THE SIGNIFICANCE OF THE GOLGI APPARATUS IN THE MORPHOGENESIS OF THREE PORCINE CORONAVIRUSES: HEV, TGEV and CV777

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Of these three porcine coronaviruses, the Transmissible Gastro-Enteritis Virus (TGEV) and the CV777 virus (I) are enteropathogenic, causing epizoötics of diarrhea in newborn piglets. The Haemagglutinating Encephalo-myelitis Virus (HEV) is the agent responsible for "vomiting and wasting" in piglets. The latter virus is essentially neuropathogenic. All three these viruses replicate in the cytoplasm of their host cells.
The purpose of the present study was to compare the virus-host cell interaction with these viruses.

For this purpose, a case of TGEV infection from a natural outbreak was selected. With the CV777 virus, a series of 16 caesarean-derived, colostrum-deprived (CDCD) piglets were infected experimentally at the age of two or three days. They were euthanatized at different time intervals after inoculation (2). For the study of the morphogenesis of HEV, a three days old CDCD piglet was infected with the VW572 strain in the stomach wall after laparotomy. This animal was euthanized when it started vomiting, after an incubation period of seven days. From all of these animals, tissue specimens were collected and fixed in glutaraldehyde, formaldehyde, cacodylate buffer. For the study of HEV, also four cell cultures of primary porcine kidney cells were fixed 24h after they were inoculated with the same strain of virus. All specimens were postfixed, block stained, and embedded in Spurr's medium for transmission electron microscopy.

With the CV777 virus, the first morphologic evidence of infection of epithelial cells was the assemblage of the virus by budding through endoplasmic reticulum (EPR) membranes. This budding started as a crescent formation with accumulation of electron-dense material parallel to an EPR membrane. Subsequently a bud was formed, and this bud was pinched-off to form a complete virus particle inside EPR cysternae. Viral particles also were seen inside Golgi sacs, where they caused a spindle-shaped distention of the lamellae. The Golgi apparatus often was hypertrophic, forming an onion-like structure. Subsequently, the viruses also were observed in the extremities of the Golgi sacs, where they were pinched off as Golgi vesicles. Such virus-filled Golgi vesicles were seen in the cytoplasm of infected cells. Similar vesicles were seen to fuse with the apical cell membrane. As a result, rows of viral particles were present between the microvilli. Electron-dense, membrane-bound cytoplasmic structures with a fine granular matrix often were seen, especially in the early phase of diarrhea. These structures frequently contained numerous viral particles. They were morphologically similar to secondary lysosomes.
With HEV, assemblage of viruses by budding through EPR membranes was frequently seen in the cell cultures. In contrast, it was rare in the infected neurons of the myenteric plexus of the stomach.
Viral particles frequently were present inside the Golgi sacs, and formation of virus-filled Golgi vesicles was seen in both the "in vivo" material and in the cell cultures. Many small virus-containing Golgi vesicles were particularly striking in the Golgi area. Virus release by fusion of small vesicles with the plasma membrane was observed only in the cell cultures, and not in the neurons of the myenteric plexus. Budding of viral particles into Golgi sacs or at the cell membrane was never seen. Electron-dense, membrane-bound, lysosome-like cytoplasmic structures were seen to contain viral particles both in the cell cultures and in the neurons in vivo.
With TGEV in the enterocytes of a naturally infected piglet, budding at EPR membranes was occasionally seen. Viral particles also were observed inside Golgi sacs and in Golgi vesicles. The Golgi apparatus often appeared vacuolated and hypertrophic. Virus-containing lysosome-like cytoplasmic structures occasionally were seen.

All three viruses had a similar morphogenetic pattern. This pattern was characteristic for coronaviridae. Nevertheless, in our material, a hitherto not described, remarkable interaction with the Golgi apparatus was observed in infected cells. Indeed, after assemblage at the EPR, the virus particles seem to be transported to the Golgi cysternae, where embedded each in a small vesicle. These viruses thus seem to use the physiologic function of the Golgi apparatus in the packing up and transporting of endogenous material. Consequently, viral particles embedded in Golgi vesicles can be released from the host cell by fusion of the vesicles with the cell membrane. This can result in virus release without destruction of the host cell.

In conclusion, piglets infected with enteropathogenic coronaviruses can excrete infectious material prior to having significant histological intestinal lesions, and possibly prior to showing clinical signs of disease. It has indeed been shown with CV777 virus that viral particles are present in the faeces of infected piglets already during the incubation period.
This study was supported by a grant of the IWONL, Brussels, Belgium.

Selected references

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