Ultrastructure of the Small Intestine in Astrovirus-infected Lambs

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SUMMARY

The ultrastructure of the small intestine of gnotobiotic lambs infected with lamb astrovirus was studied. The virus was observed from 14 to 38 h p.i. in mature columnar epithelial cells covering the apical two-thirds of villi. Crystalline arrays of virus particles with a centre to centre distance of approx. 29 nm were seen in the cytoplasm and virus particles were also observed in apical pits and tubules and in lysosomes. Macrophages containing virus particles in lysosome-like organelles were seen in the lamina propria. Virus particles were released by desquamated cells disintegrating in the gut lumen. Cuboidal cells lining villi appeared from 38 to 70 h p.i., and by 120 h p.i. the villi appeared normal.

INTRODUCTION

The name astrovirus has been suggested for viruses 28 to 30 nm in diam. with circular outlines and surface structure often arranged as a five or six pointed star (Madeley & Cosgrove, 1975). These morphologically distinctive viruses have been observed in the faeces of diarrhoeic children (Kurtz et al. 1977; Madeley et al. 1977; Ashley et al. 1978; Maass et al. 1978; Schnagl et al. 1978), lambs (Snodgrass & Gray, 1977) and calves (Woode & Bridger, 1978). Studies on the pathogenesis of astrovirus infection in lambs have shown the site of virus multiplication to be the small intestine (Snodgrass et al. 1979). No ultrastructural studies on the small intestine during astrovirus infection have been made although the appearance of human astrovirus in tissue culture has been reported (Kurtz et al. 1979). This paper describes the ultrastructure of the small intestine of astrovirus-infected lambs.

METHODS

Infection of lambs. Six 1 day-old gnotobiotic lambs were inoculated with intestinal contents from the third gnotobiotic lamb passage of lamb astrovirus (Snodgrass et al. 1979). One lamb was killed at each of the following times p.i.: 14, 23, 38, 45, 70 and 120 h. Six gnotobiotic lambs killed between 48 and 122 h of age were used as controls.

Preparation for electron microscopy. The lambs were anaesthetized and small pieces of intestine were taken from jejunum, mid-gut and ileum (Snodgrass et al. 1979). These were fixed by immediate immersion in 1% glutaraldehyde in phosphate buffer (pH 7.4) at room temperature, diced into 1 mm³, post-fixed in 1% osmium tetroxide in phosphate buffer (pH 7.4), dehydrated in graded alcohols and embedded in Araldite. Location sections 1 μm thick were stained in 10% Giemsa at 60 °C for 5 min. Ultrathin sections for electron microscopy were stained with saturated aqueous uranyl acetate followed by lead citrate and examined on a Siemens Elmiskop 1.
RESULTS

Control lambs

The villi were covered for most of their length by mature columnar epithelial cells (Fig. 1). These cells had long glycocalyx-covered microvilli at their luminal margins and numerous apical pits in the luminal membrane (Pensaert et al. 1970), particularly in cells in the apical portion of villi. The cytoplasm contained wide terminal webs, apical tubular systems, numerous mitochondria, a few lysosomes and varying quantities of endoplasmic reticulum and glycogen. Autophagic vacuoles (Moon, 1976) were present in most cells in the apical two-thirds of the villi; large vacuoles tended to be basal while smaller vacuoles were widely distributed throughout the cell. The nucleus in these cells was irregular in outline and apical or medial in the cell, while in cells at the base of villi the nucleus was regular ovoid and basal. Cells at the base of villi were incompletely differentiated with short microvilli, no apical pits or autophagic vacuoles, poorly developed apical tubules and were deficient in glycogen and cell organelles. These cells also tended to be cuboidal in shape. A few degenerate epithelial cells were seen sloughing from extrusion zones at the tips of villi. Goblet cells were scattered throughout the villus epithelium and a few lymphocytes were seen in the lateral intercellular spaces between epithelial cells.

The lamina propria was mainly filled with small blood vessels but also contained a few lymphocytes, eosinophils and macrophages; the cytoplasm of the latter often contained numerous organelles resembling lysosomes. Plasma cells were rarely seen.

Infected lambs

Virus particles were found in mature villus epithelial cells in jejunum and ileum at 14 h p.i. and in all three gut sites at 23 and 38 h p.i. At 14 h p.i. infected cells were confined to the tips of villi but by 23 and 38 h p.i., virus particles were found in most epithelial cells covering the apical two-thirds of villi.

The cells shown in Fig. 2 are typical of infected cells at all times and sites. Electron-dense aggregates were observed in the cytoplasm of cells, and at a higher magnification (Fig. 3) these were seen to consist of circular hollow-cored particles in an amorphous matrix, often with apparently partly formed particles within the same matrix. Crystalline and quasicrystalline arrays of solid or hollow-cored particles with a centre to centre distance of approx. 29 nm were also found either free in the cytoplasm, enclosed by a membrane or within secondary lysosomes (Fig. 4, 5 and 6). Virus particles in all of these forms could also be seen in autophagic vacuoles (Fig. 7). In a few infected cells, virus particles were seen in apical pits and tubules (Fig. 8). Virus particles lining the outer membrane of microvilli were observed at 23 and 38 h p.i. (Fig. 9). At these times degenerate epithelial cells, which were usually infected, were found sloughing from the apical portions of villi particularly in the mid-gut. The microvilli of these cells were disintegrating and they were deficient in ribosomes and cell organelles (Fig. 10). Release of virus particles from ruptured luminal margins of degenerating cells was not seen and virus particles were not seen in crypt cells.

By 38 h p.i. many of the columnar cells covering the villi had been replaced by cuboidal cells, particularly in the ileum (Fig. 11). These cells were similar in appearance to the immature incompletely differentiated cells seen at the base of villi in control lambs. They had short microvilli, round basal nuclei, few apical tubules and no apical pits or autophagic vacuoles. Cuboidal cells persisted at the tips of villi at 45 and 70 h p.i. but by 120 h p.i. the villi were indistinguishable from control villi. Infected cells were not seen after 38 h p.i.

From 23 to 70 h p.i. goblet cells were reduced in number and their contents were usually discharged, while increased numbers of lymphocytes were seen in the lateral intercellular spaces of epithelial cells. The lamina propria was increasingly infiltrated with lymphocytes, plasma cells and macrophages. The macrophages occasionally contained virus particles in organelles resembling lysosomes (Fig. 12).
Fig. 1. Control mid-gut, 48 h old. A typical columnar villus epithelial cell showing autophagic vacuoles (AV), glycogen (G) and apical pits (arrows).
Fig. 2. Infected mid-gut, 23 h p.i. Villus epithelial cells with apical vacuoles and several virus aggregates (arrows). Autophagic vacuoles (AV) and apical tubules (AT) can also be seen.
Fig. 3. Higher magnification of virus aggregates arrowed in Fig. 2, showing hollow-cored particles in an amorphous matrix.

Fig. 4. Infected mid-gut, 23 h p.i. A crystalline array of solid particles enclosed by a membrane.

Fig. 5. Infected mid-gut, 23 h p.i. A crystalline array of hollow-cored particles enclosed by a membrane.
Fig. 6. Infected mid-gut, 38 h p.i. Virus particles (V) are shown in secondary lysosomes (L) and also free in the cytoplasm.

Fig. 7. Infected mid-gut, 23 h p.i., showing autophagic vacuoles in the cytoplasm of an epithelial cell. Virus particles can be seen in various formations within the vacuoles.
Fig. 8. Infected mid-gut, 23 h p.i. Virus particles can be seen in apical tubules (arrows) near the luminal margin.

Fig. 9. Infected mid-gut, 38 h p.i. Virus particles are aligned along microvilli.
Fig. 10. Infected mid-gut, 38 h p.i. Degenerate epithelial cell sloughing into gut lumen. Note shedding microvilli (arrows) and virus particles (V) in secondary lysosome.
Fig. 11. Infected ileum, 38 h p.i. An immature cuboidal epithelial cell in the upper portion of a villus. Note short microvilli and lack of autophagic vacuoles, apical pits and tubules.
Fig. 12. Infected mid-gut, 23 h p.i. Cells infiltrating the lamina propria, including a plasma cell (P), lymphocyte (L) and macrophage (M). The inset shows virus particles in the lysosome-like organelle arrowed, at higher magnification.
**Discussion**

Previous histological and immunofluorescent studies of lamb astrovirus infection showed that the sole target site of virus multiplication was the small intestine (Snodgrass *et al.* 1979). Ultrastructural studies, therefore, were concentrated on this site and have now confirmed that the site of virus multiplication is the mature villus epithelial cell. The numbers of infected cells increased from 14 to 38 h p.i. and infected cells were often observed sloughing into the gut lumen at 23 and 38 h p.i. These sloughed cells were replaced by immature cuboidal cells which persisted at villus tips until 70 h p.i. By 120 h p.i. the villi appeared normal.

Kurtz *et al.* (1979) have briefly described the appearance of human astrovirus in the cytoplasm of HEK cells. This virus was seen as arrays of dense round particles with a centre to centre spacing of 28 nm. Thus, human astrovirus in tissue culture cells resembles lamb astrovirus in villus epithelial cells, but insufficient detail of the human astrovirus was given by these authors for further comparisons to be made. Furthermore, the human astrovirus produced in tissue culture cells is apparently non-infective.

Apical pits in cells of the small intestine have been shown to be the route of entry of transmissible gastroenteritis virus (TGE) in pigs (Pensaert *et al.* 1970) and an adenovirus and an adeno-associated virus in rats (Worthington & Graney, 1972a). Absorptive cells of the ileum and jejunum are capable of withdrawing intact adenovirus particles from the intestinal lumen and transporting them to degradative organelles (Worthington & Graney, 1972b). In this study astrovirus particles were found in apical pits and tubules and this was considered to be the route of entry into epithelial cells. Immature replacement epithelial cells lacked apical pits and tubules and therefore were not infected. From these tubules virus particles presumably pass into the cytoplasm where they may enter secondary lysosomes. The role of lysosomes in the multiplication of astroviruses is not clear. Virus particles lining microvilli were observed at 23 and 38 h p.i. as observed with TGE virus in pigs by Pensaert *et al.* (1970) who suggested that maturation of TGE virus occurred at this site. Astroviruses were never seen passing through the membranes of microvilli in either direction, however, and the precise reason for their presence at this site could not be ascertained. Virus particles apparently within microvilli were thought to be a sectioning artefact arising from the small size of the virus (28 nm) within a relatively thick section (50 nm approx.), resulting in distorted spatial relationships. TGE virus is released into the gut lumen through ruptured luminal membranes (Pensaert *et al.* 1970) but this was not observed with astroviruses. The presence of considerable numbers of astrovirus particles, particularly at 38 h p.i., lining microvilli was therefore presumed to be the result of disintegrating desquamated cells releasing virus.

Astroviruses are RNA viruses with genomes similar to those of picornaviruses (A. J. Herring, personal communication). The ultrastructural studies reported here and studies reported previously (Snodgrass & Gray, 1977) have shown that lamb astrovirus is similar in size to some picornaviruses and also multiplies in the cytoplasm. However, further physicochemical characterization is required before the astrovirus can be assigned to any of the virus families.

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**References**


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