The Ultrastructure of Feline Infectious Peritonitis Virus in Feline Embryonic Lung Cells

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SUMMARY

The ultrastructure of feline infectious peritonitis virus in cultured feline embryonic lung cells is reported. Feline embryonic lung cells were infected with feline infectious peritonitis virus and studied by transmission electron microscopy. The virus was not apparent in the cultured cells until 24 h after infection when it occurred in the endoplasmic reticulum, perinuclear space, Golgi apparatus, free in the cytoplasm, in large vacuoles in the cytoplasm and outside the cell membrane. The virus possessed typical coronavirus morphology and was produced by budding into the endoplasmic reticulum. There was no evidence to indicate that this virus budded through the cell membrane. Multinucleate giant cells were formed by infection of the cultured cells with the virus. The host cells were destroyed by the virus and phagocytosed by apparently healthy cells.

INTRODUCTION

Feline infectious peritonitis (FIP) is a disease of cats causing intermittent or continuous fever, loss in body weight and death (Wolfe & Griesemer, 1966). Morphological and serological evidence suggests that the causative agent is a virus and should be classified as a member of the Coronaviridae (Horzinek et al., 1977; Witte et al., 1977; Ward, 1970).

FIP virus is fastidious in its choice of host cells and has until recently (Evermann et al., 1981; Hitchcock et al., 1981; Black, 1980; O'Reilly et al., 1979) defied attempts to grow it in tissue culture. For this reason FIP virus has been extensively studied only in animals and in organ cultures (e.g. Pedersen & Boyle, 1980; Ward, 1970; Hoshino & Scott, 1978; Osterhaus et al., 1978). O'Reilly et al. (1979) succeeded in culturing FIP virus in monolayer cultures of feline embryonic lung (FEL) cells, and a complete account of the in vitro culture method has been reported (Hitchcock et al., 1981). This paper describes in detail, for the first time, the ultrastructure of FIP virus in cultured FEL cells.

METHODS

Virus culture. This has been described in detail elsewhere (Hitchcock et al., 1981). In brief, confluent monolayers of FEL cells (O'Reilly & Whitaker, 1969) were inoculated with 1 ml of infected peritoneal fluid and incubated at 37 °C for 8 days. After freezing and thawing, 1 ml of the harvest was inoculated into fresh confluent monolayer cultures. A cytopathic effect appeared after the third subculture. Further passaging was carried out every 3 to 4 days, by inoculating 3 ml of a 10³ dilution of the previous passage on to confluent cells in 150 cm² Corning flasks. For certain experiments FEL cells were grown to confluence on glass coverslips, then inoculated with FIP virus. After incubation times of 10 min, 1 h, 2 h, 8 h, 24 h, 32 h, and 48 h, the cells were rinsed in phosphate-buffered saline, then fixed for electron microscopy. A growth curve of the virus was constructed. The virus was harvested from...
tissue culture at varying times after infection and the amount of virus estimated by titration. These values were then plotted against the corresponding harvest time.

Electron microscopy. The medium from infected cultures was centrifuged at 15,000 g for 1 h. The pellets were resuspended in distilled water and negatively stained with 2% potassium phosphotungstate on 400 mesh Formvar and carbon-coated copper grids.

The corresponding cell cultures were fixed for 1 h in 2.5% glutaraldehyde in 0.05 M-sodium cacodylate buffer containing 0.05 M-CaCl₂ and 0.05 M-NaCl then post-fixed for 30 min in cacodylate-buffered 1% osmium tetroxide. After tertiary fixation in 2% aqueous uranyl acetate for 30 min the cells were dehydrated and embedded in Emix (Emscope Laboratories, Ashford, Kent, U.K.). The cells were processed either in situ (Beesley et al., 1978) or by scraping the glutaraldehyde-fixed cells from the culture vessel, pelleting them in a haematocrit and then following the same embedding schedule. Sections were cut on an LKB Ultratome III and stained with 2% aqueous uranyl acetate (15 min at 60°C) and Reynolds' lead citrate (15 min at room temperature).

Specimens on coverslips for transmission electron microscopy were initially assessed under phase contrast light microscopy. They were processed through to resin in situ on the coverslip. The glass coverslip was removed from the Araldite by immersion in liquid nitrogen and pulling apart. Suitable cells were then chosen for sectioning (Beesley, 1978). Specimens were viewed in either a Philips 300 or 301 transmission electron microscope, operating at 80 kV.

RESULTS

The results of a typical growth curve of FIP virus in FEL cells are shown in Fig. 1. The virus harvest was maximal between 32 h and 48 h but a comparatively high yield of virus was collected at 24 h. By 72 h post-infection the virus yield was declining and no further samples were taken. The characteristics of this growth curve were confirmed by electron microscopy. Negative stain examination of the medium from infected cultures showed that in the 2 h sample there were one or two pieces of viral debris. These were very degenerate and were probably the remains of the infecting inoculum. Complete virus particles, possessing characteristic coronavirus morphology were not found in the culture medium until 24 h and subsequent samples, after infection (Fig. 2 a). The virus particles were circular or oval, with numerous teardrop projections up to 21 nm long.

There were abundant virus particles in thin sections of the cultured cells in all samples after 24 h. The virions were mostly elliptical, up to 150 nm long and 106 nm wide. They were bounded by a trilaminar unit membrane inside which was an annular osmiophilic region. The central lucent region of the virus contained diffuse osmiophilic granules. Virus projections were not always clearly visible on intracellular viruses in thin sections but could be easily distinguished on extracellular particles (Fig. 2 b).

Within infected cells numerous virus particles filled the swollen endoplasmic reticulum and perinuclear space (Fig. 2 c), and were also found in the swollen uncoated vesicles of the Golgi body (Fig. 2 d). Virus particles were present in membrane-bound vacuoles, much larger than the endoplasmic reticulum (Fig. 2 e). As well as containing many viruses, some of these vacuoles contained several short, looped membranes stacked side by side. Viruses occasionally appeared to be free in the cytoplasm.

The early stages of virus infection were not observed in thin sections of FIP-infected FEL cells until profuse budding associated with the endoplasmic reticulum occurred 24 h after infection of the FEL cell culture. The mechanism of budding was assumed to be as follows. The first recognizable sign of budding was an osmiophilic thickening on the cytoplasmic face of the endoplasmic reticulum (Fig. 3 a). This appeared to invert into the duct together with some of the dense granules in the surrounding cytoplasm (Fig. 3 b to d). The osmiophilic
thickening did not form a complete annulus until very late in the budding procedure. Very large virus particles, large S-shaped particles or grossly deformed particles were occasionally observed (Fig. 3e). High numbers of viruses in rows two or three deep lined the outside of infected cells (Fig. 3f). Viruses were not observed budding from the outer cell membrane but a virus was observed in an invagination of the cell membrane (Fig. 3g). In later stages of infection at 32 h and 48 h, totally degenerate cells containing many virus particles were seen (Fig. 3h). Despite the drastic degradation of the cell, virus particles could be seen in the remains of the endoplasmic reticulum. Several of the more healthy cells appeared to be phagocytosing these degenerate remains (Fig. 4a). Observation of the sections of cells on the grid showed that 32 h after infection 8 out of 50 sections of cells showed evidence of phagocytosing large pieces of tissue such as in Fig. 4(a), whereas in control uninfected 32 h cultures only 1 in 50 cells showed similar signs of phagocytosis.

Infection of FEL cells with FIP virus caused multinucleate giant cells to form in the culture. The typical cytopathic effect was also present. The giant cells, when observed with the light microscope, possessed many nuclei, although transmission electron microscopy, with the limits of sectioning, failed to show more than three nuclei in any one cell (Fig. 4b). Cell membranes were not observed between the nuclei, and neither the nuclei nor the cells appeared to be dividing. Giant cells were not seen in control uninfected cultures.

**DISCUSSION**

The morphology and budding sequences of this feline coronavirus cultured in FEL cells are similar to those seen in feline coronavirus infections of kittens (Pedersen & Boyle, 1980; Ward, 1970) and of peritoneal cell cultures of kittens (Pedersen, 1976), and also in canine coronavirus infections of neonatal dogs (Takeuchi et al., 1976). As in this study, these authors describe the common site of virus replication as the endoplasmic reticulum, Golgi body, perinuclear space and smooth membrane-lined vacuoles in the cytoplasm (e.g. Takeuchi et al., 1976; Hamre et al., 1967). Other reports of FIP virus grown in tissue culture (Evermann et al., 1981; Black, 1980) do not report thin-sectioned observations.

Although many hundreds of virus particles were seen in association with the outside of cultured cells, budding of the virus through the host cell plasma membrane was not observed in this study. This is unlike feline leukaemia virus or feline syncytia-forming virus (Pedersen, 1976), both of which bud through the plasma membrane of the host cell. The virus found near an invagination of the plasma membrane may represent a route of secondary infection. In this study, as in many others, virus particles have been observed on the outside of the cell but we have not seen how they get from the plasma membrane to the endoplasmic reticulum.
Fig. 2. (a) Negative stain of complete FIP virus from culture fluid at 24 h. Bar marker represents 62 nm. (b to e) Transmission electron micrographs. (b) This shows complete FIP virus on outside of cell membrane. Bar marker represents 54 nm. (c) Virus particles (△) in swollen endoplasmic reticulum (★) and perinuclear space (★). Bar marker represents 0.4 μm. (d) Virus particle (△) in swollen vesicle of the Golgi body. Bar marker represents 0.13 μm. (e) Viruses (△) in membrane-bound vacuole with stacked membranes (★) and virus particles free in the cytoplasm (★). Bar marker represents 0.17 μm.

Infective virus is probably distributed partly by the breakdown and phagocytosis of infected cells. The virus particles lining the outside of individual cells may be released virus from the same or neighbouring cells. It is not clear whether the increased phagocytosis is a result of infection or merely that healthy cells scavenge dying cells.
It has been found that the ultrastructure of FIP virus in FEL cells is compatible with that found in experimental infections in vivo and in organ cultures. This model in which viruses can be passaged for many generations should prove to be a valuable asset in the study of FIP virus.
Fig. 4. Transmission electron micrographs of: (a) apparently healthy cell phagocytosing debris of a cell containing virus particles (△); (b) giant cell showing three nuclei. Bar markers in (a and b) represent 0.7 and 1.4 μm respectively.

REFERENCES


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