Electron Microscopy of Equine Rotavirus in MA-104 Cells

Hiroshi Imagawa,* Ryuichi Wada* and Kiyoshi Hirasawa*

Morphogenesis of equine rotavirus strain BI in MA-104 cells was examined. Numerous virus particles were detected within the cisternae of endoplasmic reticulum in the cytoplasm of infected cells. Three types of virus particles were found: core particles 30-40 nm in diameter, single-shelled particles 55-60 nm in diameter, and double-shelled particles 70-80 nm in diameter. The outer layer of the double-shelled particle was formed by budding from the membrane of endoplasmic reticulum. Besides, tubular and honeycomb-like structures were observed in the cytoplasm of the infected cells.

Previously, the authors1,2) reported that equine rotavirus could be isolated and passaged in MA-104 cells. Moreover, they3) pointed out that the virus possessed the same antigenic and physicochemical properties as those of the genus rotavirus. This report presents the morphogenesis of equine rotavirus strain BI1) in MA-104 (rhesus monkey kidney) cells.

The virus used was equine rotavirus strain BI which had been isolated by the authors. For the test, the virus was passaged 20 times in MA-104 cells in the presence of acetyl trypsin (type V-S, Sigma Co., Saint Louis, Missouri, U.S.A.). MA-104 cells were propagated in modified minimum Eagle's medium (MEM) (Flow Laboratories, McLean, Virginia, U.S.A.) containing 10% fetal bovine serum and antibiotics. A maintenance medium used was MEM containing 10% tryptose phosphate broth (TPB), 0.05% yeast extract, 0.1% glucose, 1 μg/ml of acetyl trypsin, and antibiotics. A cell monolayer was allowed to form in a bottle (5×5×12 cm) at 37 °C for 1 day. It was washed three times with MEM and infected with strain BI at a multiplicity of approximately two per cell attached. After adsorption at 37 °C for 60 min, it was washed three times with MEM. Then 10 ml of maintenance medium was added and incubation was continued at 37 °C for 24 h.

The infected monolayer was fixed in 2.5% glutaraldehyde in 0.1 mol/l phosphate buffer (pH 7.2) at 4 °C for 30 min, and postfixed in 1% osmium tetroxide in the same buffer for 30 min. The fixed cells were scraped with a rubber policeman and pelleted by centrifugation at 2,000 rpm for 5 min. The resultant pellet was dehydrated in an ethanol series and
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Fig. 1. Electron micrographs of MA-104 cell 24 h after infection with equine rotavirus strain BI
A: Numerous virus particles are present within dilated cisternae of endoplasmic reticulum. N is nucleus. Bar represents 1 μm. B: Double-shelled particles (most solid arrow), single-shelled particles (second most solid arrow), and core particles (slender arrow) are found within a vesicle of endoplasmic reticulum. Bar represents 100 nm.

Fig. 2. Electron micrographs of MA-104 cell 24 h after infection with equine rotavirus strain BI
A: Outer capsid layer is formed by budding from the membrane of endoplasmic reticulum (arrow). N is nucleus. Bar represents 1 μm. B: Magnified budding process. Bar represents 100 nm.

Numerous virus particles were observed in infected cells 24 h after infection. They were present within the cisternae of endoplasmic reticulum in the cytoplasm (Fig. 1-A). There were three morphological types of virus particles, double-shelled, single-shelled, and core particles (Fig. 1-B). Double-shelled particles were 70 to 80 nm in diameter and formed from core and inner and outer layer. The outer layer was formed by budding from the

embedded in epon B12. Sections were cut with glass knives, stained with 2% uranyl acetate followed by lead citrate, and examined under an electron microscope (Hitachi H-600 type).
membrane of the endoplasmic reticulum (Fig. 2-A, B). Such findings were seen in MA-104 cells infected with human rotavirus strain Wa,⁴ and in the duodenal and jejunal mucosa of horses infected with equine rotavirus.⁵ Single-shelled particles were 55 to 60 nm in diameter and contained a core at the center. Core particles were 30 to 40 nm in diameter.

Tubular structures approximately 80 nm in diameter were frequently observed in the cytoplasm and sometimes gathered around double-shelled particles (Fig. 3-A). They were seen in both nucleus and cytoplasm of MA-104 cells infected with human rotavirus⁶ or in the cytoplasm of cells in the jejunal mucosa of infants infected with rotavirus.⁶ Honeycomb-like structures were very rarely observed in the vesicle of endoplasmic reticulum (Fig. 3-B). No virus particles were seen in the nucleus.

In this study, no detailed morphogenesis of equine rotavirus in MA-104 cells could be made clear, since no cells were examined serially with the lapse of time after infection. The results of this study, however, agreed basically with those obtained from MA-104 cells infected with human rotavirus strain Wa. As a result, it is suggested that equine rotavirus may show the same morphogenesis as human rotavirus.

The authors wish to thank Dr. M. Tajima, director, Nippon Institute for Biological Science, for valuable advice regarding electron microscopy. They wish to thank Professor Omori, of the College of Agriculture and Veterinary Medicine, Nihon University, for advice and guidance extended to this study. Thanks are also due to Mr. T. Yamakawa and Mrs. K. Ito, of the authors' station, for technical assistance.

Literature Cited

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**MA-104 細胞における馬ロタウイルスの電子顕微鏡的観察**（短綱）：今川 浩*・和田隆一*・平澤澄*（* 日本中央競馬会競走馬総合研究所栃木支所）——馬ロタウイルスの MA-104 細胞における増殖形態について検討した。無数のウイルス粒子が感染細胞の細胞質の拡散した小胞体内に認められた。ウイルス粒子は直径 30–40 nm の core 粒子、単層の殻を有する直径 55–60 nm の粒子、二層の殻を有する直径 70–80 nm の粒子の三つの型のものが認められた。二層の殻を有する粒子の外層の殻は小胞体の膜を用いた出芽によって形成された。さらに、感染細胞の細胞質には管状構造物および蜂の巣様構造物が認められた。