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Distribution of 28.5 kDa antigen in the tegument of adult *Fasciola gigantica*

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Abstract

Monoclonal antibody (MoAb) specific to 28.5 kDa tegumental antigen (TA) was used to localize this antigen in various tissues of adult *Fasciola gigantica* by means of indirect immunofluorescence, immunoperoxidase and immunogold techniques. The indirect immunofluorescence and immunoperoxidase detections revealed that this antigen was concentrated in the tegument particularly in its outer rim, tegumental cells and their processes, epithelial linings of the oral sucker and the proximal part of digestive tract. It was also detected at a moderate concentration in spermatogenic cells in the testes, cells of Mehlis' gland, oocytes within the ovary, and ovum within the egg of adult parasites. At TEM level, the immunogold detection showed deposit of gold particles specifically in G_2 tegumental granules and on the surface membrane. Thus, this antigen is expressed in the tegument and associated structures of adult parasites, and it could be a major component of the G_2 granules which are shown to fuse with the surface membrane and contribute material to replace the casted-off membrane. This process is a part of membrane turnover that prevents the parasite from being attacked by the host immune effector cells.

Keywords: *Fasciola gigantica*; Tegument; 28.5 kDa antigen; G_2 tegumental granule; Ultrastructure; Monoclonal antibody; Immunoelectron microscopy

1. Introduction

In liver flukes, the tegument is the interface layer that helps the parasite to maintain their homeostasis which is essential for their survival, for examples, in the absorption and exchange of nutritive and waste molecules, regulation of osmolarity as well as ionic equilibrium, and protection from hostile host immune response. The tegument is the major site from which antigens are released to stimulate the host immune response (Hanna and Jura, 1977; Sobhon et al., 1998; Fairweather et al., 1999). The tegument was also shown to be the target of several anthelminthic drugs (Sobhon and Upatham, 1990; Apinhasmit and Sobhon, 1996; Meaney et al., 2002, 2003, 2004) and a few vaccine candidates (Waine et al., 1999).

The tegument-associated antigens are among the principal antigens released into the excretion–secretion (ES) materials of the trematode parasites. In schistosome species, especially adult *Schistosoma japonicum* and *S. mekongi*, analysis by immunoblotting showed that there were 15–20 bands of tegument-associated

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antigens at MW 26–205 kDa (Sobhon and Upatham, 1990). *Schistosoma mansoni* also showed common surface antigens with these species at MW 97, 86, 68, 50 and 38 (Taylor et al., 1981), and the other tegumental antigens at 25–27 kDa which might be surface membrane antigens (Fallon and Doenhoff, 1995). In *Fasciola hepatica*, monoclonal antibodies (MoAbs) were raised against antigens present in the tegumental syncytium of the juvenile parasite and immunoprecipitation analysis showed that the MoAbs could react with 50 and 25–40 kDa tegumental antigens. Using immunohistochemical method, these antigens could be localized specifically in the glycocalyx coating the surface membrane (Hanna and Trudgett, 1983).

In adult *Fasciola gigantica*, the immunoblotting of ES antigens with naturally infected cattle serum exhibited two groups of molecules (Sobhon et al., 1996). The first group consisting of molecules at 66, 64, 58 and 54 kDa was believed to be the tegumental antigens, which were more species-specific than the second group which included molecules at 27 and 26 kDa that may be released from the caecum (Viyanant et al., 1997a,b). Among these tegumental antigens, the 66 kDa antigen was specifically localized by the corresponding MoAbs at high concentration in the outer part of the tegument cytoplasm and the surface membrane (Krailas et al., 1999). Another *F. gigantica* specific tegumental antigen at 28.5 kDa was detected by Chaithirayanon et al. (2002) using a corresponding monoclonal antibody (MoAb). In this study, we have regenerated this MoAb and used it to further localize the distribution of 28.5 kDa antigen in the tegument and associated structures of adult *F. gigantica* by means of indirect immunofluorescence, immunoperoxidase and immunogold techniques.

2. Materials and methods

2.1. Collection of adult parasites and antigen preparation

Adult *F. gigantica* were collected from gall bladders and intrahepatic bile ducts of infected cattle or water buffaloes killed for consumption at the local abattoirs. They were washed several times with 0.85% NaCl solution and used immediately.

To obtain tegumental antigens (TA), adult worms were extracted with a non-ionic detergent (1% Triton X-100 in 0.05 M Tris buffer, pH 8.0, 0.01 M EDTA, 0.15 M NaCl) for 20 min at room temperature. Shedding of the tegument was monitored under a light microscope, and at the end of extraction the supernatant was collected and devoided of contaminating eggs by centrifugation at 5000 × *g* for 20 min. The supernatant that contained soluble TA was collected and dialyzed against 0.01 M phosphate buffered saline (PBS) overnight at 4 °C, using Spectra/Por dialysis membrane (Spectrum Medical Industries, Los Angeles, CA, USA) with molecular weight cut off at 6–8 kDa. The protein concentration was determined by Lowry's method (Lowry et al., 1951), and the protein solution was stored at −70 °C until use in later experiments.

2.2. Preparation of MoAb against 28.5 kDa tegumental antigen

Four inbred female BALB/c mice 6- to 8-week-old were immunized according to the method described by Chaithirayanon et al. (2002). The hybridoma clone expressing MoAb against 28.5 kDa antigen was revived and this MoAb was regenerated and used in the present study.

2.3. Specimen preparations

For indirect immunofluorescence detection, freshly collected adult *F. gigantica* were cut into blocks and fixed in 4% (w/v) paraformaldehyde in 0.1 M PBS pH 7.4 at 4 °C for 4 h. After washing, they were immersed in 5% (w/v) sucrose in 0.1 M PBS at 4 °C for 24 h and then transferred into 30% (w/v) sucrose in 0.1 M PBS at 4 °C for 1–2 weeks. The tissue blocks were embedded in the Tissue Tek O.C.T. medium (Miles Inc., Elkhart, USA) at −20 °C and cryo-sectioned at 5 μm thickness using Leica CM 1800 cryostat, mounted on 3-aminopropyltriethoxy-saline (APES) (Sigma–Aldrich Co.) coated slides.

For immunoperoxidase detection, the fresh worms were cut into blocks and fixed in 10% buffered formalin, dehydrated through graded series of alcohol, embedded in paraplast, cut at the thickness of 5 μm and placed on APES-coated slides.

For immunogold detection, the fresh worms were cut into small blocks and fixed immediately in a solution containing 0.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M PBS pH 7.4 at 4 °C for 1 h. The worms were dehydrated in 70%, 80%, 90% ethanol, and the tissue blocks were placed into the 90% ethanol-diluted L.R. White (London Resin Co., Thule, UK), at ratios of 1:2 and 2:1 for 1 h each. Then, the tissue blocks were transferred to pure L.R. White, kept at 4 °C overnight, followed by one change of pure L.R. White for 1 h, and finally embedded at 50 °C for 24 h. The thin sections were cut and collected on formvar-coated 300-mesh nickel grids.