IMMUNOHISTOCHEMICAL LOCALIZATION OF *GNATHOSTOMA SPINIGERUM* LARVAL ANTIGENS BY MONOCLONAL ANTIBODIES: II. ELECTRON MICROSCOPY

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Abstract. Immunocytochemical localization of antigens in advanced third-stage larvae of *Gnathostoma spinigerum* (GsAL3) was studied by immunogold labeling method using seven *G. spinigerum* specific monoclonal antibodies (MAbs), FS-3D11, SS-5H5, SK-6C4, SK-4E1, SK-7G6, SK-8D4 and SA-9B5. All these MAbs belong to the IgG_1 subclass and only FS-3D11 and SS-5H5 recognize carbohydrate epitopes. The paraformaldehyde-fixed GsAL3 were embedded in Lowicryl K4M medium, and the gold colloidal particles used were 15 nm in size. When the worm sections were probed with FS-3D11, the gold particles appeared to concentrate specifically on the intestinal brush border. When SS-5H5 was applied, the particles were scattered densely over the brush border and in the cytoplasm of epithelial cells. The rest of the MAbs which recognize protein determinants exhibited a lack of labeling. The results suggested that the carbohydrate antigenic determinants recognized by the two MAbs are the most stable and most abundant particularly in the intestine of GsAL3. These results also confirmed the previous finding that the most antigenic site of GsAL3 is the intestine.

INTRODUCTION

G. spinigerum, a nematode parasite of cats and dogs, is the most important etiologic agent causing human gnathostomiasis (Daengsvang, 1980; Miyazaki, 1991). Little is known concerning the specific antigens responsible for host immune response in gnathostomiasis. However, Tapchaisri *et al* in 1991 have reported that the *Mr* 24-kDa component of GsAL3 (infective larvae) is a specific antigen with diagnostic potential for use in the serodiagnosis of human gnathostomiasis. Furthermore, Nopparatana *et al* (1991, 1992) demonstrated that this *Mr* 24-kDa component is a protein with a pI of 8.5, and is anatomically located in the body fluid, esophagus and intestine of the larvae. Morakote *et al* (1989) also demonstrated that the target antigens of the hyperinfected rabbit or human gnathos-tomiasis sera are in the anterior part of the esophagus, the surface of the cuticle and in the cytoplasmic granules of the intestine.

In our previous study on the immunohistochemical localization of antigens in GsAL3 by indirect enzyme immunostaining-light microscopy using 7 *G. spinigerum* specific MAbs, FS-3D11, SS-5H5, SK-6C4, SK-4El, SK-7G6, SK-8D4 andSA-9B5 (Rojekittikhun *et al*, 1993), we demonstrated that FS-3D11 is bound primarily to the intestinal brush border while SS-5H5 reacted with various tissues of the larvae. SK-6C4, SK-8D4 andSA-9B5 stained predominantly the muscle, the cuticle and the brush border, and the pseudocoel, respectively, whereas, SK-4El and SK-7G6 showed no labeling. These results suggested that antigens sharing common epitopes are present in various structures of the larvae with the intestine being the most antigenic site. The data from this report also suggested that certain GsAL3 antigens recognized by these MAbs are sensitive to formalin fixation and/or paraffin embedding (Rojekittikhun *et al*, 1993). This paper reports our further study on localization of the antigens in GsAL3 by immunogold electron microscopy using the same MAbs.

MATERIALS AND METHODS

Parasite preparation

GsAL3 were obtained from naturally infected eels. They were washed with phosphate buffered saline (PBS) and fixed with 4% paraformaldehyde containing 0.2% glutaraldehyde in 0.1 M PBS, pH

7.2. After dehydration, they were infiltrated with Lowicryl K4M and embedded into the beem capsule under UV light at -20°C for 24 hours. Ultrathin sections (100-120 nm thick) were then made, and the sections were mounted onto a nickel grid.

Monoclonal antibody production

Production and characterization of the 7 MAbs specific to *G. spinigerum* were previously described (Rojekittikhun *et al,* 1991, 1993).

Immunogold labeling method

GsAL3 sections were first blocked with 0.5% bovine serum albumin (BSA), and were then left to react with undiluted culture supernatant containing MAbs for 2 hours at room temperature. After washing 3 times in PBS, they were probed with 1:50 diluted sheep anti-mouse IgG coupled to 15 nm gold colloidal particles (E-Y Laboratories, USA) for 30 minutes, and washed 5 times in PBS. The sections were post-fixed with 2% glutaraldehyde in 0.1 M phosphate buffer for 10 minutes and rinsed 5 times in distilled water (DW). They were then sufficiently dried, stained with 2% uranyl acetate for 3 minutes, and rinsed thoroughly in DW. The sections were finally immersed in Reynolds lead solution for 25 seconds, rinsed thoroughly in DW, and then dried.

RESULTS

MAbs FS-3D11 and SS-5H5 exhibited strong labeling on the GsAL3 sections, whereas the rest. SK-6C4, SK-4E1, SK-7G6, SK-8D4 and SA-9B5, which recognized protein determinants, showed an opposite pattern - a lack of labeling. When FS-3D11 was applied to the section, the gold particles were concentrated specifically on the intestinal brush border as shown in Fig 1. When the section was probed with SS-5H5, the particles were labeled predominantly over the brush border (Fig 2) and in the cytoplasm of epithelial cells (Fig 3). Fig 4 shows negative immunolabeling of the section when it was treated with any of the five MAbs recognizing protein epitopes or with the negative control (culture supernatant of X63-Ag8.653 myeloma cells).

DISCUSSION

The 7 MAbs used in this study exhibited only two immunolabeling patterns - strong or none. FS-3D11 and SS-5H5 showed intense labeling on the intestine of the sectioned GsAL3, whereas the rest: SK-6C4, SK-4E1, SK-7G6, SK-8D4 and SA-9B5 showed a lack of labeling. The results agree with our previous study (Rojekittikhun *et al,* 1993) that FS-3D11 stained primarily the intestinal brush border while SS-5H5 reacted strongly with almost all organs, especially the intestinal brush border, epithelial cells and intestinal contents. The present data, therefore, confirm the previous findings that the intestine is the most antigenic site of GsAL3 (Nopparatana *et al,* 1992; Rojekittikhun *et al,* 1993). FS-3D11 and SS-5H5 are anti-carbohydrate antibodies and the rest of the 5 MAbs recognize protein determinants. These five MAbs showed, however, moderate to weak or even no reactivity to some structures of the larvae except the intestine in the immunoperoxidase staining, but completely lost their binding activity in the immunogold labeling. This reflects the antigenicity, stability and quantity of the antigens present in the GsAL3 sections, or the affinity of the MAbs themselves. It is likely that GsAL3 antigenic epitopes, which are carbohydrate moieties, are the most stable and the most abundant epitopes existing particularly in the intestine of the larvae. Moreover, the 5 MAbs which recognize protein moieties may have poor affinity, or their target molecules may be present in limited amounts or within small organs normally absent from the sections (Hanna *et al,* 1988).

In conclusion, the major foci of antigenic expression in infective larvae of *G. spinigerum* are shown to be the surface of the cuticle, the esophagus and the intestine (Morakote *et al,* 1989; Rojekittikhun *et al,* 1993), with the latter being the most antigenic site (Rojekittikhun *et al,* 1993). This is closely analogous to some other tissue-migrating/dwelling nematodes reported by other investigators, that the surface antigens and the intestinal antigens of *T. spiralis* muscle larvae are localized in the epicuticle and in the brush-border microvilli, respectively (McLaren *et al,* 1987); and that the two primary somatic locations of ES antigens of *T. canis* infective larvae are the esophageal glands and the large branched secretory cells (Page *et al,* 1992).

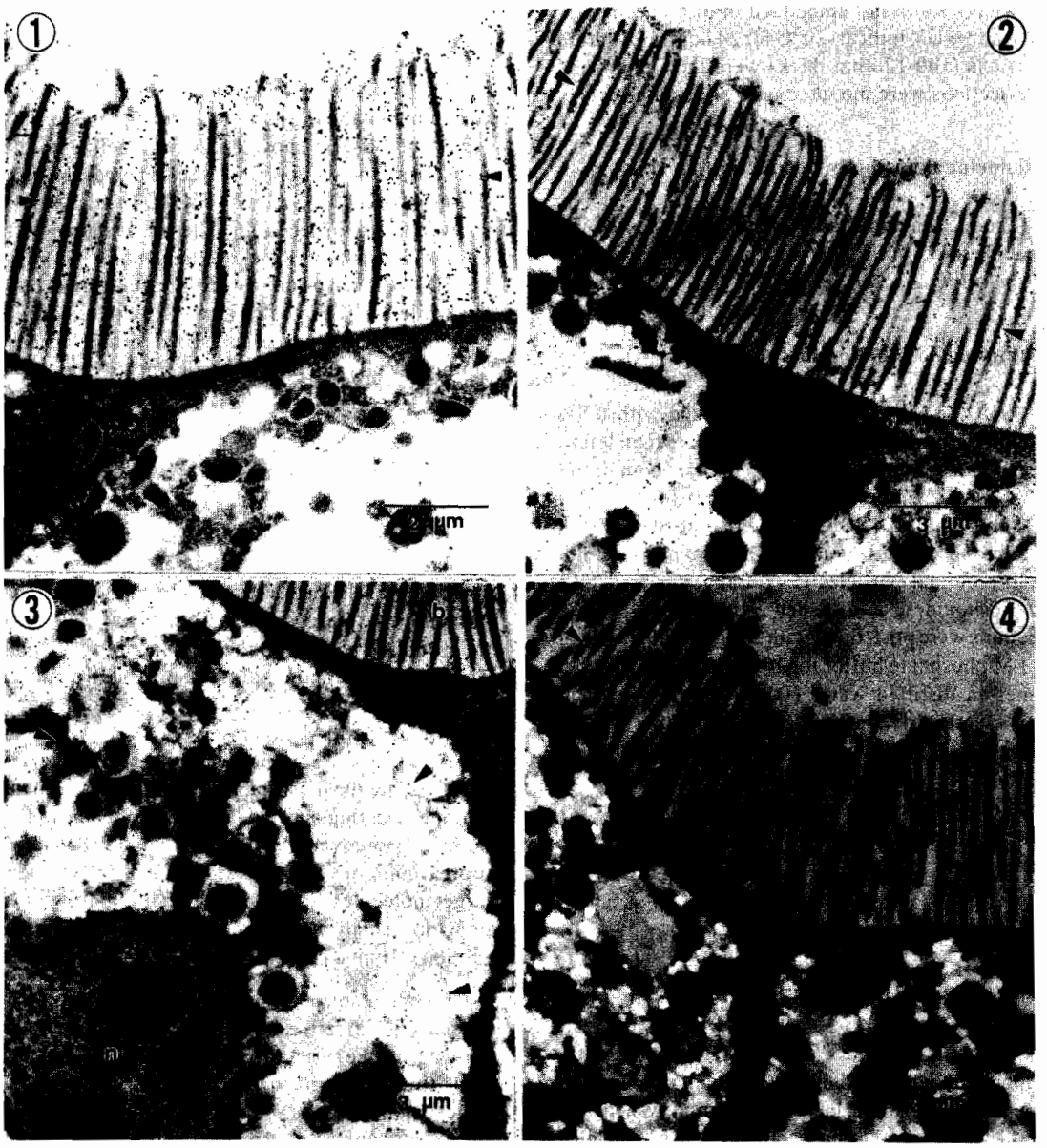

Fig 1–Electron micrograph of immunogold localization of antigens in an untrathin section of *G. spinigerum* advanced third-stage larva by MAb FS-3D11. The gold particles are specifically concentrated on the intestinal brush border as shown (arrow head).

Fig 2–Localization of antigens in *G. spinigerum* larva by MAb SS-5H5. Gold particles are predominantly labeled on the intestinal brush border as shown (arrow head).

Fig 3–The gold particles were densely scattered over the cytoplasm of epithelial cells (arrow head) when the section was probed with MAb SS-5H5. b represents brush border; n, nucleus.

Fig 4–Negative immunoglod labeling in a section of *G. spinigerum* larva when probed with the culture supernatant of X63 myeloma cells (negative control). Note the clear intestinal brush border (arrow head).

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