through 0.8% agarose containing 2.2 M formaldehyde. For Southern blots, high molecular mass *S. mansoni* DNA from adult worm pairs was isolated (17), restriction endonuclease-digested, and run on a 0.6% agarose gel. Transfer to nylon membranes, prehybridization, and hybridization procedures for RNA transfer and Southern blots followed the Zeta-Probe manual (Bio-Rad). RNA transfer blots were probed with 1 × 10^7 cpm of complementary transcripts and Southern blots were probed with 5 × 10^7 cpm of T7 polymerase-promoted transcript from the recombinant plasmid. All probes were labeled to a specific activity of about 1 × 10^8 cpm/μg.

Electrophoretic Transfer Blot Analysis. Fusion protein was obtained by infecting *E. coli* strain Y1090 (r$^-$, m$^+$) with 2.5 × 10^5 recombinant phages. Infected bacteria were plated on a 150-mm L plate supplemented with 2 mM isopropyl β-D-thiogalactopyranoside and 100 μg of ampicillin per ml. After incubation for 6 hr at 40°C, plates were overlaid with 10 ml of 10 mM Tris·HCl (pH 8.0) with 2 mM MgSO$_4$, and the proteins were eluted by incubation overnight at 4°C with gentle agitation. Overlay buffer was centrifuged at 8000 × *g* for 10 min at 4°C to remove bacterial debris. Aliquots of lysate were electrophoresed and transferred to nitrocellulose (18). Filters were blocked and incubated in a 1:1000 dilution of rabbit anti-β-galactosidase antibody (Cappel Laboratories, Cochranville, PA), 1:75 dilution of polyclonal antibody 654, 1:100 dilution of mAb 654B2/1 ascites fluid, or 1:50 dilution of sera from vaccinated mice. Bound antibody was detected by incubation with biotinylated anti-rabbit or anti-mouse IgG followed by avidin-conjugated alkaline phosphatase (Vector Laboratories).

RESULTS

Identification of the Antigenic Target Recognized by mAb 654B2/1. mAb were prepared using spleen cells of mice vaccinated twice with radiation-attenuated cercariae. These antibodies were screened by radioimmunoprecipitation assays using ^{125}I-labeled surface polypeptides of newly transformed schistosomula. One mAb, 654B2/1, was reactive with a single surface-labeled polypeptide of 18 kDa (Fig. 1). Sera of patently infected and vaccinated mice were each reactive with more than five schistosomular surface-labeled polypeptides, including the 18-kDa polypeptide. More importantly, the immunogenicity of the 18-kDa polypeptide was enhanced in vaccinated mice (Fig. 1; compare lanes 1 and 2).

The 18-kDa polypeptide was isolated by mAb immunoaffinity chromatography from concanavalin A-binding glycoproteins of adult worms. Final purification of the glycoprotein was achieved by high-pressure liquid chromatography.

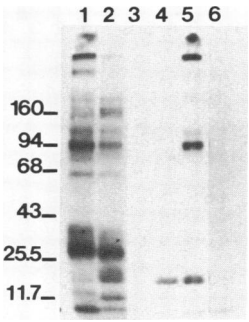

FIG. 1. Identification of surface-radioiodinated polypeptides of newly transformed schistosomula reactive with mAb 654B2/1 and polyclonal rabbit anti-654 serum: autoradiographs after one-dimensional 5–15% NaDodSO$_4$/PAGE. ^{125}I-labeled polypeptides were immunoprecipitated with sera of chronically infected mice (lane 1), sera of vaccinated mice (lane 2), sera of noninfected mice (lane 3), mAb 654B2/1 (lane 4), polyclonal rabbit anti-654 serum (lane 5), and serum of a noninfected rabbit (lane 6). Molecular masses are given in kDa.

The elution profile showed a single major peak corresponding to the 18-kDa glycoprotein and two very minor peaks not reactive with mAb 654B2/1. A polyclonal antiserum (anti-654) prepared against the purified 18-kDa glycoprotein immunoprecipitated this antigen from surface-labeled polypeptides of newly transformed schistosomula and also reacted with polypeptides of 94, 100, and >300 kDa (Fig. 1, lane 5). The only protein nonspecifically precipitated was a low molecular mass component (Fig. 1, lane 6).

Schistosomular proteins that had been metabolically labeled with [^{35}S]methionine were also tested as an antigen source (Fig. 2*A*). Sera from chronically infected mice immunoprecipitated many schistosomular polypeptides of >300 to <10 kDa. In contrast, sera from mice vaccinated twice were predominantly reactive with high molecular mass polypeptides. mAb 654B2/1 and anti-654 polyclonal antiserum immunoprecipitated an 18-kDa polypeptide; anti-654 was also reactive with polypeptides of 94 and 100 kDa.

When [^{35}S]methionine-labeled adult worm polypeptides were examined (Fig. 2*B*), mAb 654B2/1 and anti-654 polyclonal serum immunoprecipitated only the 18-kDa glycoprotein. Sera of patently infected mice were reactive with many polypeptides of >300 to <10 kDa, whereas sera of vaccinated mice precipitated fewer antigens, one of which was the 18-kDa glycoprotein. The 18-kDa antigen was a more predominantly labeled component of adult worms than of schistosomula. Two-dimensional NaDodSO$_4$/PAGE analysis revealed that the 18-kDa glycoprotein immunoprecipitated from schistosomular and adult worm extracts either by mAb 654B2/1 or by sera of vaccinated mice was focused as several isomorphs spanning a pH range of 6.5–5.5 (data not shown).

Immunofluorescence Studies. Since mAb 654B2/1 and the anti-654 polyclonal serum could precipitate surface-labeled polypeptides of newly transformed schistosomula, it was important to ascertain whether the epitopes recognized by these antisera were exposed on the surface of newly transformed schistosomula and/or later developmental stages. Indirect immunofluorescence studies on live newly transformed schistosomula, lung-stage schistosomula, 4-week-old liver-stage worms, and adult worms showed no surface fluorescence using mAb 654B2/1 (not shown). In contrast, incubation with anti-654 resulted in strong surface fluorescence on newly transformed and lung-stage schistosomula (Fig. 3) but no surface staining of liver-stage or adult worms (not shown).

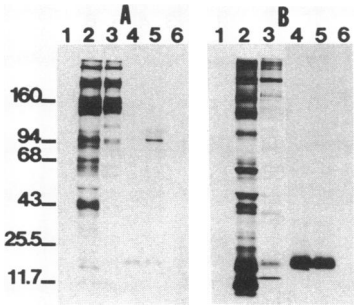

FIG. 2. Identification of newly transformed schistosomular and adult worm polypeptides metabolically labeled with [^{35}S]methionine and reactive with mAb 654B2/1 and polyclonal rabbit anti-654 sera: fluorograph after one-dimensional 5–15% NaDodSO$_4$/PAGE. ^{35}S-labeled polypeptides of newly transformed schistosomula (*A*) or adult worms (*B*) were immunoprecipitated by sera of noninfected mice (lanes 1), sera of chronically infected mice (lanes 2), sera of vaccinated mice (lanes 3), mAb 654B2/1 (lanes 4), polyclonal rabbit anti-654 serum (lanes 5), and serum of a noninfected rabbit (lanes 6). Molecular masses are given in kDa.

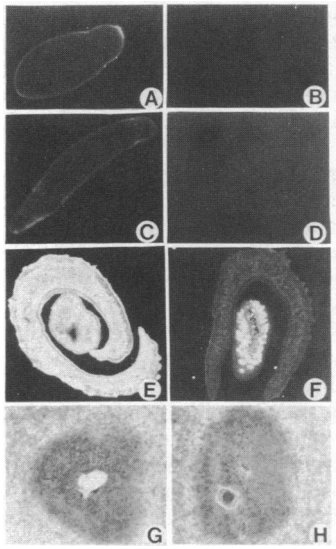

FIG. 3. Indirect immunofluorescence localization of the target antigen(s) of mAb 654B2/1 and polyclonal rabbit anti-654 on various developmental stages of *S. mansoni*. Polyclonal rabbit anti-654 showed strong staining of the surface of newly transformed schistosomula (*A*) and of the more elongated lung-stage schistosomula (*C*); no surface staining of these stages was observed with mAb 654B2/1 (not shown) or with serum of a noninfected rabbit (*B* and *D*). mAb 654B2/1 and polyclonal anti-654 showed intense staining within the tissues of male and female adult worms (*E*) and in schistosome eggs but not in the egg granuloma (*G*); no specific staining was observed with sera of noninfected mice, but autofluorescence of the female eggshells was seen (*F* and *H*). (×100.)

The distribution of the target epitope of mAb 654B2/1 was also investigated by immunofluorescent staining of frozen sections of newly transformed schistosomula and adult worms and livers from infected hamsters. The epitope was present throughout the tissues of schistosomula (not shown) and adult worms (Fig. 3). The epitope was also expressed in the egg stage but was not detected within the egg granuloma (Fig. 3). Serum from a noninfected rabbit did not show any specific fluorescence; however, autofluorescence of the female's egg shells was observed.

Identification of cDNA Clones Reactive with Rabbit Anti-654 Serum. The anti-654 polyclonal serum prepared against the 18-kDa glycoprotein was used to screen an adult worm cDNA expression library constructed in λgt11. Screening of 20,000 plaques yielded five positive clones. Physical mapping showed that each of these recombinant clones contained an insert of 0.52 kb and that all five clones had similar restriction maps (data not shown). Clone 654.2 was further characterized.

RNA Transfer, Southern, and Coding Strand Analyses. Clone 654.2 contained a cDNA insert lacking an *Eco*RI site at the left arm. This damage could have been caused by endonuclease star activity occurring during *Eco*RI digestion of cDNA in library construction (19). We therefore subcloned a 1.54-kb *Eco*RI/*Kpn* I fragment of clone 654.2 containing 1.02 kb of phage DNA into a recombinant plasmid containing SP6 and T7 polymerase promoters. Single-stranded RNA transcripts from either strand of cDNA were produced using either SP6 or T7 polymerase. When duplicate blots of poly(A)$^+$ RNA from adult worm pairs were probed with either transcript, only the probe synthesized using SP6 polymerase hybridized to an mRNA species of ≈1.2 kb (Fig. 4*A*); the T7 transcripts must therefore be derived from the coding strand. Southern blots probed with ^{32}P-labeled T7 transcripts showed hybridization to single bands of 3.5, 7.5, 6.6, and 3.1 kb in adult worm DNA digested with *Eco*RI, *Xba*

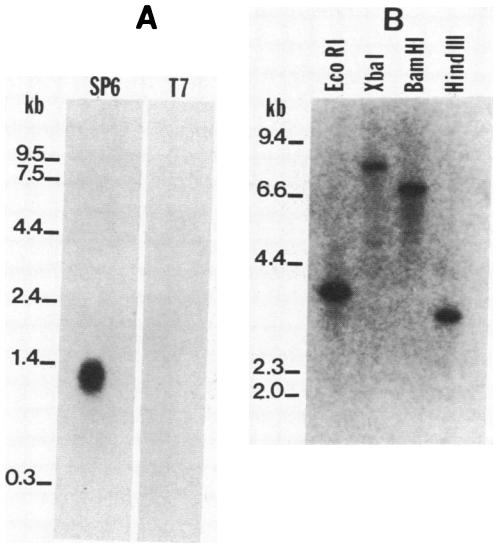

FIG. 4. RNA transfer and Southern blots. (*A*) RNA transfer blot analysis of *S. mansoni* adult worm poly(A)$^+$ RNA (4 μg) was performed and blots were probed with RNA transcripts of the cDNA insert of clone 654.2 synthesized using either SP6 or T7 polymerase. A Bethesda Research Laboratories RNA ladder was used for molecular size markers. (*B*) *S. mansoni* adult worm genomic DNA was endonuclease-digested with *Eco*RI, *Xba* I, *Hin*dIII, and *Bam*HI and run on a 0.6% agarose gel. After transfer the blots were probed with an RNA transcript of the cDNA insert of clone 654.2 synthesized using SP6 polymerase.

I, *Bam*HI, and *Hin*dIII, respectively (Fig. 4*B*). These data confirmed the presence of a homologous sequence of DNA in the genome of *S. mansoni*.

Characterization of Recombinant Fusion Protein. Lysates of bacteria infected with recombinant phage were tested for immunoreactivity following electroblotting using anti-β-galactosidase, mAb 654B2/1, and polyclonal anti-654 sera. A fusion protein of 125 kDa was detected with each of the antisera (Fig. 5). Similar results were obtained with sera of mice vaccinated twice with radiation-attenuated cercariae; in contrast, there was no detectable reactivity of the fusion protein with sera of chronically infected mice (data not shown). As expected, the anti-β-galactosidase antiserum detected parental β-galactosidase of 116 kDa in this lysate (Fig. 5). The difference in molecular size between β-galactosidase (116 kDa) and the recombinant fusion protein (125 kDa) indicated that the schistosome peptide portion of the fusion protein was about 9 kDa.

DISCUSSION

Epitopes exposed on the surface of newly transformed and lung-stage schistosomula appear to be the target of the protective humoral immunity observed in mice vaccinated with radiation-attenuated cercariae (1). In this study, we describe the approach used to identify and then clone a cDNA partially encoding an 18-kDa polypeptide expressed on the surface of schistosomula. This antigen exhibited an enhanced immunogenicity in mice protected against *S. mansoni* infection by vaccination with radiation-attenuated cercariae as compared to patently infected mice and may therefore represent a potential prophylactic immunogen.

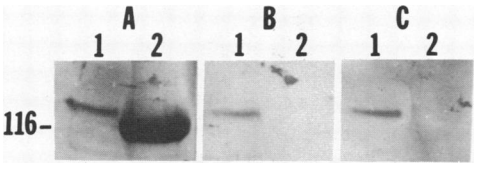

FIG. 5. Electrophoretic transfer blot analysis of recombinant fusion protein. Lysates of *E. coli* Y1090 (r^-, m^+) infected with recombinant (lanes 1) or parental phage (lanes 2) were subjected to 7.5% $NaDodSO_4$/PAGE before electroblotting to nitrocellulose. Filters were probed with rabbit anti-β-galactosidase (*A*), mAb 654B2/1 (*B*), or polyclonal rabbit anti-654 (*C*). Molecular mass is given in kDa.

The 18-kDa polypeptide was isolated by mAb immunoaffinity chromatography from concanavalin A-binding glycoproteins of adult worms. Isolation of sufficient purified antigen for vaccination trials would require a prohibitively large number of infected mice. We therefore used the purified 18-kDa glycoprotein to prepare a polyclonal antiserum for use in screening a cDNA expression library. The reactivity of this polyclonal antiserum with ^{35}S-labeled adult worm polypeptides was indistinguishable from that of mAb 654B2/1; both precipitated a single component of 18 kDa. The polyclonal antiserum immunoprecipitated additional ^{125}I-surface-labeled polypeptides of 94, 100, and >300 kDa from newly transformed schistosomula and the 18-, 94-, and 100-kDa antigens from schistosomula metabolically labeled with ^{35}S. In contrast, mAb 654B2/1 reacted only with the 18-kDa antigen, irrespective of the stage of parasite development examined. The immunoprecipitation of multiple schistosomular polypeptides by the polyclonal antiserum did not appear to be a result of either degradation or coprecipitation, since the mAb should also have precipitated the high molecular size components, and this was not observed. Nor is it likely due to the presence of antibodies generated against minor contaminants, since this polyclonal antiserum did not precipitate multiple polypeptides of adult worms, the stage from which the 18-kDa glycoprotein was isolated. The most likely interpretation of these observations is that the 94-, 100-, and >300-kDa polypeptides each share an epitope with the 18-kDa glycoprotein. Such cross-reactivity between schistosomular surface components has been observed for carbohydrate (20, 21) and peptide epitopes (20).

Immunofluorescence assays using the polyclonal anti-654 showed that an epitope(s) contained within the 18-kDa polypeptide is expressed on the surface of newly transformed and lung-stage schistosomula. Because the 18-, 94-, 100-, and >300-kDa polypeptides are all accessible to surface iodination and share an epitope(s), they may each contribute to the fluorescence observed. In the case of liver-stage and adult worms the epitopes recognized by anti-654 were not accessible at the surface and may be masked by host molecules (22). This correlates with the insusceptibility of schistosomula to immune-dependent elimination following migration from the lungs (23).

The expression of the 18-kDa polypeptide was not restricted to the surface of the organism but was detected by immunofluorescence studies throughout the tissues of schistosomula and adult worms. The antigen was also detected in the egg but not within the granulomata and therefore may not play a role in the egg-induced pathology of the disease.

Several other schistosomular surface antigens have been identified that are similar in molecular size to the 18-kDa polypeptide described here (24–26). Based on comparisons of their isoelectric points, concanavalin A binding, surface-labeling properties, and mobility, these polypeptides appear to differ from the 18-kDa glycoprotein reactive with mAb 654B2/1.

Several lines of evidence demonstrate that we have isolated a clone that encodes part of the 18-kDa polypeptide: (*i*) the clone was isolated from a cDNA library prepared using mRNA of adult worms, which do not detectably express the 94-, 100-, and >300-kDa polypeptides; (*ii*) anti-654 as well as mAb 654 recognized in adult worms only the 18-kDa poly-

peptide; and (*iii*) the fusion protein was reactive with mAb 654. Transfer blot analysis of poly(A)$^+$ RNA from adult worms detected an mRNA of 1.2 kb that was homologous to clone 654.2. Since only about 500 nucleotides are needed to code for a protein of 18 kDa, it appears that the native 18-kDa protein undergoes posttranslational modification or, alternatively, that the mRNA contains a large proportion of untranslated sequence. Since the schistosome portion of the fusion protein is 9 kDa, approximately half of the 0.52-kb cDNA insert is expressed by clone 654.2, suggesting the presence of a termination codon in the middle of the cDNA. Still, the fusion protein most likely represents >50% of the glycosylated native 18-kDa polypeptide.

Several groups have reported the cloning and expression of cDNAs coding for antigenic proteins from *S. mansoni* (2–5). One of these clones encodes a 22.6-kDa protein present within the adult tegument but not expressed in schistosomula (3). Lanar *et al.* (4, 5) have cloned several cDNAs coding for schistosome proteins of 27, 70, and 97 kDa, the last shown to be paramyosin. None of these proteins is expressed on the surface of schistosomula. Cordingley *et al.* (14) have identified cDNA clones encoding schistosomular surface antigens; however, these clones were not expressed in bacteria, and the native proteins have not been characterized. Clone 654.2 represents cloning and expression of a cDNA encoding a characterized schistosomular surface antigen. Recognition of the recombinant fusion protein by sera of vaccinated mice but not by sera of patently infected mice suggests that this protein contains epitopes that may be important in the induction of a protective immune response. This hypothesis is supported by the work of Omer-Ali *et al.* (27), who showed that the antibody response of vaccinated mice is directed toward surface peptide epitopes of schistosomula, whereas that of infected mice is directed predominantly against carbohydrate moieties. Future studies will assess the antigenicity and immunoprophylactic potential of the purified fusion protein.

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