Ultrastructural Changes in the Small Intestinal Epithelium of Suckling Pigs Affected with a Transmissible Gastroenteritis (TGE)-Like Disease

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With 9 Figures
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Summary

The small intestine of piglets collected during a sudden outbreak of diarrhoeal disease resembling transmissible gastroenteritis (TGE) was examined by light and electron microscopy. The principal histopathological changes were moderate infiltration by mononuclear cells in the lamina propria of the villi and cytoplasmic vacuolation. These were most pronounced in the epithelial cells covering the villous tips. By scanning electron microscopy, the intestinal villi were swollen and the transverse furrows disappeared. Microvilli were reduced in number leaving denuded areas on the brush border of the villous epithelial cells.

The ultrastructural changes were restricted to the cytoplasm of affected villous epithelial cells. The cell organelles were missing in rounded areas leaving cleared areas in the cytoplasm. Parallel fascicles and bundles were seen in these areas. Viral particles with an average diameter of 70 nm were found within the dilated apical tubulo-vesicular system, free in the cytoplasm, among the microvilli or lying free in the intestinal lumen. Viral particles surrounded a non-membrane bound viroplasm in some cases. The negatively stained particles showed a typical coronavirus morphology. These particles were found to be distinct from the known coronaviruses of swine, TGE virus and hemagglutinating encephalomyelitis virus by immune electron microscopy.

Introduction

Transmissible gastroenteritis (TGE) is a highly contagious enteric disease of swine of all ages causing important economic losses, especially in dense swine populations. The disease was first described by Doyle and Hutchings (9) in the United States, in 1946. In Hungary, Szent-Iványi et al. (20) reported the occurrence of TGE in 1964. Following the elaboration of methods for the routine
laboratory diagnosis of the disease (7), Csontos and Benyeda (6) turned their attention in 1970 to a new TGE-like epizootic diarrhoea of swine in which the causative role of TGE virus was excluded on the basis of virological examinations. Clinical observations and the experimental reproduction of diarrhoea in colostrum-deprived pigs with bacteria-free intestinal material have also supported the viral etiology of this diarrhoecal disease (1, 11).

In the past few years, an increased frequency of newborn pig diarrhoea caused by rotavirus was reported (3, 8, 12, 13, 16, 22, 24). However, in a number of instances, the significance of rotavirus could not be established (23). Finally, in Belgium Penxaert and De Bouck (15) and in England Chasey and Cartwright (4) detected coronavirus-like particles in the feces and intestinal epithelium of pigs affected with a TGE-like epizootic diarrhoea.

The present report describes the ultrastructural changes in the small intestinal epithelium and the detection of a virus morphologically similar to the coronaviruses in the intestinal content and epithelium of pigs affected with a TGE-like disease, which has occurred periodically in Hungary since 1970.

Materials and Methods

Animals

Three to seven days old piglets were collected from 3 large Hungarian swine-breeding farms during sudden outbreaks of diarrhoeal disease affecting animals of all ages. All the pigs showed a watery diarrhoea. The etiological role of TGE coronavirus was excluded by the negative result of direct immunofluorescence tests carried out on scrapings of the small intestinal mucosa, by unsuccessful virus isolation attempts on secondary swine thyroid cell cultures and by the absence of TGE virus-neutralizing antibodies in the acute- and convalescent-phase sera (14). Direct immunofluorescence (21), counter-immunoelectrophoresis (19), electron microscopic (18) and immune electron microscopic examinations (17) for rotavirus were also negative and no rotavirus antibodies were found by counter-immunoelectrophoresis (5) in acute- and convalescent-phase sera from the recovered animals.

Histopathology

Intestinal segments were removed from six different regions of the small intestine of sacrificed piglets and fixed in buffered formalin. The sections were stained with hemotoxylin-eosin (HE).

Scanning Electron Microscopy

Disc-shaped portions of intestinal wall, approximately 1.5 cm in diameter were stretched on a dental wax sheet. They were fixed in 2.5 per cent glutaraldehyde at pH 7.3 and postfixed in 1 per cent phosphate-buffered osmium tetroxide, pH 7.3. They were dehydrated in graded acetone and vacuum-dried. Finally, tissue blocks were vapor coated with gold in an Edwards’ vacuum-evaporator. The preparations were examined under a JEOL SM 15 scanning electron microscope using an acceleration potential of 21 kV.

Electron Microscopic Methods

Small segments were removed from six sites of the small intestine, adjacent to those taken for histopathology. The segments were fixed in Karnowsky’s paraformaldehyde or in 2.5 per cent glutaraldehyde at pH 7.3 and post-fixed in 1 per cent phosphate-buffered osmium tetroxide at pH 7.3. Tissue blocks were dehydrated in graded ethanol and embedded in Durecupan ACM. The sections were cut with a Reichert Om U3 ultramicrotome. Semi-thin sections were stained with toluidine blue and thin sections with uranyl acetate and lead citrate.