NOTE

Electron Microscopic Observations on the Intestine of a Cow with Johne's Disease

Masanori KUBO, Masashi MORIWAKI, and Hiroshi WATASE

Kyushu Branch Laboratory, National Institute of Animal Health,
2702 Chuzan-cho, Kagoshima, Kagoshima 891-01

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Johne's disease is an insidious disease in ruminants. *Mycobacterium paratuberculosis* is the causative agent and it multiplies in macrophages or epithelioid cells [5, 6]. The interaction between the bacteria and macrophages, however, is not clear. We examined the intestine of a cow with naturally occurring Johne's disease by electron microscopy.

The cow was female holstein, 5 years of age. She was killed at the Nansatsu Livestock Hygiene Service Center, Kagoshima, Japan, because she was diagnosed as Johne's disease by complement-fixation test and detection of *M. paratuberculosis* from the feces.

Grossly some parts of the mucosa of the ileum, cecum and colon showed slight thickening. Histologically the characteristic lesions of Johne's disease were observed in the jejunum, ileum, cecum, mesenteric lymph nodes and liver.

Samples for electron microscopic examination were taken from the intestines. They were fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4) and postfixed in 1% osmium tetroxide in the same buffer. After dehydration in a series of alcohols, samples were embedded in Epon 812. Sections were stained with uranyl acetate and lead citrate and examined with at H-300 (Hitachi) electron microscope.

M. paratuberculosis was found in macrophages which infiltrated into the lamina propria of the intestine (Fig. 1). *M. paratuberculosis* had a triplelayered cell wall, i.e., inner and outer electron-dense membranes and an electron-transparent zone (ETZ) (Figs. 2 and 3). ETZ was about 12 nm in width. Cytoplasmic membrane was seen beneath the inner electron-dense membrane. Ribosomes were present at the peripheral portion of the bacterial cell. Fibrillar nuclear material was seen in the central part of the bacterium. Mesosomes were observed in some bacteria. Many bacteria lay directly in the cytoplasm of the macrophages. They were not surrounded by a limiting membrane (Fig. 2). Some bacteria were seen in the lysosomes (Fig. 3). However, most of them had an intact appearance.

Macrophages were observed in the lamina propria of the intestine. Macrophages in which a large number of *M. paratuberculosis* were seen had a small number of organelles. On the other hand, those in which a small number of *M. paratuberculosis* were seen had many lysosomes (Fig. 1). Collagen fibers were seen on one side of the macrophages, but none of them were found between the macrophages. Eosinophils and lymphocytes infiltrated around the macrophages (Fig. 1).

Bendixen *et al.* [3] reported electron microscopic observations on Johne's disease. They did not describe whether the bacteria were seen directly in the cytoplasm or not. In our report, *M. paratuberculosis* lay directly in the cytoplasm, i.e., it was not surrounded by a limiting membrane. Some bacteria were engulfed in lysosomes. The lysosomes had a unit membrane, so it was possible to distinguish the bacterial cell wall from the limiting membrane.

Experiments using *M. tuberculosis* showed that *M. tuberculosis* was found in phagosome of macrophages [1, 2, 4], and that acid-fast bacilli hinder phagosome-lysosome fusion [1, 2]. But in this study, *M. paratuberculosis* was found directly in the cytoplasm and nearby or in the lysosomes. The phagosome-lysosome theory did not explain why *M. paratuberculosis* could survive in the cytoplasm of macrophages. Further studies are needed to explain how *M. paratuberculosis* invades or is phagocytized by macrophages.

M. paratuberculosis had a triple-layered cell wall, which was the same as that of *M. leprae*, *M. aurum* and *M. avium* [7, 8], and was different from that of *M. tuberculosis* [8]. This difference in cell wall might

reflect the difference in host-parasite relationship between *M. paratuberculosis* and *M. tuberculosis*.

It might be difficult for Epon 812 to penetrate into the ETZ, so that many ETZs were broken during the preparation of thin sections, leaving pores around the bacteria (Fig. 1). These pores were frequently observed by many workers studying acid-fast bacilli electron microscopically [1–4]. ETZs were rather characteristic of acid-fast bacilli, and it might play some role in anti-phagocytizing effects.

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EXPLANATION OF FIGURES

Fig. 1. Macrophages infiltrating into the lamina propria of the cecum. *M. paratuberculosis* is seen in the cytoplasm of macrophages. M: macrophage; E: eosinophil. $\times 1,750$.

Fig. 2. *M. paratuberculosis* seen directly in the cytoplasm. It has a triple-layered cell wall, i.e.,

inner and outer electron-dense membranes and electron-transparent zone (arrows). $\times 77,500$.

Fig. 3. *M. paratuberculosis* seen in a lysosome. Lysosome has a unit membrane (arrowheads). $\times 77,500$.

要 約

ヨーネ病牛の腸の電顕的観察(短報): 久保正法・森脇 正・渡瀬 弘(家畜衛生試験場九州支場)——鹿児島県下で発症した牛ヨーネ病の腸病変を電顕的に観察した。*Mycobacterium paratuberculosis* は粘膜固有層に浸潤したマクロファージ細胞質内に限界膜に包まれずに多数存在し、明瞭な3層の細胞壁構造を有していた。ライソゾーム内にも若干の菌がみられたが、多くは構造に異常を示さなかった。