PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST PARTIALLY PURIFIED SURFACE TEGUMENT ANTIGENS OF *FASCIOLA GIGANTICA*

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Abstract. Monoclonal antibodies (MAbs) were produced by the *in vitro* fusion of Balb/C mice spleen cells immunized with partially purified surface tegument antigens of *Fasciola gigantica* . The surface membrane and tegument antigens were purified by using gel filtration chromatography. SDS-PAGE performed on the processed proteins demonstrated that the proteins had molecular weights of 20 to 97 kDa. In this study, fifteen monoclonal antibody clones were selected from the hybridoma clones, namely: 1B7, 1B11, 1B12, 1C9, 1D4, 1G2, 1H7, 2B6, 2C3, 2C9, 2D11, 2F11, 2G2, 2G5, and 2G11. They were evaluated by immunoblot assay and were differentiated into two groups. In the first group were found 60 and 38 kDa proteins; in the second group were found 66, 60, and 38 kDa proteins. All were found to secrete IgM, kappa light-chain antibodies. These MAbs were tested for their cross-reactivity with other trematodes commonly found to infect cattle and man. All of these MAbs showed some degree of cross-reactivity with other trematode species.

INTRODUCTION

Fascioliasis caused by *Fasciola gigantica* infection is one of the tropical diseases that imposes a significant global economic burder. In Thailand, it causes economic damage by affecting meat and milk production; the damage is valued at 300-350 million bath (1US$ = 25 baht) per year. The current method of diagnosis in cattle is based on the microscopic detection of eggs in feces. Convenient and reliable immunodiagnostic methods based on the detection of either antibodies or antigens in the blood of infected animals should be used for epidemiological studies. One source of antigen, as with many other helminths, such as the genus *Schistosoma* Bilharz 1852, is the tegument proteins, whose covering membrane and associated antigens turn-over rapidly and are released into the host's circulatory system (Smither, 1982; Smither and Doenhoff, 1982). *Fasciola gigantica* has its tegument-associated antigens concentrated in the tegument surface membrane, the outer rim of the tegument cytoplasm and tegument cell (Krailas *et al*, 1999).

In this study, monoclonal antibodies (MAbs) were produced by the *in vitro* fusion of Balb/c mice spleen cells immunized with partially purified surface tegument antigens of *F. gigantica*. The monoclonal antibodies will be used for immunodiagnostic assay during further studies of fascioliasis.

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MATERIALS AND METHODS

Preparation of surface membrane and tegument antigens

Adult *F. gigantica* were removed from the bile duct and gall bladders of bovine livers obtaind from a local slaughterhouse. The parasites were washed several times with Hank's balanced salt solution containing 100 U/ml penicillin and 100mg/l streptomycin. The worms were then extracted with a non-ionic detergent solution (1% Triton X-100 in Tris-HCl buffer, pH8, at room temperature, for 30 minutes; the degree of extraction of the tegument was monitored by microscopy). The solution was collected, centrifuged, and kept at -20˚C until use. The protein content of all antigens were determined by the method of Lowry *et al* (1951).

Partially purified surface tegument antigens

The surface tegument antigens were fractionated by Sephadex G-100 gel filtration (Phamacia, Sweden). Briefly, 5.45mg of protein in 500µl 0.01M PBS, pH 7.4, were applied to the gel (BIO-RAD,USA; column 2.5cm diameter, 500 cm^3, 4˚C) followed by 0.01M PBS (flow rate = 2ml/ minute). Fractions (2ml) were collected and analyzed at 280nm using a spectrophotometer (Shimadzu UV-VIS UV-160A). Verification of the void volume was accomplished with

vitamin B12. Bovine serum albumin (BSA) was applied to the column and used as the standard protein. The standard protein was fractionated and compared with the peaks of the antigen fractions. The protein concentration was determined by the method of Lowry *et al* (1951).

Native gel electrophoresis and SDS-PAGE

Nondenaturing PAGE and sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) were conducted in order to characterize the *F. gigantica* surface tegument antigen fractions. The discontinuous buffer system of Laemmli (1990) was used. The gels were stained with Coomasie blue and Silver stain.

Production of monoclonal antibodies

Balb/c mice were immunized by subcutaneous inoculation with 100μg of partially purified surface tegument (ST) antigens of *F. gigantica*, mixed with an equal amount of complete Freund's adjuvant. The second and third immunizations were given at two week intervals by the same route. The last immunization (200μg antigens) was given via the intraperitoneal route two weeks after the third. The antibody titers were determined by enzyme-liked immunosorbent assay (ELISA). Hybridomas were produced by the fusion of spleen cells from immunized mice and P3/x63-Ag8 murine myeloma cells. The hybridoma clones were examined for the production of specific antibody against ST antigens by ELISA. The monoclonal antibodies were typed by ELISA using a Mouse MonoAb Screen Kit (Zymed, USA).

Characterization of the monoclonal antibodies

The tegument antigen samples were separated in SDS-PAGE and their antigenicity was determined by enzyme-linked immunoelectrotransfer blot (EITB). SDS-PAGE was carried out according to the method of Laemmli (1990). The separated proteins were electrophoretically transferred onto a nitrocellulose paper and probed with monoclonal antibody. The monoclonal antibodies which showed high ELISA activity were also used for characterization of the specific immunogenic molecules in the ST antigens.

Cross-reactivity of MAbs with other parasite antigens

Indirect ELISA was used for studying the cross reactivity of MAbs. The antigens used in this study were antigens from adult worms commonly found to infect cattle and man: *Fasciola gigantica*, *Paramphistomum* sp, *Schistosoma japonicum*, *S. mansoni,* and *S. mekongi*. The parasite antigen (1μg) was used for coating the microtiter plate, and then allowed to react with supernatant culture fluid of MAbs.

RESULTS

Partially purified surface tegument

One major peak of *F. gigantica* surface tegument antigen was obtained on a Sephadex G-100 column (Fig 1). Consequently, the fractions were characterized by native gel electrophoresis and SDS-PAGE. Only one band of protein was observed in nondenaturing PAGE (Fig 2). The protein profiles of these fractions, after electrophoretic separation by SDS-PAGE are shown in Fig 3. All fractions from the major peak presented proteins with molecular weights of 97, 66, 54, 47, 38, 29, and 27 kDa respectively; the non-purified antigens presented proteins with molecular weights of 97, 66, 54, 47, 38, 29, 27, 17, and 15kDa.

Monoclonal antibodies

Fifteen monoclonal antibodies which were highly reactive to surface tegument antigens were characterized further by EITB. They are were: 1B7, 1B11, 1B12, 1C9, 1D4, 1G2, 1H7, 2B6, 2C3, 2C9, 2D11, 2F11, 2G2, 2G5, and 2G11. They were differentiated into two groups. The first group was found to contain 60 and 38kDa. The second group (1B7, 1B11, 2G11) was found to contain 66, 60 and 38kDa (Figs 4 and 5). All were found to secrete IgM, kappa light-chain antibodies.

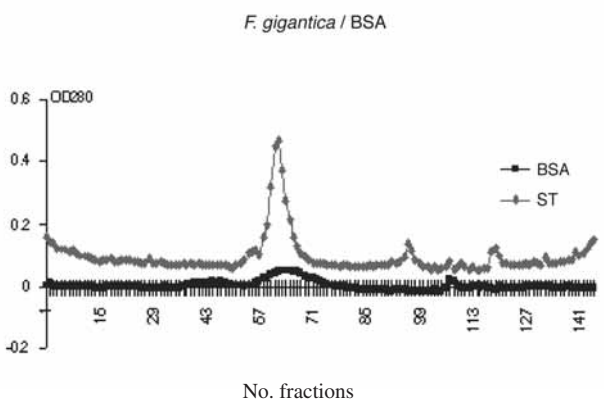

Fig 1- Elution profile of *Fasciola gigantica* surface tegument antigens on Sephadex G-100. One major peak was obtained in the fractions: BSA = Bovine serum albumin; ST = surface tegument antigen of *F. gigantica.*

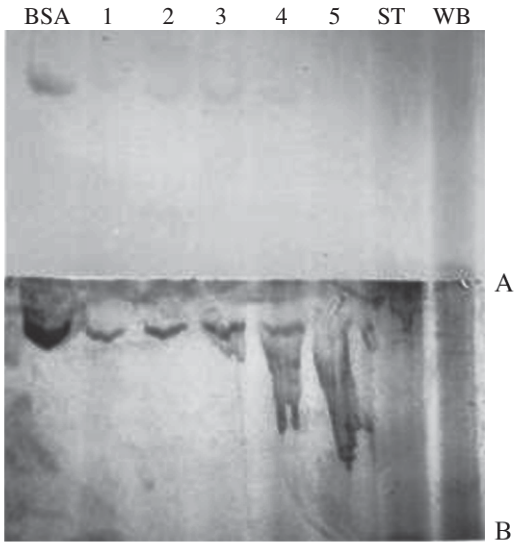

Fig 2 - Nondenaturing analysis of *Fasciola gigantica* proteins present in 5 fractions. Protein fractions were separated by electrophoresis on 10% and protein bands were detected by Coomassie brilliant blue (A) and Silver staining (B). BSA = Bovine serum albumin (10μg); ST = crude surface tegument antigen (10μg); WB = Whole worm antigens (10μg); 1, 2, 3, 4, 5 = 61, 62, 63, 64, 96 fractions (10μg).

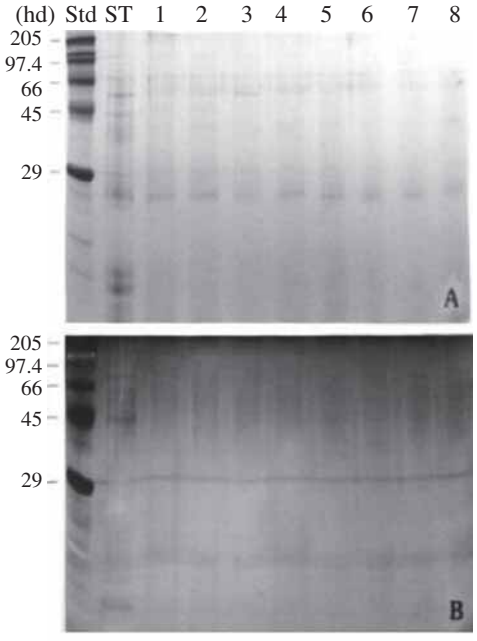

Fig 3 - SDS-PAGE analysis of *Fasciola gigantica* proteins present in 8 fractions. Protein fractions were separated by electrophoresis on 12% and protein bands were detected by Coomassie brilliant blue (A) and Silver staining (B). SD = Standard marker (10μg); ST = crude surface tegument antigen (10μg); 1-8 fraction (2μg).

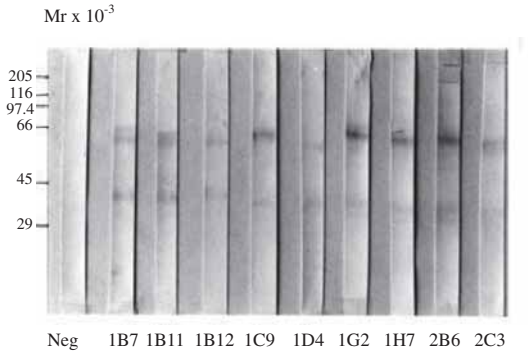

Fig 4 - Immunoelectrotransfer blot of 1B7, 1B11, 1B12, 1C9, 1D4, 1G2, 1H7, 2B6, and 2C3 monoclonal antibodies. Neg = Negative control.

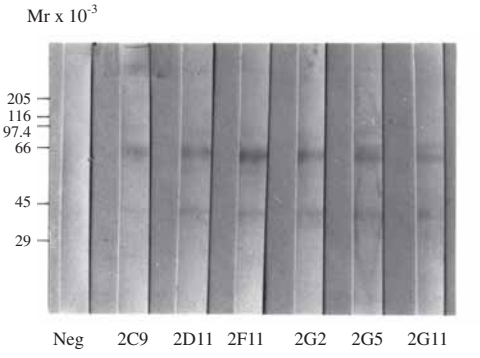

Fig 5 - Immunoelectrotransfer blot of 2C9, 2D11, 2F11, 2G2, 2G5, and 2G11 monoclonal antibodies. Neg = Negative control.

Cross-reaction of MAbs with other trematode antigens

The MAbs were tested for their cross-reactivity to other trematodes commonly found to infect cattle and man. All of the MAbs showed some degree of cross-reactivity (Fig 6A and B).

DISCUSSION

The immunodiagnosis of animal fascioliasis by the detection of circulating antigens has not yet been thoroughly investigated. Fegbemi *et al* (1995) successfully used rabbit antibodies against the 88kDa

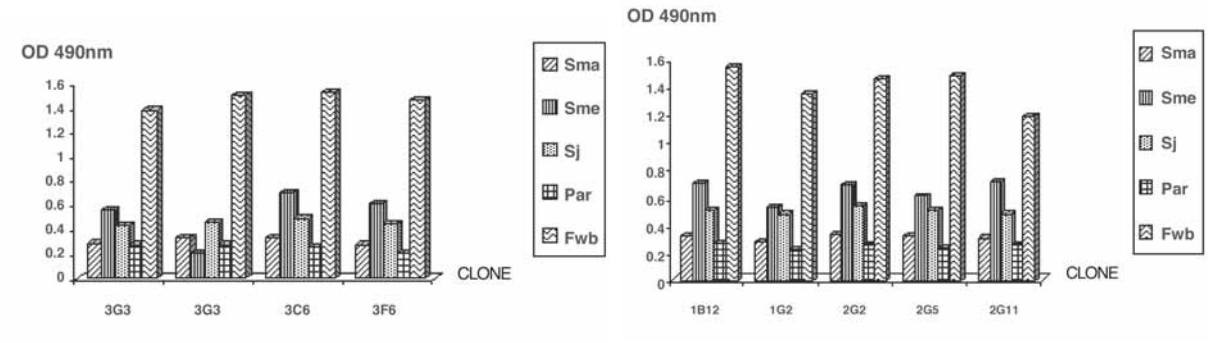

Fig 6 A,B - ELISA values of cross-reactivity studies of the MAbs and the antigens of various other trematodes.
Sma = *Schistosoma mansoni*; Sme = *Schistosoma mekongi;*
Sj = *Schistosoma japonicum*; Par = *Paramphistomum* sp;
Fwb = *Fasciola gigantica* whole body

antigen of adult *F. gigantica* for the detection of circulating antigens in experimentally-infected cattle sera. More recently, Viyanant *et al* (1997) studied a monoclonal antibody specific to a 66kDa antigen of *F. gigantica* for the detection of circulating antigens in experimentally- and naturally-infected cattle: they reported that circulating antigens could be detected as early as the second and third weeks after infection; these antigens was associated with the crude surface tegumental of the parasite.

Sobhon *et al* (1996) analyzed the proteins from the homogenized whole body of *F. gigantica:* it was found that there were approximately 21detectable bands, ranging in molecular weight from 17 to 110kDa. Eleven of the bands at 97, 86, 66, 64, 58, 54, 47, 38, 35, 19, and 17 kDa, were present in the tegument antigen which was extracted from the parasites' bodies by Triton X-100.

The present study aimed to improve the immunodiagnosis of *F. gigantica*. We purified surface tegument antigens of *F. gigantica* by gel-filtration chromatography. The fractions were characterized and studied; proteins with molecular weights ranging from 27 to 97kDa were present in the fractions of purified antigens. In an earlier study, Youssef and Mansour (1991) purified adult *F. gigantica* by Sephadex G-200. They found 4 distinct protein peaks and used peaks II and III for further antigen study. Electrophoretic analysis of fraction 10 of the two peaks showed a protein band of between 10 and 66kDa. This indicates that the purification of the surface tegument antigen of *F. gigantica* is a sound method of antigen production.

We differentiated the MAbs produced in this study by EITB. The first group recognized epitopes present in 60 and 38kDa; the second group had protein of 66, 60, and 38kDa. By immunoblotting analysis, Sobhon *et al* (1996) found that 14 of the 21 bands of the whole body fraction were antigenic, while all 11 bands of the tegument-associated proteins of the ST fraction were antigenic: the major antigens were 4 bands at 66, 58, 54, and 47kDa. In comparison, there has been considerable study of *F. hepatica*. Itagaki *et al* (1995) found that the major antigens of adult *Fasciola* sp were at 64-52, 38-28, 17, 15, 13, and 12kDa; it was also reported that the antigens at 66, 58, and 54kDa were more species-specific, they might be possible candidates for serodiagnosis of fascioliasis in cattle.

We found that MAbs against purified surface tegument antigens of *F. gigantica* showed some degree of cross-reactivity with antigens extracted from *Paramphistomum* sp, *S. japonicum*, *S. mansoni* and *S. mekongi*. Our results also showed that MAbs against crude surface tegument of *F. gigantica* have cross-reactivity with antigens from other trematodes commonly found to infect cattle and man (Viyanant *et al*, 1997). It can be concluded that the MAbs against partially purified antigens and those against crude antigens could be used to develop the immunodiagnosis of fascioliasis.

ACKNOWLEDGEMENTS

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