LOCALIZATION OF ANTIGENIC MOLECULES OF ADULT *FASCIOLA GIGANTICA* USING MONOCLONAL ANTIBODIES AGAINST THE PARASITE TEGUMENTAL ANTIGEN

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Abstract. In this study, monoclonal antibodies were developed from the partially purified surface tegumental antigens of *F. gigantica*. Nine MoAbs: 2G11, 1G2, 1B12, 2G2, 2G5, 3C6, 3G2, 3G3 and 3F6 were used for anatomical localization of adult *F. gigantica*. The reaction was demonstrated by Avidin-Biotin method. The results revealed that among the sections stained with non-immune sera and control group, there were no reaction products on either the tegument or the cecal epithelium. The only brownish areas were the vitelline glands. In the sections stained with immune sera, brownish reaction products appeared on the surface membrane, the spine membrane, the cecal lumen and its epithelial cells. The experiment sections of nine monoclonal antibodies revealed that the reaction occurred mainly on the tegument of the adult worm which covered its surface and spine.

INTRODUCTION

Fascioliasis, caused by *Fasciola gigantica* infection in ruminants, produces a great economic loss throughout the world. In Thailand it causes economic damage in terms of meat and milk production. The current method for the diagnosis of fascioliasis is demonstration of the presence of *F. gigantica* eggs in feces. However, the fecal examination technique is unpleasant, tedious, time-consuming, and requires a well-trained microscopist for interpretation, which may not be practical in remote areas. Immunodiagnostic assays, either for circulating antigen or antibodies, should be the methods of choice (Fu and Carter, 1990; Fegbemi *et al*, 1995). Although demonstration of circulating antibodies has been used for epidemiological studies, the presence of antibodies is not a direct indicator of active infection, and cross-reactivity with other parasites. The detection of circulating antigens rather than antibodies is considered to be a more reliable method (Viyanant *et al*, 1997). Monoclonal antibodies were developed from the partially purified surface tegumental antigen of *F. gigantica* and used for immunological assay.

In this study, nine monoclonal antibodies were used for the anatomical localization of specific antigens. Cryostat sections of adult *F. gigantica* were performed. The reaction was demonstrated by Avidin-Biotin method.

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MATERIALS AND METHODS

Monoclonal antibodies

Hybridomas were produced by fusion of spleen cells of a mouse immunized with partially purified *F. gigantica* surface tegumental antigens and mouse myeloma cells (P3/x63-Ag8). Nine monoclonal antibodies were used in this study, 2G11, 1G2, 1B12, 2G2, 2G5, 3C6, 3G2, 3G3 and 3F6.

Non-immune sera and immune sera

The immune sera were obtained from a mouse immunized by intramuscular injection of 200 μg partially purified surface tegumental antigens of *F. gigantica* emulsified in Freund's complete adjuvant. Two weeks post-infection, the mouse was given a second immunizing dose similar to the first, with the antigen emulsified in Freund's incomplete adjuvant. The third immunizing dose was given two weeks after the second dose. The final boost of antigen was given intravenously with 800 μg of the antigens without adjuvant. Mouse antisera to *F. gigantica* were collected one week after the final boost and kept at -20^{o}C until use. Non-immune sera were obtained from an uninfected mouse.

Tissue preparations for cryostat sections

Adult *F. gigantica* were collected by dissecting livers and gall bladders of infected cows, killed at local abattoirs. The flukes were washed carefully to remove contaminating blood and tissue debris with several changes of Hank's balanced salt solution. Pieces of adult worms were immediately fixed with 2% paraformaldehyde in 0.1M phosphate buffer saline, pH 7.4 for 2 hours, then embedded in Tissue Tek OCT medium at -30^{o}C. The frozen tissues were cut 10 to 12 μm thick in a cryostat microtome, and the sections were

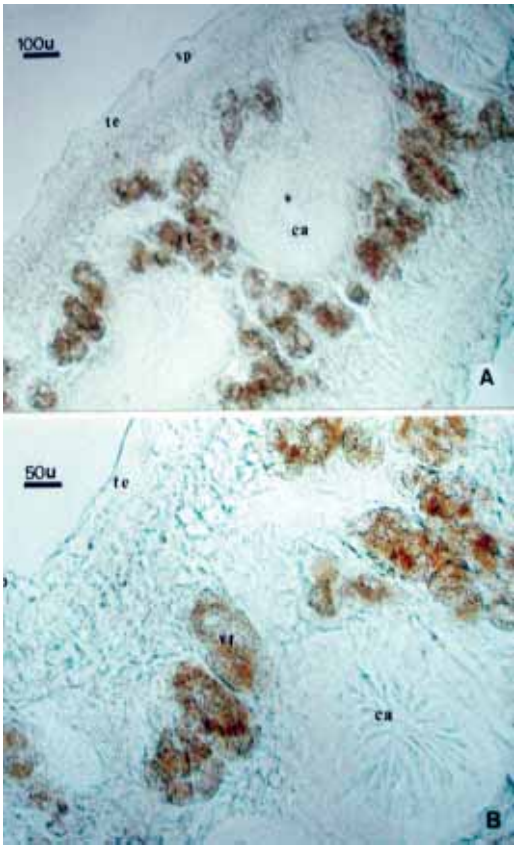

Fig 1- The control cryosections of adult *Fasciola gigantica*, in which staining with the primary antibody is omitted. There is no staining on either the surface of the tegument (te) or the intestinal cell (ca). However, brownish areas were observed in the area of the vitelline glands (vt).

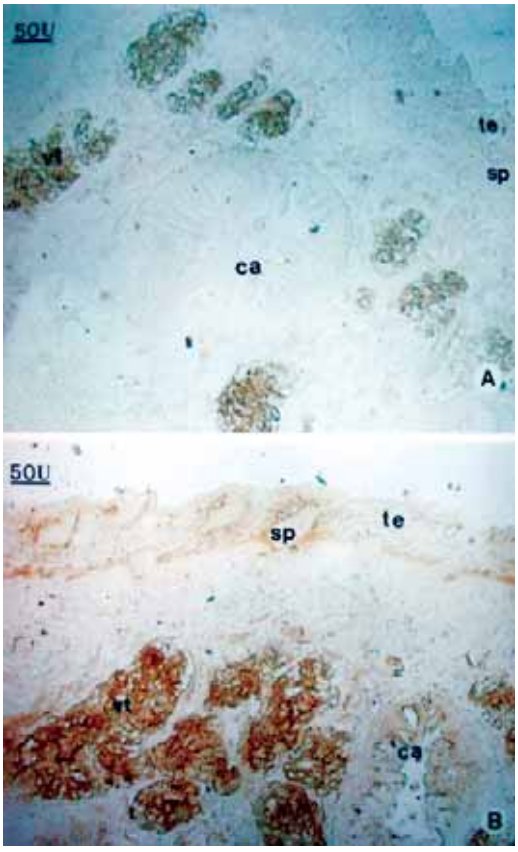

Fig 2- Immunoperoxidase staining of adult *Fasciola gigantica* frozen sections as detected by immune sera. A. Section stained with non-immune sera. There is no staining on either the surface of the tegument (te) or the intestinal cell (ca); B. In the section stained with immune sera, the brownish reaction products appeared on the tegument (te), the spines (sp), the cecal lumen (ca) and the vitelline glands (vt).

transferred to gelatin-coated slides.

Localization of the immunoreactive molecules

The nine MoAbs were used for reaction with the specific antigenic molecules in the cryosections. The sections of *F. gigantica* were stained with immunoperoxidase, as follows. The cryosections were treated with 3% H_2O_2 in methanol to inhibit endogenous peroxidase and then stained with non-immune sera, immune sera or MoAbs. Staining with primary antibodies was followed by biotinylated rabbit anti-mouse IgG (1:200), Z-avidin (1:200), biotinylated peroxidase complex (1:200) and DAB solution (3,3´-diaminobenzidine + H_2O_2 in 0.1 M PBS pH 7.4).

RESULTS

The results of anatomical localization of adult *F. gigantica* revealed that among the sections stained with non-immune sera and the control group, there was no reaction on either the tegument or the spine (Figs 1, 2A, 3A). Brownish areas were observed in the area of the vitelline gland (Fig 1). In sections stained with immune sera, the brownish reaction products appeared on the surface membrane, the spines, the cecal lumen and its epithelial cells (Fig 2B). The reaction of nine MoAbs (2G11, 1G2, 1B12, 2G2, 2G5, 3C6, 3G2, 3G3 and 3F6) were similar, and clearly demonstrated the presence of their specific antigenic molecules. The experiment revealed that the reaction occurred mainly

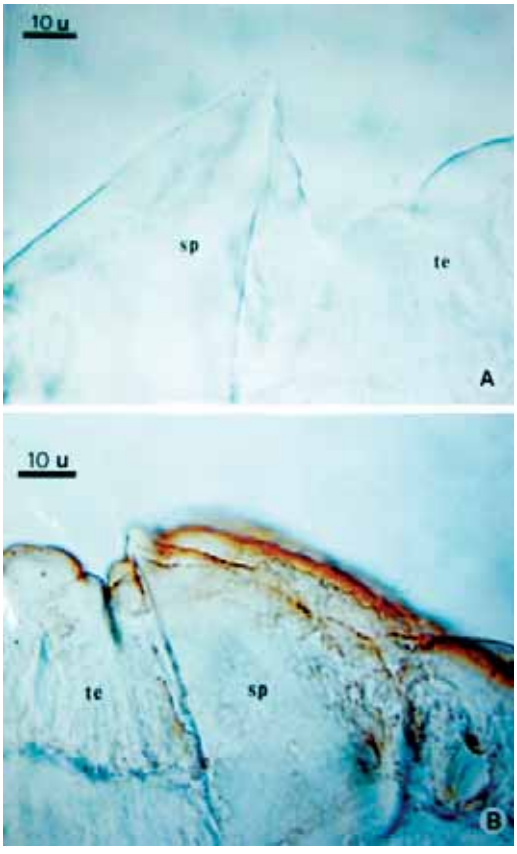

Fig 3- Immunoperoxidase staining of adult *Fasciola gigantica* frozen sections, as detected by MoAb. A. Control crysections in which primary antibody staining is omitted. There is no staining on the surface of the tegument or the spine. B. Cryosection demonstrating intense staining on the surface membrane covering the spine (sp).

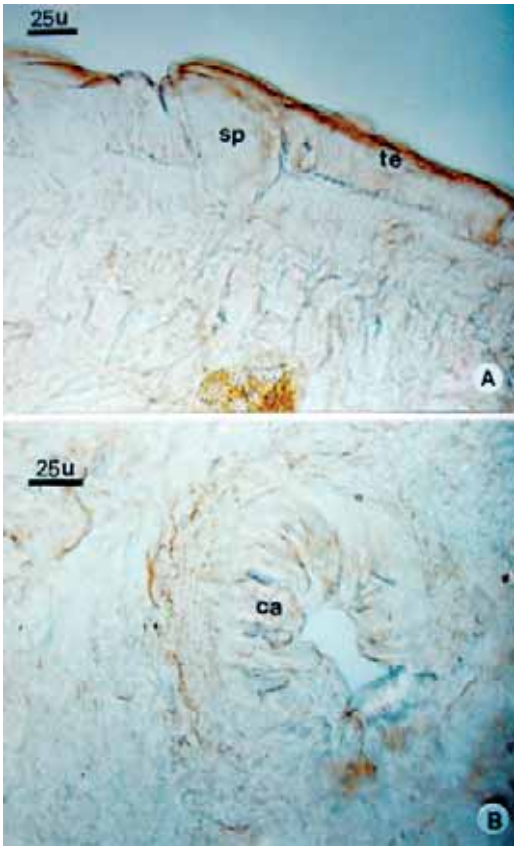

Fig 4- Immunoperoxidase staining of adult *Fasciola gigantica* frozen sections, as detected by MoAb (1B12). A. High-power magnification light micrograph of cryosection showing intense staining of tegumental cytoplasm (te); B. Cryosection demonstrating staining in the epithelium cell of the ceca (ca).

on the tegument of the adult worm which covered its surface and spine (Fig 4A). In addition, intense staining was found only on the surface membrane covering the spine, but not in the spine crystalline matrix (Fig 3B). The immuno-staining of the sections, as detected by the nine MoAbs, demonstrated that antigens were concentrated in the luminal content as well as in the cecal epithelial cells (Fig 4B).

DISCUSSION

The localization of antigenic molecules of adult *F. gigantica* was studied by using immune sera to detect the sites of antigen formation. The deposit of brownish reaction products implied that the major antigen-producing tissues were the tegument and the cecum. The experiment revealed that the reaction occurred mainly on the tegument of the adult worm which covered its surface and spine. Intense staining was found on the surface membrane covering the spine, but not in the spine crystalline matrix. This implies that the spine materials were not antigenic by nature, or more likely, that spines were not shed and turned over like the surface membrane, thus their contents were not released into the host's circulation to stimulate antibody production (Krailas *et al*, 1999). In the control section, where MoAbs were not used in place of primary antibody, there was no reaction on either the tegument or the spine (Fig 3A). Brownish areas were observed in the area of the vitelline glands (Fig 1),

where there might be endogenous peroxidase that could not be completely inhibited by 3% H_2O_2 in the conduct of the immunohistochemical techniques (Sobhon *et al*, 1998).

The result of this experiment indicated that there was an intense staining of the narrow zone of cytoplasm just beneath the surface membrane. This might represent the zone of tegumental cytoplasm where tegumental granules were observed to be highly concentrated. These granules were believed to contribute to the synthesis of the surface membrane. There was some staining of the membrane lining of the cecal epithelial cells (Fig 4B). This suggested that an antigenic epitope of MoAb was also present in the membrane of the cecal epithelial cells (Krailas *et al*, 1999).

From the results, it can be concluded that the reactions of the nine MoAbs were similar and the pattern of antigens in *F. gigantica* have two major sources of antigens released from the tegument and the cecum.

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