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Research Note

Immuno-electron Microscopic Observation of Excretory Cell of *Toxocara canis* Larva

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Excretory-secretory (ES) products of *Toxocara canis* larvae have been considered as a functional antigen in immune response of toxocariasis, and it has been demonstrated that a large quantity of the ES products would be contained in the excretory cell of the larva (de Savigny, 1975; Maizels *et al.*, 1982; Sugane and Oshima, 1983; Akao *et al.*, 1983). However, little information of morphological feature of the excretory cell of *T. canis* larva has been given, excepting the description by Nichols (1956) with light microscope. The present paper dealt with detailed finding of the excretory cell of *T. canis* larva, in particular, refered to the localization of the antigen by means of electron microscope.

T. canis larvae collected by method of Kondo *et al.* (1981) were incubated in Eagle's MEM for one week according to the method of de Savigny (1975). Larvae used for electron microscopic observation were fixed with 2.5% glutaraldehyde for overnight and 1% osmium tetroxide for 3 hours. The fixed larvae were dehydrated with an ethanol series, and embedded in Epon. The thin sections by utlramicrotome were stained with uranyl acetate and lead citrate.

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The larvae were fixed for 12 hours at 4°C in PLP fixative, and were embedded in OCT compound to make the frozen sections of 5 μm thick by cryostat. The sections were incubated for overnight with HRP-conjugated rabbit anti-toxocara IgG Fab that diluted by 1:100 in PBS containing 10% normal goat serum. After repeating of wash, peroxidase activity was revealed by incubation in 3-3′ diaminobenzidine-4HCl, and supplemented with H_2O_2. The specimens were fixed with 2.5% glutaraldehyde for overnight and 1% osmium tetroxide for 3 hours, the thin sections by ultramicrotome were prepared for the examination with electron microscope (Akashi LEM-2000, AKASHI CO. LTD., Japan) at accelerating voltage of 100 KV.

The anti-toxocara rabbit serum obtained from rabbit experimentally infected with 100,000 embryonated eggs of *T. canis* 26 weeks after infection was kept at −80°C until use. Antibody of anti-toxocara IgG Fab for direct immuno-staining was purified from anti-toxocara rabbit serum by Sepharose column chromatography. The Fab was conjugated to HRP by the methods of Wilson and Nakane (1978). The specimens for light microscopic observation were made according to the method of Akao *et al.* (1983).

The longitudinal section of anterior-middle portion of *T. canis* larva by electron microscope is shown in Fig. 1. The outer layer of the larva is composed of cuticle, excretory pore and

lateral alae. The hypodermis and muscle layer are located beneath the cuticle. The esophagus, nerve ring, two column-like excretory cells, intestine and ventral chord are seen in the pseudocoel. The sizes of two trasnsverse sections at middle portion of the larva are 17.1 or 17.4 μm in diameter. The cuticle has 0.18–0.22 μm thick, hypodermis measuring 0.23–0.31 μm thick with waved fashion is seen under the cuticle. Muscle layer is divided into four groups by two lateral chords, dorsal and large ventral chords. There are 7 or 8 muscle cells per quadrant. Excretory cells are large oval shaped. The cytoplasm of the excretory cell is filled with numerous round vesicle-like structures (Fig. 2). The net-like architectures in the excretory cells were more minutely observed with immunoenzymatic staining by light microscopic observation (Fig. 3). These structures were composed of numerous vesicles with high electron density (Fig. 4). In the vesicles, there were smaller vesicles of granules with high electron density of various sizes measuring approximately 0.1–0.06 μm which seemed to the protruded or excreted in the lumen of the large vesicles.

The paired excretory cells of the larva were large oval shaped in transverse section and were column-like or elongated form in longitudinal section, as pointed out by Nichols (1956), though we cannot see the canaliculi in the central part of the excretory cell. As far as vesicle-like structure of excretory cell is concerned, Bird (1971) described excretory system of the adult female worm of *Meloidehyne javaenica*. However, the relation between the structures and function of excretory system has been retained unsolved. Our study using electron microscope combined with immunoenzymatic staining suggested that ES antigen might be possibly produced or excreted from the excretory cells of the larva of *T. canis*.

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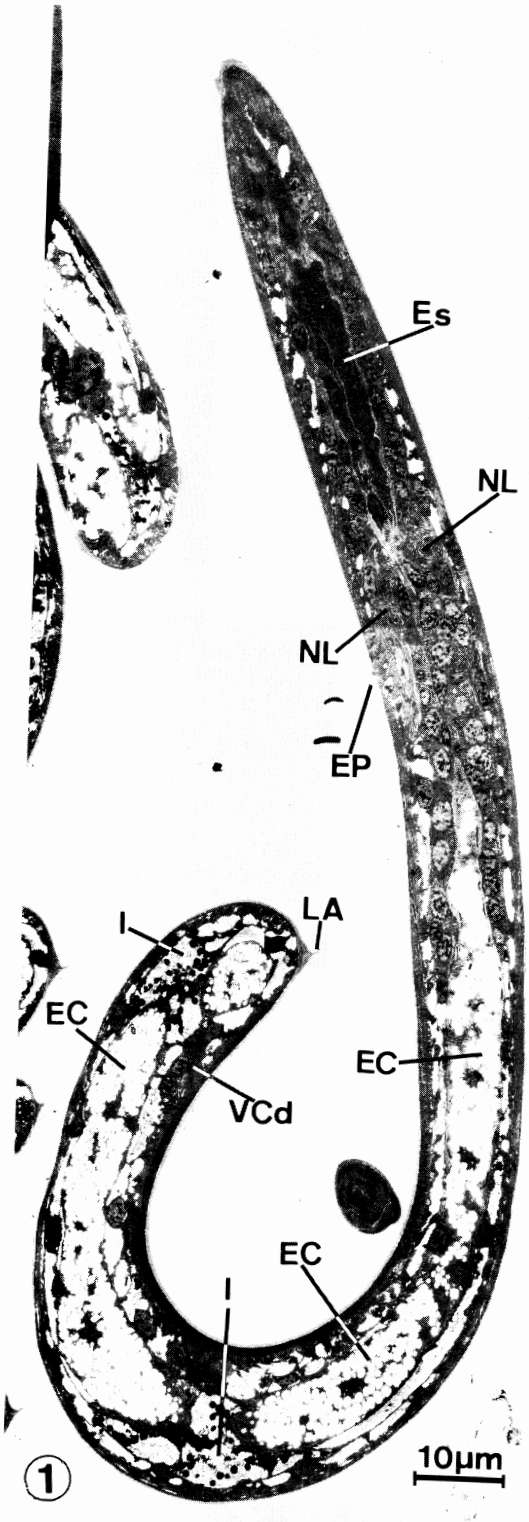
Fig. 1 The feature of longitudinal section at anterior-middle portion of the second stage larva of *Toxocara canis* (×1,200).

Fig. 2 The transverse section at middle portion of larva (×4,600).

Fig. 3 Immunoenzymatic staining of transverse section at the middle portion of larva. Note the localization (dark stained) of antigen in the excretory cell (×2,400).

Fig. 4 The localization of antigen in the excretory cell of larva. MRP-conjugated anti-toxocara IgG Fab and peroxidase activity were revealed by electron microscope (×2,500).

Cu: Cuticle, DCd: Dorsal chord, EC: Excretory cell, I: Intestine, LA: Lateral ala, LCd: Lateral chord, MCl: Muscle cell, VCd: Ventral chord, VEC: Vesicle of excretory cell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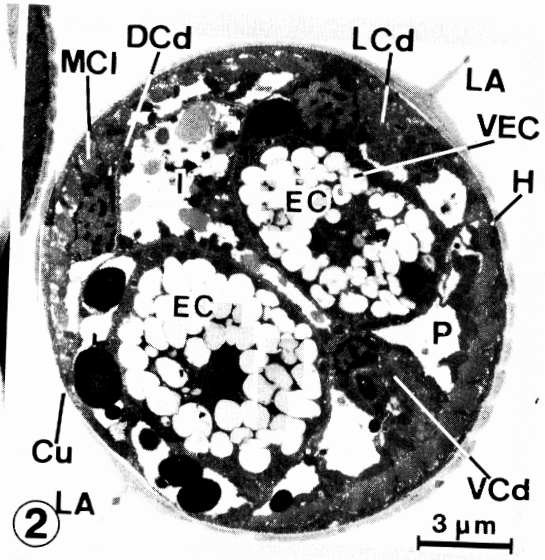
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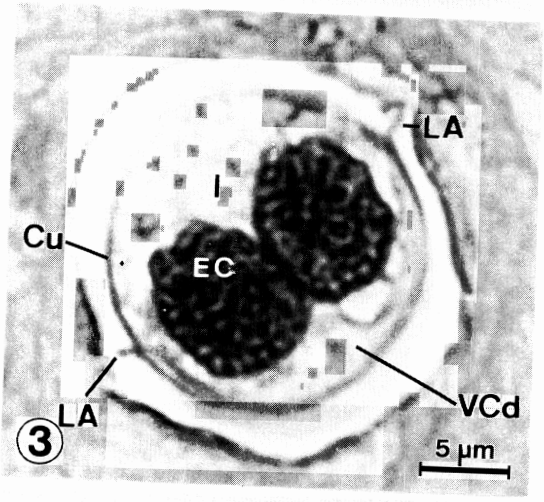
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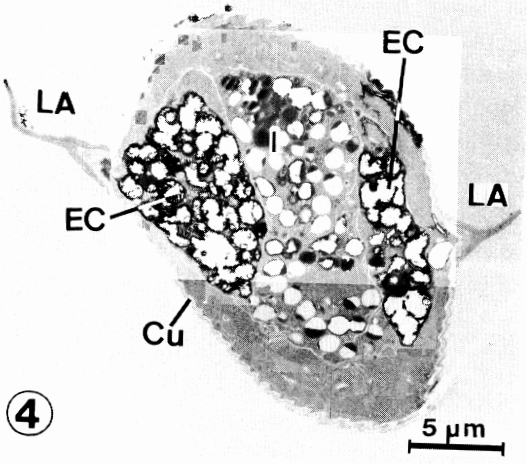
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