The Ultrastructure of Cell Cultures Infected with Border Disease and Bovine Virus Diarrhoea Viruses

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SUMMARY

The morphology of border disease virus and bovine virus diarrhoea virus in infected bovine embryonic testis cells was examined by electron microscopy. Particles which appeared to be mature virions of both viruses were similar, being roughly circular and approximately 46 nm in diameter with a 20 to 25 nm core. Virus replication took place totally within the cytoplasm in association with structures formed from modified endoplasmic reticulum.

INTRODUCTION

Border disease is a congenital disorder of lambs characterized by low birth weight, poor viability, tremor and fleece changes (Barlow & Patterson, 1982). The aetiological agent, border disease virus (BDV), is related serologically to bovine virus diarrhoea virus (BVDV) and hog cholera virus (HCV) and all three have been grouped in the genus Pestivirus in the family Togaviridae (Westaway et al., 1985). The pestiviruses have been shown, from studies of comparative buoyant density and sedimentation coefficients, to form a homogeneous group unrelated to the other genera of the togavirus family (Laude, 1979). Morphological homogeneity has, however, not been convincingly demonstrated.

Both BVDV and HCV have been shown by negative staining to be enveloped spherical particles 40 to 60 nm in diameter with 27 to 29 nm diameter cores (for review, see Horzinek, 1981) but no convincing ultrastructural details of BDV have yet been published. One previous study of BDV and BVDV in tissue sections has been made (Scott et al., 1977) but later studies of BVDV from the same laboratory by Chasey & Roeder (1981) concluded that the particles described were non-viral. Other studies of BVDV in thin sections have been made by Ritchie & Fernelius (1969) and Ohmann & Bloch (1982) who described BVDV virus as spherical particles approximately 50 nm in diameter. Most studies of BVDV have utilized suspensions of virus and negative staining (Hafez et al., 1968; Maess & Retzko, 1970; Horzinek et al., 1971; Stott et al., 1974; Chu & Zee, 1984), and have variously reported the virus as roughly spherical and from 30 to 100 nm or more in diameter.

This report describes the morphology of cytopathic strains of BDV and BVDV in bovine embryonic testis (BET) cells.

METHODS

Cell cultures. Secondary cultures of BET cells were grown in medium 199 supplemented with 0.5% lactalbumin hydrolysate (LAH), 0.2% glucose, and 10% heat-inactivated calf serum in 80 cm² plastic flasks. Newly confluent monolayers were washed three times with phosphate-buffered saline and either mock-inoculated or infected with virus at a m.o.i. of 1. After 2 h adsorption at 37 °C, inocula were removed and replaced by maintenance medium consisting of medium 199 supplemented with 0.5% bovine serum albumin, 0.1% LAH, 0.1% yeast extract and 3% heat-inactivated horse serum.

Viruses. Cytopathic BVDV (NADL strain), derived from the American Type Culture Collection (ATCC VR-534), which had been passaged a total of seven times in either bovine kidney or bovine turbinate cells was kindly supplied by Dr P. Roeder, Central Veterinary Laboratory, Weybridge, Surrey, U.K. This virus was amplified by three further passages in bovine embryonic kidney cells at a low m.o.i.
The BDV was isolated from a pool of brain (IIB brain pool) from newborn lambs clinically affected with BD (Barlow, 1972). The virus was cytopathic in cell cultures (Vantsis et al., 1976) and was used following four passages at a low m.o.i. in foetal lamb kidney cells.

Electron microscopy. Uninfected control cells and cells infected with BDV or BVDV, which showed c.p.e. involving 20% of the monolayer, were harvested simultaneously, usually at 72 h post-inoculation. After pre-fixation on the flask with 1% glutaraldehyde in 0.1 M-phosphate buffer pH 7.2, for 5 min at room temperature the cells were removed and centrifuged at 4800 g for 10 min. The resulting pellets were resuspended in fresh 1% glutaraldehyde for 10 min at room temperature and then washed three times in 0.1 M-phosphate buffer containing 2% dextrose. The cells were post-fixed in 1% osmium tetroxide in 0.1 M-phosphate buffer pH 7.2, for 20 min at room temperature, washed as before, dehydrated in graded ethanols and embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop 1a or a Siemens 102 electron microscope.

RESULTS

Uninfected BET cells

Uninfected cells usually had an irregular outline due to cytoplasmic projections. The cytoplasm contained numerous mitochondria, was often vacuolated and also contained smooth and rough endoplasmic reticulum (SER and RER) which was occasionally distended. The nucleus was irregular and was usually surrounded by an abundant cytoplasm. Degenerating cells with either a dark or pale cytoplasm and few organelles were frequently seen and these often contained many circular profiles of RER and a degenerating nucleus.

The cytoplasm of the uninfected cells occasionally contained pleomorphic electron-dense organelles but no virus-like particles were seen.

Border disease virus-infected cells

Cultures infected with BDV showed rounding and a reduction in background ribosomes giving the cell a paler appearance. The cells often contained several profiles of ER and many dense lamellar bodies (Fig. 1). At higher magnification the lamellar bodies appeared to be formed from alternating light and dark bands of material of varying length which, when sectioned transversely, appeared as multiple rows of tubules 33 nm in diameter (Fig. 2). These lamellae were often found in association with RER and on one occasion virus-like particles appeared to be budding within it (Fig. 3). Some cells contained roughly circular, electron-dense, virus-like particles surrounded by a granular matrix within distended RER (Fig. 4), and a few enveloped particles were seen (Fig. 5). Most virus-like particles were poorly defined and those showing detail were seen only after examination of hundreds of cells. The total diameter range of 30 virus-like particles was 33 to 52 nm and 12 enveloped, cored particles had a mean diameter of 46 nm with a 24 nm diameter core. Virus-like particles were not seen free in the cytoplasm, nor were any seen budding through internal host cell membranes or passing through the cytoplasmic membrane.

Bovine virus diarrhoea virus-infected cells

Cells infected with BVDV contained RER modified into tubules. When examined at higher magnification (Fig. 6) these were seen to be similar in diameter but varied in length. One end was occasionally closed and the other end frequently terminated in a vesicle of SER or RER from which they appeared to originate. Tubules were either smooth-sided or covered with ribosome-like material. Within the tubules and vesicles roughly circular, electron-dense virus-like particles were present with the occasional tubule containing as many as 14. Virus-like particles were also observed in irregular-sized circular profiles of RER. These particles ranged from 33 to 50 nm in diameter and enveloped particles had an approximate mean diameter of 46 nm with a 20 to 25 nm diameter core. Virus-like particles were not seen free in the cytoplasm, nor were any seen budding through internal host cell membranes or passing through the cytoplasmic membrane.

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Tubules and virus-like particles were also observed in dense necrotic cells and in paler degenerating cells but no virus particles were seen budding through membranes or being released from cells. Cells containing virus-like particles were seen about ten times more frequently in the BVDV-infected cultures than in those infected with BDV, and higher numbers
Ultrastructure of BDV- and BVDV-infected cells

Fig. 1. BDV-infected BET cell. Note smoother outline and distended ER containing virus-like particles (V) and lamellar bodies below nucleus (arrows). Bar marker represents 2 \( \mu \)m.

of particles were seen. However, for both viruses, particles showing detail were very difficult to find. Pleomorphic enveloped organelles were observed in the cytoplasm of infected and uninfected cells.

**DISCUSSION**

This ultrastructural study has revealed for the first time the appearance of virus-like particles in cells infected with BDV which showed morphological similarities with cultures of BVDV. Replication of both viruses appears to take place in the cell cytoplasm and is closely associated
Fig. 2. BDV-infected BET cells. Higher magnification of lamellar bodies in transverse and longitudinal section showing banded and circular appearance. Bar marker represents 200 nm.

Fig. 3. BDV-infected BET cell. Lamellar bodies and virus-like particles (V) in association with ER. Two apparently budding particles are shown (white arrow). Bar marker represents 200 nm.
Ultrastructure of BDV- and BVDV-infected cells

Fig. 4. BDV-infected BET cell. Virus-like particles surrounded by amorphous granular material within RER. Bar marker represents 200 nm.

Fig. 5. BDV-infected BET cells. Several enveloped, cored, virus-like particles are shown in distended RER close to the membrane but no attachment is seen. Bar marker represents 200 nm.
Fig. 6. BVDV-infected BET cell. Higher magnification of an area of modified RER containing virus-like particles. Note continuity between RER and tubules (black arrow) also enveloped, cored particles (white arrow). Bar marker represents 200 nm.
Ultrastructure of BDV- and BVDV-infected cells

with ER. The association of BDV and BVDV with ER and associated structures is an observation frequently made in cells infected with togaviruses (Murphy, 1980) but as yet their role in viral morphogenesis has not been determined.

The enveloped, cored, virus-like particles in modified ER observed in both BDV- and BVDV-infected cells were similarly sized at 46 nm in diameter and were considered to be the mature virions. The smaller particles observed were most likely to be precursor stages in virus maturation, possibly cores. Both types of particle were occasionally observed in close association with internal cell membranes but no budding through the membranes was observed. Maturation of both viruses appeared to take place in modified ER and the apparent budding of BDV from lamellar structures was not observed frequently enough for any firm conclusion to be drawn. Detailed information on the morphogenesis of the viruses was not attempted in this study; virus release was not observed but was presumed to occur when the cell disintegrated or when remnants of ER were released. The pleomorphic electron-dense enveloped organelles seen in both uninfected and BVDV-infected cultures closely resembled the type II particle observed by Scott et al. (1977). These particles were reported by Chasey & Roeder (1981) as having no significance and were considered to be non-viral. Our findings support this observation.

The various structures described as BVDV by several authors in sections or negative-stained preparations have resulted in a wide size range of 20 nm to more than 100 nm being attributed to the virus (Horzinek et al., 1971; Chu & Zee, 1984; Ward & Kaeberle, 1984). This range contrasts with the size reported here of 46 nm which is in close agreement with the diameter of 50 nm reported by Ritchie & Fernelius (1969), Chasey & Roeder (1981) and Ohmann & Bloch (1982). Chasey & Roeder (1981) reported the presence of tubules in infected cells but unlike the present study did not observe virus particles within them. The nuclear cores described by Ward & Kaeberle (1984) were comparable in appearance to the mature virions enclosed by tubules or ER described in this paper. The masking of fine detail by immunoperoxidase (Ward & Kaeberle, 1984) could explain the difference in size and appearance of mature virions in the two studies. A further possible explanation of the wide size range attributed to BVDV could be related to the recognition that many cytopathic isolates of BVDV contain a mixture of cytopathic and non-cytopathic viruses (Brownlie et al., 1984; McClurkin et al., 1985).

A considerable number of studies have been made on the morphology of togaviruses but the only members of the group with convincing detail of their morphogenesis are the alphavirus subgroup. Many members of this subgroup have been shown to produce nucleocapsids which bud through host membranes (particularly the plasma membrane) to form complete virions 55 to 58 nm in diameter. The outer layer of mature virions occasionally shows surface projections (Murphy, 1980).

Most studies of the pestivirus genus have concerned HCV (Cheville & Mengeling, 1969; Scherrer et al., 1970; Schulze, 1971) but no clear mode of morphogenesis could be ascertained. Scherrer et al. (1970) often found HCV in very close association with host cell membranes but never saw budding of a nucleocapsid through the cell membrane. The BDV and BVDV particles described in this study were more irregular than HCV particles (Murphy, 1980) but our observations support the continued inclusion of these viruses within the Pestivirus genus.

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REFERENCES


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