Intestinal Pathology in Human Metagonimiasis with Ultrastructural Observations of Parasites

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A human case of intestinal metagonimiasis that was incidentally found during the histological examination of a resected segment of jejunum was described. The small adults trematode of Metagonimus yokogawai were found free in jejunal lumen as well as impacted in intervillous spaces. Histologically intestinal lesions were massive lymphoplasmacytic and eosinophilic infiltration in stroma, erosion of nearby enterocytes, goblet cell depletion and occasional villous edema.

Scanning electron microscopy revealed the worm of spatulate appearance with rake-shaped tegumental spines. By transmission electron microscopy, the syncytial integument with dense discoidal bodies, basement membrane, muscle cells and subtegumental cells were observed. Characteristic junctional complex was demonstrated between subtegumental and parenchymal cells as well as between parenchymal cells.

Key Words: *Metagonimus, electron microscopy, parasite, human parasitism, intestinal parasite, helminth, trematode*

INTRODUCTION

Metagonimiasis is one of the endemic parasitic diseases in Korea (Seo et al, 1981). The trematode parasite *Metagonimus yokogawai* resides in the small bowel and causes clinical manifestations of fatigue, diarrhea and abdominal pain (Chai et al, 1977). Peripheral eosinophilia is associated especially in early phase. Experimental infection of *Metagonimus yokogawai* in rats and cats (Chai, 1979; Lee et al, 1981) showed that the pathology of intestine explained well the clinical symptoms in man. The main lesions are confined in mucosa of upper small intestine but the lesions may extend to ileum particularly in heavy infections. Though animal studies of its pathology have been done, the human intestinal le-

sions in metagonimiasis was seldom the subject of study because of its rare availability. By the same reason the worm parasite in the human mucosa has rarely been studied for its ultrastructures under scanning or transmission electron microscopy.

We found incidentally a human *Metagonimus* infection in an intestinal segment that was removed surgically for treating intestinal perforation associated with malignancy. In this paper, the pathologic findings of human metagonimiasis were discussed in relation with animal study results, and ultrastructures of the worm parasites are described.

MATERIALS AND METHODS

The intestinal segment removed from a 40 years old man was immediately fixed in 10% neutral formalin. The main and extended lesions of malignant histiocytosis were carefully selected. Because the patient was known to be infected with *Metagonimus yokogawai* by stool examination the remaining tissues

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Fig. 1. Photomicrograph of the intestinal mucosa with an adult *Metagonimus* in the Lieberkuehn's crypt. The oral sucker is facing the depth of the crypt. Surrounding epithelium shows compression (arrows). Goblet cells are depleted. The propria mucosae shows a marked small round cell infiltration together with a number of eosinophils. The villi are still delicate. The worm shows mature spermatozoa and eggs in the body. H&E $\times 300$

were minced for worm collection. Grossly normal tissue was cut and embedded in paraffin. The tissue sections were processed routinely and stained with hematoxylin and eosin. The collected worms from intestine were fixed using 2.5% glutaraldehyde buffered with phosphate and processed as routine procedure. Epon sections were stained with uranyl acetate and lead citrate, and examined in a Hitachi H-600 transmission electron microscope. For scanning electron microscopy the fixed and osmium-treated trematodes specimen was dehydrated in graded acetone, and also processed as routine procedure. It was examined with a Hitachi S-520 scanning electron microscope.

RESULTS

1. Intestinal lesion

The original intestinal disease was malignant

Fig. 2. *Metagonimus* is flat and slight by concave ventrally. Oral sucker (OS) is seen on the top, but ventral sucker is inconspicuous. Interrupted line measures 176 μm . SEM

histiocytosis (histiocytic medullary reticulosis) where the histiocytes showed varying degree of anaplasia and occasional erythrophagocytosis. These lymphoreticular infiltrate involved the mucosa, submucosa and muscularis in isolated fashion or transmurally. In one area the infiltrate was complicated by perforated bowel wall resulting in acute fibrinopurulent peritonitis. In the midst of these neoplastic and infectious processes there were scattered foci of parasitic infestation. The trematodes were found predominantly in the villo-crypt junction. The worms were cut in various directions and found occasionally free in the bowel lumen. The crypts with impacted parasites were often distended and the lining enterocytes were slightly eroded. Goblet cells were definitely decreased in these areas. The worms did not penetrate the epithelial layer but resided in the propria mucosae. The rostral portions of the worms directed to depths of crypts and often revealed oral sucker. The villi were relatively well preserved without atrophy or fusion. However, there were definite inflammatory cellular infiltrate in the propria, comprising chiefly of eosinophils, plasma cells and lymphocytes. The eosinophils were particularly prominent

Fig. 3. The surface of the worm is diffusely covered with fairly regular scales. SEM. Interrupted bar measures 43 μ m.

throughout with or without adjacent worm impaction (Fig. 1). The degree of edema was variable by the presence of neoplastic cell infiltration and distance from the perforation site. Most worms found in the sections were adults with characteristic hermaphroditic structure. Numerous eggs and mature sperm flagella were seen in the worm body cut in various directions. The vitellaria and excretory ducts were also seen. The tegument showed numerous spines that were closely approximated.

2. Scanning electron microscopic findings:

The worms were flat and narrowed anteriorly (Fig. 2). The oral sucker had the opening measuring about $27 \times 14 \mu$ m and the lip was thick with numerous wrinkles over the surface (Fig. 5). The ventral sucker was rather inconspicuous with small lip and located at about one-third the body length from the anterior end. The tegument of the worms was covered with velvety cytoplasmic processes (Fig. 3). The spines showed 7-9 pointed large scale-like appearance. Round swelling of cytoplasmic ridges was often seen around the ventral sucker. The eggs showed well formed opercular and finely granular external surface (Fig. 6). The

Fig. 4. Higher magnification of Plate 3, showing well developed multi-teethed spines. These spines are teathed with 7 to 9 and distribute all over the integument except for areas around oral and ventral suckers. SEM. Interrupted bar measures 3.0 μ m.

spines were fan-shaped without stalk. The base of the spines were fixed with slightly protruded tegumental tissue like a button-hole. They were arranged roughly in rows without any strict regularity (Fig. 4). The spines measured 1.8 μ m in wide and 1.8 μ m long. The size of spines was fairly uniform but those around the oral sucker were smaller. They appeared slightly concave in direction of posterior body. The spines were divided equally by deep furrows into 7-9 teeth or digits. The individual digit therefore measured 0.3 to 0.5 μ m wide and had sharp apex. The number of digits varied considerably in different portions of the body.

3. Transmission electron microscopic findings:

The integument consisted of a surface layer and a nucleated area. The surface layer was syncytial and without nuclei (Fig. 11). It rested on basement membrane and a thick layer of interstitial material which enclosed the muscle layers. It contained numerous mitochondria, endoplasmic membranes, large vacuoles and dense spines as well as a large number of

Fig. 5. Close up view of the oral sucker, showing an ovoid opening and prominent lips. Note the spines near the oral sucker are short and sparse. SEM. Interrupted bar measures 23.1 μ m.

Fig. 6. An egg of *Metagonimus* is shown. The ovoid egg shows the prominent operculum. The remaining area shows finely granular appearance. SEM. Interrupted bar indicates 7.5 μ m.

Fig. 7. Syncytial integument was provided with spines (S) and rested on the basement membrane (BM). Under the basement membrane are muscle bundles (M) that are running in various directions. One tegumental cell with its nucleus (N) is seen under the muscle layer, together with somewhat loosely textured parenchyma. TEM $\times 15,000$.

Fig. 8. The spines are often cut to show multiple teeth. These spines rest on the basement membrane. However this tangential section shows one spine (S) embedded in the muscle bundle. $\times 15,000$.

dense discoidal bodies. The tip of the spine was diverged like a bamboo rake. The spine was located in integument almost regularly and diverged into 7-9 at the tip (Fig. 8). The rootlets of the spines were located on the basement membrane. The basement

Fig. 9. Close up view of a tegumental cell nucleus located deep in the parenchyme under the basement membrane. Among many cytoplasmic organelles free ribosomes are seen. One area (arrow) shows junctional complex between this cell and the parenchymal cell (PC). Inset shows clear dense line between two cytoplasmic membranes. TEM $\times 50,000$.

Fig. 10. A portion of oral sucker showing a well developed longitudinal and circular muscle bundles among which numerous glycogen particle and other organelles are scattered. CL: central lumen. TEM $\times 6,250$.

Fig. 11. A portion of testis showing many germinal epithelial cells (GE) and sperm flagella (sterisk). $\times 7,500$.

Fig. 12. Cross section of the spermatozoa showing characteristic outer doublets, radial spokes and central singlet. Dynein arms are well shown. TEM $\times 200,000$.

membrane was rather undulating (Fig. 7). The cytoplasm of the subtegumental cells composed of fine granules and fibrils interspersed with clear areas.

It contained endoplasmic reticulum, mitochondria and various inclusion bodies (Fig. 9). Groups of small cisternae were also noted. The mitochondria distributed quite irregularly, somewhere crowded and other areas scanty. The inclusion bodies were glycogen and

lipid droplets. The subtegumental cells were separated from each other and from other organ systems by an interstitial material, which was composed of fibers embedded in a homogeneous matrix. In localized areas the interstitial material was absent and the plasma membranes of adjacent subtegumental cells were separated only by a narrow intercellular space bisected by a dense line (Fig. 9).

All junctional complexes contained the central dense line. The plasma membranes of the complex lost the unit membrane structure seen in the plasma membrane elsewhere, and each became compressed into a single dense line. The muscle bundles were well developed near the oral and ventral suckers (Fig. 10). Myofibrils measured approximately 15 μ m in diameter. Numerous mitochondria and glycogen particles were seen between muscle fibers. In seminal receptacles and testicular tissue rows of germinal epithelium and many mature flagellated spermatozoa were seen (Fig. 11). The flagella disclosed characteristic $9+2$ doublets structure with central singlet and radial spoke (Fig. 12).

DISCUSSION

Human tissue with *Metagonimus* infection is not easily available for study because the intestinal symptoms such as abdominal pain or diarrhea are seldom the subjects of surgical intervention. Incidental findings of parasitic infection in the segment of intestine that is removed for other reason are only rare chance for studying parasitism in human. In addition, when worms are harvested in the stool after antihelminthic treatment, ultrastructural changes such as vesicle or bullae formation and degeneration of sensory papillae were found (Mehlhorn *et al.*, 1983). In this regard this case is revealing human intestinal pathology with parasites without such potential artefacts related to the drug.

As described, this patient underwent segmental resection because of the intestinal perforation due to diffuse neoplastic cell infiltration of the whole layer of intestine. Therefore in this case infective process should be taken into consideration other than the parasite-related lesion. We believe that the mucosal changes particularly those of villi in the region where they was no simultaneous malignant histiocytosis involvement are, however, directly related to the parasites. It was interesting to note that histological alterations of infected mucosa in this case were not exactly same as described in experimentally infected animals. Villous atrophy, fusion, marked edema, or altered villus/crypt ratio were not seen. It was reported

by Chai (1979) that some mucosal lesions in experimental infected rats resolved naturally within a month. Lee *et al.* (1981) also observed that the pathology of small intestine in heavily infected cats also tended to restore spontaneously after 4 weeks of infection even though the adult worms remain viable. For this Kang *et al.* (1983) studied intestinal lesions of experimentally reinfected dogs with *Metagonimus*, and found that the recovering intestinal lesions were aggravated temporarily by reinfection. The pathologic findings seen in this human case were comparable with those in recovering phase of infection in experimental animals.

The parenchyme of trematodes is a syncytial meshwork enclosing fluid-filled spaces. They are considered more than a mere packing function. Since flukes are without a vascular system metabolites must pass from one to other organ system through parenchymal tissue. The relationship between tegument and the subtegumental cells has been considered very important since it is the mechanism of metabolic transport (Gallagher and Threadgold, 1966). It was well demonstrated in this case that there were unique junctional complexes among these cells. The junctional complex was thought to be analogous to the tight junction seen in vertebrate host. It has been shown in *Fasciola hepatica* by Threadgold and Gallagher (1966, 1977).

The findings of TEM in this case were comparable to those described by Inatomi *et al.* (1968). Findings of tegument of *Metagonimus yokogawai* on scanning electron microscope has been described by Lee *et al.* (1984). They observed the chronological changes in the tegumental structure of excysted metacercariae, juveniles and adults of 2 days to 4 weeks old. The tegumental structures of this case were roughly parallel to those of 4 weeks or older worms.

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