Vesicular Exocytosis of Foot-and-Mouth Disease Virus from Mammary Gland Secretory Epithelium of Infected Cows

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SUMMARY

Foot-and-mouth disease virus particles were observed by electron microscopy in the cytoplasm of alveolar secretory cells of the bovine mammary gland after contact exposure of uninfected cows to pigs with foot-and-mouth disease. Virus, contained in membrane-limited vesicles, was released from the basal and perinuclear portions of the cell into the intracellular and extracellular spaces by an exocytotic mechanism similar to that of the release of the milk-fat globule. Virus was released into the lumen from the apical portion of the cell both by membrane-limited vesicles and by the merocrine exocytosis of casein-associated virus. The lytic release of virus was observed in 20% of the preparations observed.

Foot-and-mouth disease (FMD) is a disease of livestock which spreads rapidly through susceptible animal populations, causing severe economic losses (Blackwell, 1980). The disease is highly contagious and can be transmitted through infected animals and their products (Blackwell, 1980).

The virus can survive in the milk of infected cows after high temperature-short time (HTST, 71.7 °C for 15 s) pasteurization (Hyde et al., 1975) as well as in dairy products such as butter, casein, cheese and sweet whey after various manufacturing processes (Blackwell, 1981). When FMD virus is added to milk or buffer solutions it does not exhibit this degree of stability (De Leeuw et al., 1980; Bachrach et al., 1957).

The detection of FMD virus in milk samples from infected cows was the basis for a report by Burrows et al. (1971) on the replication of the virus in the mammary gland. Their findings were corroborated by those of J. H. Blackwell & T. Yilma (1981) who reported the observation of FMD virus in fluorescent antibody-labelled and haematoxylin and eosin (HE)-stained secretory cells from the mammary gland of FMD virus-infected lactating cows.

The current studies report on the electron microscopic (EM) examination of secretory epithelial cells from mammary glands of cows infected with FMD virus by contact exposure.

Lactating first calf grade heifers or dairy cows were exposed to 13- to 27-kg Tamworth pigs infected by intradermal inoculation with 7·0 log_{10} p.f.u. O1 subtype, strain brugge (Br) of FMD virus into the foot pad of each front foot. The cows remained in contact with the pigs for the duration of the study, a minimum of 15 days.

Milk samples were collected at 24-h intervals after exposure of the cattle to infected pigs and were assayed in secondary BK cell culture as described previously (Blackwell & Hyde, 1976). The cows were examined daily for vesicular lesions and other signs of FMD (Callis, 1973).

The FMD virus type O1 (Br) virus-specific hyperimmune serum was prepared in adult albino rabbits inoculated with virus purified by centrifugation in a caesium chloride gradient. The rabbits were given three subcutaneous injections of virus mixed with incomplete Freund's adjuvant at 1-week intervals. Serum was collected 10 days after the last injection.

The cows were tranquilized with 50mg xylazine (Rompun, Cutler Laboratories, Shawnee, Kansas, U.S.A.), given intravenously, and 0·2 g lidocaine (Schein Inc. Flushing, N.Y., U.S.A.), local anaesthesia given subcutaneously.
Samples for biopsy were collected sequentially from one-quarter of a region dorsal to the mammary gland cistern at 0, 3, 10 and 14 days after exposure and the surgical procedure was performed as described by Oxender et al. (1971). Approx. 3 mm$^2$ samples for EM examination were fixed as rapidly as possible after collection, ideally within 20 s in Sorensen’s buffer (SB) pH 7.2, containing either 2% glutaraldehyde or 3% formaldehyde solution. Tissues in glutaraldehyde were transferred to SB after overnight storage at 4 °C. Tissues were then washed with SB, post-fixed for 1 h in 2% osmium tetroxide (in SB), dehydrated in a graded series of ethanol–propylene oxide–Epon mixture, embedded in Epon, and sectioned with an MT2B ultramicrotome. The sections were examined unstained or stained with uranyl acetate in combination with Reynold’s lead citrate in a Philips 201 electron microscope.

The specificity of FMD virus antigens was determined by a modification of a procedure by Dubois-Dalcq et al. (1977) for labelling with immune-protein A–horseradish peroxidase (PrA–Po). Frozen tissue sections, 20 μm thick, were collected from the cryostat knife blade with a 1.25 cm nichrome loop containing a chilled solution of 3% formaldehyde in SB. The tissue section was then placed directly on to a 0.45 μm membrane filter disc (HA, Millipore) contained in a 13 mm well of a 24-well disposable plate (Costar Plastics). A second membrane was placed on top of the tissue to hold it in place during the remainder of the labelling procedure. Wash fluids and reagents were added and removed from the well without disturbing the tissue section. After a 15 min fixation, the tissue section was washed twice with SB. Tissue sections were then stained sequentially at 24-h intervals at room temperature with a 1:100 dilution of rabbit serum hyperimmune to bovine O$_1$ (Br) FMD virus and a 1:40 dilution of Staphylococcus aureus protein A cell wall hydrolysate (Pharmacia), purified on a Sephadex G-100 (Pharmacia) column and linked to type VI horseradish peroxidase (Sigma). Tissues were washed twice in SB for 15 min after each adsorption and then treated for 45 min at room temperature with a solution of 0.1% 3,3’-diaminobenzidine (DAB) freshly prepared in SB containing 0.01% hydrogen peroxide. The labelled tissues were then lifted from the well and embedded as described above. The propylene oxide step dissolved the membrane.

Control preparations consisting of uninfected mammary gland tissue sections, infected tissue sections adsorbed with heterologous immune gamma globulin, and infected tissues adsorbed with DAB alone, were also examined.

Each cow became infected upon exposure to FMD virus-infected pigs. However, the onset of clinical signs of disease and virolactias was variable.

Virus particles were observed only in secretory epithelial cells. These particles were spherical, 23 to 25 nm in diam. and were observed only in the cytoplasm but were larger than the ribosomes present (Fig. 1a). These particles were observed mainly in the basal portions of the cell and to a lesser degree in the apical portion.

Vesicles approx. 700 nm long, and containing virus particles, were present in the extracellular spaces close to foot-like cytoplasmic processes between the plasmalemma and the basement membrane (Fig. 1b). In the basal portion of the cell, inclusion formation appeared to be a preliminary to the exocytotic release of virus from secretory cells in membrane-limited vesicles (Fig. 1a). Virus particles were also observed in the extracellular spaces completely enveloped by the plasmalemma and held by a thin cytoplasmic process. Virus-containing vesicles were observed at the luminal (Fig. 1c, d) and basal portions (Fig. 1a, b) of the cell but much more frequently in the latter. Labelled viral antigens were also observed with high frequency in membrane-limited vesicles within Golgi-like vacuoles in the luminal portion of the cell.

Alveoli with sloughed epithelium were observed more frequently when mammary gland tissues were collected after virolactia. The release of virus particles into the extracellular spaces during the lytic stage is shown in Fig. 1(e). This type of release was observed in 20% of the preparations.
Fig. 1. Electron photomicrographs of ultrathin sections of secretory epithelial cells of the bovine mammary gland after exposure to pigs with foot-and-mouth disease. Tissues were obtained 3 days after exposure and 1 day before the onset of virolactia and clinical signs (a) or 12 days after exposure and 4 days after onset of virolactia and clinical signs (b to e). Sections were stained with uranyl acetate/lead citrate. Bar markers represent 0.5 µm. (a) Basal portion of cell. Viral inclusions (V) are present close to (1), and budding into the plasmalemma (2) and in the process of being 'pinched off' as a membrane-limited vesicle (MLV) into the extracellular space (ES). [Note difference in size between ribosomes (arrows) and viruses.] (b) Viral inclusions (V) are seen close to cytoplasmic ‘foot-process’ (FP) and also contained in membrane-limited vesicles (MLV) in the intercellular spaces. (c) Apical portion of cell. Membrane-limited vesicle containing virus-like particles in close proximity to milk fat globule (MFG), microvilli (Mv) and casein micelles (C). (d) Virus-like particles contained in membrane-limited vesicles were observed free (1) and in an exocytotic state (2) before release into the lumen (L). Lumen contains synthesized milk components as well as a solitary mononuclear cell (Mn). Microvilli (Mv) and a milk fat droplet (MFD) are also observed. (e) Basal portion of cell. Lysis of plasmalemma (PI) with release of virus (V) into the extracellular space is observed. Mitochondria (M) and cytoplasmic contents appear stressed and damaged.
Fig. 2. Electron photomicrographs of ultrathin sections of secretory epithelial cells of bovine mammary gland after exposure to pigs with foot-and-mouth disease. Tissues depicted were obtained 3 days after exposure and 1 day before onset of virolactia and clinical signs. Sections were labelled with immune-protein A–horseradish peroxidase or treated in combination with uranyl acetate/lead citrate; bar markers represent 0.5 μm. (a) Basal portion of cell. Labelled viral antigens (v) are present in compact clusters close to plasmalemma (Pl) just below nucleus (N), and (b) observed within the cytoplasm of a membrane-limited vesicle (MLV) free in the extracellular space (ES) and close to ‘foot process’ (FP). (c) Apical portion of cell. Labelled antigens are observed in the cytoplasm close to the plasmalemma (I) and in apparent membrane-limited vesicles (2). Membranes and background cytoplasm of secretory epithelium surrounding the lumen (L) are barely discernible. (d) Large Golgi vacuole containing labelled antigens (arrows) and surrounded by microvesicles (mv). The membrane of the vacuole has fused with the plasmalemma (Pl) before the release of Golgi contents into the lumen (L). Inset: antigens are labelled with combination immune horseradish peroxidase uranyl acetate/lead citrate stain (arrows).

The viral specificity of the observed particles was determined by positive reactivity of viral antigens with immune-PrA–Po label. Neither uninfected mammary gland tissue sections nor infected sections incubated with heterologous serum or with DAB alone reacted. In positive reactions, the antigens appeared as electron-opaque particles that were quite distinct from cytoplasm and organelles that took up only an equivocal amount of label. Two forms of antigen array, diffuse and compact, were observed. Both forms were found as free inclusions or in membrane-limited vesicles (Fig. 2a to c). Labelled antigens were observed associated with Golgi vesicles but not with the nucleus or other cellular organelles. In these associations, viral inclusions ringed with Golgi vesicles were present in large Golgi vacuoles (Fig. 2d).

The release of virus particles, free or present in membrane-limited vesicles, from viable cells in cell culture systems has been reported by a number of authors (Reissig et al., 1956; Melendez, 1959; Khera & Dhillon, 1962; Dunnebacke et al., 1969; Yilma et al., 1978). Yilma
and co-workers (1978) proposed that the release of FMD virus from BK cell culture monolayers in `cytoplasmic blebs' may be a general release mechanism for the picornavirus group. However, in the present study, FMD virus was observed not only in apparently normal bovine mammary gland cells in vivo, but also in intimate association with secretory cell release mechanisms for casein and lipid.

The release mechanism for the latter synthetic product empirically involves the enveloping of a lipid droplet with the unit membrane of the synthesizing cell, pinching off of the now milk fat globule (MFG) from the cell, and subsequent release of the MFG into the central cavity of the alveolus (Patton & Jensen, 1976; Wooding, 1977).

Wooding (1971, 1977) and others (Christie & Wooding, 1975; Brooker, 1978) reported the occurrence of MFG-containing fragments of cytoplasm in the milk of bovine and caprine species. Based upon these findings, we proposed and later demonstrated an exocytotic model for the release in the same manner of membrane-limited cytoplasm containing virus particles.

Given the mechanisms for the synthesis of casein (Mepham, 1977; Hopkins, 1979; Larson, 1979), we can also speculate that the attachment of FMD virus to either the filamentous or folded form of the casein micellar subunit would eventually internalize the virus within the micelle, thus protecting the virus from environmental inactivants.

Although FMD virus particles were found associated with Golgi vesicles close to the cell membrane in the luminal portion of the cell, they were not observed in association with other cytoplasmic organelles (Fig. 2 d). The virus was observed much more frequently in the basal portion than in the apical portion of the cells. This observation, plus the basal localization of rough endoplasmic reticulum and the extraction of precursor substances from the blood of the interalveolar capillaries into the foot processes of the basal membrane of the secretory epithelial cells (Bargmann & Welsch, 1969; Larson, 1979), suggests that this portion of the cell is the site of virus production.

At least three virus-release mechanisms appear to operate in mammary gland secretory cells infected with FMD virus. One mechanism involves the lytic release of virus particles. The other two utilize the exocytotic mechanisms of the cell for the production and release of milk products, casein and lipid.

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REFERENCES


Short communications


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