MONOCLONAL ANTIBODIES AGAINST *SCHISTOSOMA MEKONGI* SURFACE TEGUMENTAL ANTIGENS

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Abstract. Monoclonal antibodies were produced from naturally infected BALB/c mice. Thirteen hybridomas which were found to produce monoclonal antibodies against surface tegumental antigens of *Schistosoma mekongi* by ELISA assay were used in this study. The antigen specificities of hybridomas reactive with surface tegumental antigens were characterized and localized by immunoblotting analysis and Avidin-Biotin method. Of the 13 hybridomas, only three produced monoclonal antibodies to the single epitopes in the surface tegumental antigens. These epitopes (125 kDa, 97 kDa and 38 kDa) have been found to be the major antigenic components of the surface tegument of *S. mekongi*. The 38 kDa antigen was found to associate with the surface tegumental layers, the muscular layers lying just beneath the tegument, as well as in the gut surface. The 97 and 125 kDa antigens were detectable only in the surface tegumental area. The biochemical identity of these proteins or glycoproteins is unknown. However, these antigens have also been described in *S. japonicum* and *S. mansoni*.

INTRODUCTION

The isolation and characterization of specific schistosome antigens, as well as the use of such antigens and antibodies for immunodiagnostics are obvious fields of application of monoclonal antibodies (MAb). A hybridoma derived mouse IgG2a antibody directed against a 23 kDa antigen of the adult *Schistosoma japonicum* worm has immunodiagnostic potential in schistosomiasis japonica in the Philippines (Cruise *et al*, 1983). Moreover, MAb against 26 kDa glutathione S-transferase has been successfully used for detection of circulating antigen in the infected individuals (Davern *et al*, 1990). At the present time, more MAb have been produced and used by many investigators for detection of circulating antigens in infected hosts (Ripert *et al*, 1988; Deelder *et al*, 1989a,b ; Barsoum *et al*, 1990; Fu and Carter, 1990). Circulating antigens may represent interesting tools in the diagnosis of parasitic infections because they can induce specific immune responses of patients and may serve as markers for the intensity and severity of the disease or may indicate the efficiency of chemotherapy.

In the present study, we have produced 13 MAb against the surface tegument of *S. mekongi*. These MAb were characterized in terms of their epitopes and localization of the corresponding antigens.

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MATERIALS AND METHODS

Preparation of the surface tegumental antigens (STA)

Fifty to seventy *S. mekongi* cercariae from infected *Neotricula aperta* were applied directly to the shaved and wetted abdominal skin of Nembutal-anesthetized Swiss albino mice by the hair-loop method. Seven to eight weeks after infection, adult worms were collected by portal perfusion with 0.15 M sodium chloride in 0.05 M sodium citrate (Smithers and Terry, 1965). Two hundred to five hundred freshly obtained adult male worms were washed three times with Minimum Essential Medium (MEM) (GIBCO, USA) and another three times with 50 mM Tris-HCl pH 8.0 to remove any contamination of the host blood. After the final wash, the worms were placed in 0.5 ml of 50 mM Tris-HCl pH 8.0 and freeze-thawed on dried ice for 15 cycles. The shed pieces of surfaced tegument in the supernatant were aspirated into an Eppendorf tube. The worms were rewashed with 0.1 ml of 50 mM Tris-HCl pH 8.0 and the supernatant was aspirated into the same tube. The tegumental suspension was centrifuged at 100X*g* for 10 minutes at 4°C, and the supernatant was collected and kept at − 20°C until use. The protein content of the tegumental suspension was

determined by Lowry's method (Lowry *et al*, 1951).

Production of monoclonal antibodies against *S. mekongi*

Monoclonal antibodies were produced by fusion of the spleen cells from *S. mekongi* infected BALB/c mice with mouse myeloma cells according the method of Kohler and Milstein (1975). In this experiment, a total of 388 hybridoma clones were produced and grown successfully in culture. These hybridoma clones were examined for the production of specific antibodies against tegumental antigens of *S. mekongi* by ELISA technique. Of the 388 clones, 53 clones were selected for recloning by limiting dilution method. Thirteen stable hybridoma clones which were found to produce MAb directed against STA of *S. mekongi* were expanded and the culture supernatants were collected for analysis of the monoclonal antibodies. The monoclonal antibody isotypes and subisotypes were determined by ELISA using a hybridoma subisotyping kit (Behring Diagnostic, USA).

Characterization of monoclonal antibodies by SDS - PAGE and immunoblotting analysis

SDA - PAGE was carried out according to the method of Laemmli (1990). The tegumental antigen samples and standard proteins were mixed with the sample buffer and bromophenol blue and heated in a boiling water bath for 3 minutes. The tegumental antigen samples were loaded at the concentration of 50 microgram/well into 12.5% SDS - PAGE gel. High molecular weight standard proteins comprising myosin (205 kDa), betagalactosidase (116 kDa), phosphorylase B (97.4 kDa), albumin, bovine (66 kDa), albumin egg (45 kDa) and anhydrase (29 kDa) were run in parallel with the antigen samples in the same gel for molecular weight determination. Electrophoresis was performed at 100 V 30 mA from cathode to anode. It was terminated when the bromophenol blue marker had reached the bottom of the gel.

The separated proteins were electrophoretically transferred onto a nitrocellulose paper and probed with the monoclonal antibodies. The monoclonal antibodies that reacted with tegumental proteins were detected by incubation with peroxidase-conjugated rabbit anti-mouse Ig and visualized by further incubation in DAB and H_2O_2 (Towbin *et al*, 1979).

Characterization of monoclonal antibodies by the Avidin-Biotin method

Frozen sections of adult male *S. mekongi* were incubated with 0.6% H_2O_2 in methyl alcohol to quench any peroxidase activity that may be present in the tissue. The slides were rinsed with distilled water and washed three times with 0.05M Tris-buffer saline pH 7.6 (TBS) for 10 minutes each. Normal rabbit serum diluted 1 : 10 in TBS was then applied to the section for 30 minutes at room temperature and then replaced with undiluted monoclonal antibodies for 1 hour at room temperature. After washing with TBS, biotinylated rabbit anti-mouse immunoglobulins (DAKOPATTS, Denmark) diluted 1 : 200 in TBS was applied to the section and incubated at room temperature for 30 minutes. The washing step was repeated three times before applying ABComplex/HRP (DAKOPATTS, Denmark) to the section for 30 minutes at room temperature. The slides were washed again before diaminobenzidine substrate solution was applied for 10 minutes at room temperature. Finally the slides were washed three times in distilled water before mounting on the glass slides. The slides were examined under a light microscope. Immune mouse serum diluted 1 : 50 in TBS and 10% normal rabbit serum were used as positive and negative controls, respectively.

RESULTS

Of the 13 hybridoma clones 10 were found to secrete IgM antibody, two to secrete IgG_1 antibody and one to secrete IgG_3 antibody (Table 1). The monoclonal antibodies produced by these hybridomas were used for characterization of *S. mekongi* surface tegumental antigens by immunoblotting method.

Fig 1 shows that the hybridomas which produced 2D5 - 8 - 3 and 2F10 - 2 - 4 MAb recognized similar antigenic patterns. The most intense staining band was found at Mr 63 kDa and many minor bands were detected, ranging from 125 to 30 kDa. Monoclonal antibodies 5D4 - 2 - 1, 6B2 - 1 - 19 and 1B10 - 4 - 4 recognized single epitope at Mr 38 kDa, 97 kDa and 125 kDa, respectively. Weak staining bands were observed with IF10 - 7 - 3 and 3E8 - 2 - 10 MAb at Mr 125 and 120 kDa and 125, 120 and 47 kDa, respectively. The other six MAb (4D11 - 3 - 2, 4D11 - 3 - 5,

Table 1

Optical density (OD) of ELISA and subisotypes of the MAb from 13 hybridoma clones.

Clone	OD	Subisotype
2D5 - 8 - 3	2.393	IgM
2F10 - 2 - 4	2.494	IgG1
5D4 - 2 - 1	0.452	IgM
6B2 - 1 - 19	0.188	IgM
1B10 - 4 - 4	1.187	IgM
1F10 - 7 - 3	0.408	IgM
3E8 - 2 - 10	0.306	IgM
4D11 - 3 - 2	2.543	IgG1
4D11 - 3 - 5	2.525	IgM
6D2 - 2 - 2	2.535	IgM
6D2 - 2 - 3	2.508	IgM
7E7 - 1 - 1	2.083	IgM
2F5 - 1 - 4 - 9	2.608	IgG3

6D2 - 2 - 2, 6D2 - 2 - 3, 7E7 - 1 - 1 and 2F5 - 1 - 4 - 9) have been found to bind to rather heterogeneous epitopes ranging from Mr 125 to 30 kDa (Fig 2).

Localization of the antigens in cryostat section by MAb and ABComplex/HRP system showed that MAb reactive with the 97 and 38 kDa proteins could identify components associated with the surface tegumental system of adult worms. The 5D4 - 2 - 1 MAb which reacted to the 38 kDa molecule recognized the antigens in the surface tegumental area, the muscular layers lying just beneath the tegument as well as in the gut surface (Fig 3). The 6B2 - 15 and 1B10 - 4 - 4 MAb which reacted to the 97 and 125 kDa molecules recognized the antigens only in the surface tegumental area. The other MAb which immunoprecipitated a wide range of antigen fractions identified almost all structures of the worms. Particularly strong reaction was observed with tegument, subtegument muscle and with decreased intensity through the bulk of the internal structure of the worm.

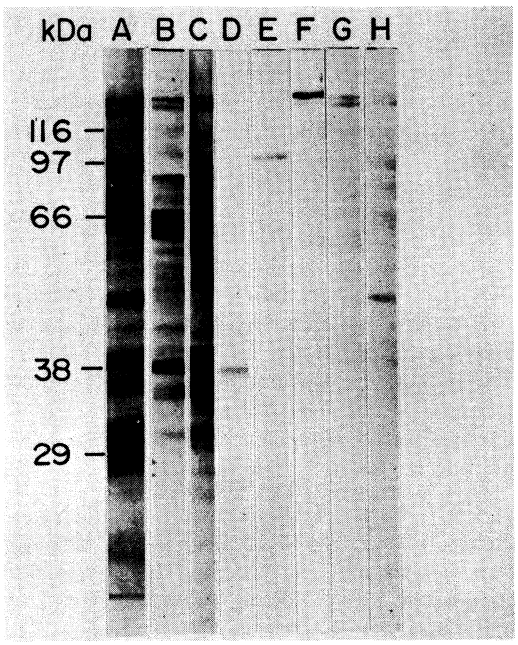

Fig 1—Immunoblotting patterns between STA of *S. mekongi* and various MAb. Immune mouse serum served as positive control (A), MAb 2D5 - 8 - 3 (B), MAb 2F10 - 2 - 4 (C), MAb 5D4 - 2 - 1 (D), MAb 6B2 - 1 - 19 (E), MAb 1B10 - 4 - 4 (F), MAb 1F10 - 7 - 3 (G) and MAb 3E8 - 2 - 10 (H).

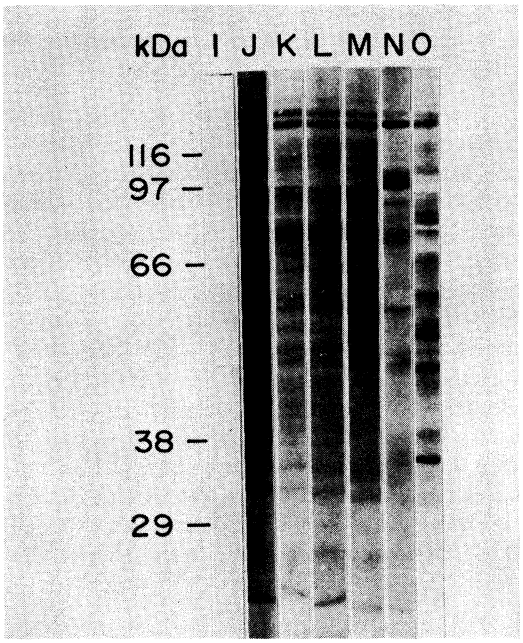

Fig 2—Immunoblotting patterns between STA of *S. mekongi* and various MAb. Myeloma conditioned medium served as negative control (I), MAb 4D11 - 3 - 2 (J), MAb 4D11 - 3 - 5 (K), MAb 6D2 - 2 - 2 (L), MAb 6D2 - 2 - 3 (M), MAb 7E7 - 1 - 1 (N) and MAb 2F5 - 1 - 4 - 9 (0).

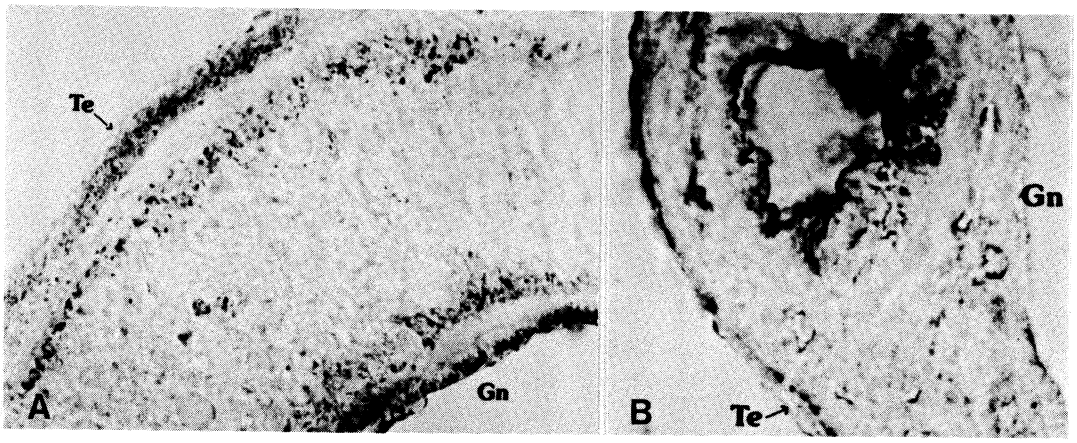

Fig 3—Anatomical localization of the *S. mekongi* antigenic target recognized by MAb 5D4 - 2 - 1. Specific reaction on the surface tegument (Te) of the dorsum and gynecophoral canal (Gn) and on the muscular layers beneath the tegument (A). Strong reaction in the gut (B).

DISCUSSION

In the present study, the surface tegument of *S. mekongi* was obtained by repeated freeze-thawing of adult male worms. This method has been found to give clean separation of the surface tegument from the basement membrane and underlying connective tissue (Sobhon *et al,* 1992). When freeze-thawed tegument antigens were immunoblotted with primary mouse immune sera against *S. mekongi,* there appeared to be two intense staining bands at the Mr 97 kDa and between 64 - 68 kDa and other diffused staining bands at Mr 125, 120, 86, 56, 47, 38 and 30 kDa (Figs 1, 2).

Immunoblotting analysis of the 13 MAb revealed that three MAb recognized single epitope at 125 kDa (1B10 - 4 - 4), 97 kDa (6B2 - 1 - 19) and 38 kDa (5D4 - 2 - 1) (Fig 1). The other 10 MAb were found to react to more than one major antigenic bands as demonstrated by immunoblotting between STA and primary mouse immune sera (Figs 1, 2). The MAb which immunoprecipitated the 97 and 38 kDa antigens are of great interest, since these immunogenic molecules have been reported as the major antigenic components of *S. mekongi, S. japonicum* and *S. mansoni.* Cross-reaction of these antigenic molecules between *S. mekongi, S. japonicum* and *S. mansoni* also has been observed (Sobhon *et al,* 1992). The stage specific MAb against 38 kDa and the species specific MAb against 97 kDa molecules have been produced from schistosomula of *S. mansoni* and *S. japonicum,* respectively (Dissous *et al,* 1982; Kojima *et al,* 1987).

In our hands MAb against 97 and 38 kDa were produced from surface tegumental molecules of *S. mekongi.* These molecules have been reported to be the major antigenic components of adult worms of all three species of schistosomes. The universally recognized MAb may prove to have some diagnostic value for the screening of schistosomiasis. By Avidin-Biotin method, all these three MAb showed the expected localization to the surface membrane of the tegument. In addition, this antigen was largely stripped from the surface by simple freeze-thaw technique and the antigen released from denuded worms gave a strong immunoblot reaction with these 3 MAb. Since it is well known that schistosome parasites are continuously shedding their surface tegument into the host circulation, an antigen-capture assay using single or combination of these MAb may be utilized for diagnosis of schistosomiasis. This possibility is being investigated in our laboratory at present.

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REFERENCES

Barsoum IS, Colley GG, Kamal KA. *Schistosoma mansoni :* Detection of circulating antigens in murine schistosomiasis by antigen-capture sandwich ELISA using a monclonal antibody. *Exp Parasitol* 1990; 71 : 1 - 7.

Cruise KM, Mitchell GF, Garcia EG, *et al.* Sj23, the target antigen of *Schistosoma japonicum* adult worms of an immunodiagnostic hybridoma antibody. *Parasite Immunol* 1983; 5 : 37 - 46.

Davern KM, Tiu WU, Samaras N, *et al. Schistosoma japonicum:* Monoclonal antibodies to the Mr 26,000 schistosome glutathione S-transferease (Sj26) in an assay for circulating antigen in infected individuals. *Exp Parasitol* 1990; 70 : 293 - 304.

Deelder AM, de Jonge N, Boerman OC, *et al.* Sensitive determination of circulating anodic antigen in *Schistosoma mansoni* infected individuals by an enzymelinked immunosorbent assay using monoclonal antibodies. *Am J Trop Med Hyg* 1989a; 40 : 268 - 72.

Deelder AM, de Jonge N, Fillie YE, *et al.* Quantitative determination of circulating antigens in human schistosomiasis mansoni using an indirect haemaglutination assay. *Am J Trop Med Hyg* 1989b; 40 : 50 - 4.

Dissous C, Grzych JM, Capron A. *Schistosoma mansoni* surface antigen defined by a rat monoclonal IgG2a. *J Immunol* 1982; 129 : 2232 - 4.

Fu C, Carter CE. Detection of circulating antigen in human schistosomiasis japonica using a monoclonalantibody. *Am J Trop Med Hyg* 1990; 42 : 347 - 56.

Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975; 256 : 495 - 7.

Kojima S. Janecharut T, Hata H, Niimura M. Role of a mouse monoclonal IgE antibody in passive transfer of immunity to *Schistosoma japonicum* infection. *Memo Do Inst Oswaldo Cruz* 1987; 82 : 237 - 41.

Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1990; 227:680 - 5.

Lowry OH, Rosenbrough NJ, Farr SL, Randal RJ. Measurement with the folin phenol reagent. *J Biol Chem* 1951; 193 : 265 - 75.

Ripert C, Comb A, Daulouede S, *et al.* Detection with a monoclonal antibody of an antigen, characteristic of the genus *Schistosoma,* excreted in the urine. *Trop Med Parasitol* 1988; 39 : 131 - 5.

Smithers SR, Terry RJ. The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of the adult worms. *Parasitology* 1965; 55 : 695 - 700.

Sobhon P, Upatham ES, Viyanant V, *et al.* Identification and localization of surface antigens in adult *Schistosoma japonicum* and *Schistosoma mekongi.* In : Aquaculture and Schistosomiasis. Proceeding of a network meeting BOSTID National Research Council. Manila, Phillipines August 6 - 10, 1991. 1992 : 178 - 95.

Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets : Procedure and some applications. *Proc Natl Acad Sci USA* 1979; 76 : 4350 - 4.