In vivo Morphogenesis of a New Porcine Enteric Coronavirus, CV 777

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With 9 Figures

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Summary

The morphogenesis of a new porcine enteric coronavirus, CV777, in intestinal epithelial cells of experimentally infected newborn piglets is described. The virus shows a morphogenetic pattern characteristic for members of the Coronaviridae family. It is formed by budding through membranes of the endoplasmic reticulum. Some specific aspects of this morphogenesis are discussed.

Introduction

In 1978, a new coronavirus-like agent (CVLA), was demonstrated in the faeces of piglets from outbreaks of diarrhoea (16). The clinical findings after natural and experimental infection have been described (7). The virus has been provisionally classified as a coronavirus, based on its morphologic aspect with negative staining. It has been shown to differ serologically from other known porcine coronaviruses, transmissible gastro-enteritis virus (TGEV) and haemagglutinating encephalo-myelitis virus (HEV) (16).

The pathogenesis of the experimental oronasal infection has been studied by immunofluorescence (8). The accompanying histological and ultrastructural lesions will be reported elsewhere (4, 11). The results of these studies show that virus replication occurs in the cytoplasm of absorptive epithelial cells covering the small intestinal villi and the large intestine. The intracellular virus replication results in alterations of cellular organelles and cell desquamation.

The present study deals with the morphogenesis of the CVLA in the intestinal epithelial cells of piglets after experimental oronasal infection.

Materials and Methods

Sixteen caesarean-derived colostrum-deprived piglets were inoculated oronasally on the second or third day of life with 10^4 pig infective doses of a virus stock, obtained
as described elsewhere (DEBOUCK et al., to be published). One piglet was kept as a control. The pigs were sacrificed at different time intervals after the inoculation (12, 18, 24, 30, 32, 36, 38, 41, 48, 60, 72, 96 and 120 hours).

Tissues were collected from the duodenum, middle jejunum and colon as described elsewhere in more detail (DUCATELLE et al., to be published). The specimens were fixed in 2 per cent paraformaldehyde, 2.5 per cent glutaraldehyde in 0.1 molar cacodylate buffer. Postfixation was done with 1 per cent OsO4. The blocks were stained with uranylacetate dehydrated in acetone in a vacuum chamber and then embedded in Spurr medium. Semi-thin sections were stained with toluidine blue; ultra-thin sections were stained with lead citrate.

Results

In the watery phase of diarrhoea, numerous viral particles were detected in the sections of the small intestinal villi.

Occasionally, the particles were seen in close apposition to, and between the microvilli of uninfected cells. More frequently, rows of virus particles were seen between the microvilli of heavily infected cells (Fig. 1). These cells showed an electron translucent cytoplasm and short, irregular microvilli. Cell free viral particles ranged in diameter from 60 to 90 nm. They were round and consisted of an inner electron dense core of 40 to 70 nm. This core sometimes showed a central clearing halo, but was completely electron-dense in other particles. It was separated by a narrow translucent ring from an outer unit-membrane.

Virions were sometimes seen in the intercellular space between epithelial cells. They were frequently present inside caveolae or apical pits between the microvilli

Fig. 1. Interaction of the virus with the apical cell membrane: 1 Viral particles in small cytoplasmic vesicles. 2 Rows of viral particles between the microvilli of an infected cell. × 23,275; 36 hours post infection (hpi)