The Morphogenesis and Cytopathology of Bovine Parainfluenza Type 3 Virus

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SUMMARY

The structure of bovine parainfluenza type 3 virus (strain SF-4) was found to be indistinguishable from that of other paramyxoviruses. Negatively stained virus particles had an overall diameter ranging from 80 to 580 nm. and a nucleocapsid diameter of 17 to 19 nm. By light microscopy, SF-4-infected LLC-MK2 cells showed eosinophilic cytoplasmic inclusions, followed later by eosinophilic nuclear inclusions. By electron microscopy, aggregates of nucleocapsid-like filaments were seen in the cytoplasm and nucleus. Maturation of virus particles occurred at the plasma membrane, in a manner similar to that observed with other paramyxoviruses. Some cells containing inclusions exhibited an unusual type of particle formation in dilated portions of the endoplasmic reticulum. Bovine parainfluenza 3 virus appears to be unique among parainfluenza viruses in that its cytopathology and morphogenesis more closely resemble that of the unrelated measles subgroup of paramyxoviruses than that of the antigenically related parainfluenza viruses.

INTRODUCTION

Bovine parainfluenza virus type 3 (SF-4 strain) is a paramyxovirus isolated from calves afflicted with the respiratory disease known as shipping fever (Reisinger, Heddleston & Manthei, 1959). Initially, this virus appeared to be serologically identical to a human strain of parainfluenza virus recovered from children with pneumonia and upper respiratory illness (Chanock et al. 1958). Subsequently, slight antigenic differences between the bovine and human strains were demonstrated (Abinanti & Huebner, 1959; Jensen, 1961; Ketler, Hamparian & Hilleman, 1961; Spurrier & Robinson, 1965; Abinanti et al. 1961; Fischmann & Bang, 1966).

The most striking difference between bovine strains of parainfluenza 3 virus and all other parainfluenza viruses is seen in the cytopathic effect. Whereas parainfluenza viruses characteristically produce eosinophilic cytoplasmic inclusions, bovine type 3 strains have been found to cause both nuclear and cytoplasmic inclusions (Reisinger et al. 1959; Reczko & Bögel, 1962; Churchill, 1963; Inaba et al. 1963; Dawson, 1964; Omar, 1965). Both types of inclusion appear to contain abundant protein, some RNA and no DNA or polysaccharides (Kasten & Churchill, 1966).

Only two electron microscopical studies have been reported on ultrastructural changes in cells infected with bovine parainfluenza 3 virus; both describe changes in the cytoplasm, but...
make no reference to nuclear changes. Reczko & Bögel (1962) described cytoplasmic inclusions consisting of thread-like structures with a diameter of 5 to 10 nm. and Ané (1967) reported that the cytoplasmic inclusions, with filaments measuring about 20 nm. in diameter, were similar in appearance to those seen with other paramyxoviruses (Howe et al. 1967; Kuhn & Harford, 1963; Prose et al. 1965). It is now accepted that these aggregates of filaments represent the virus nucleocapsid, consisting of virus RNA and protein (Bonissol, 1966; Kasten & Churchill, 1966; Ané, 1967).

The presence of both nuclear and cytoplasmic inclusions in cells infected with bovine parainfluenza 3 virus sets this virus apart as a unique member of the parainfluenza virus group. Unlike other parainfluenza viruses, its cytopathic effect resembles that of the antigenically unrelated measles-rinderpest-distemper subgroup of paramyxoviruses (Waterson, 1965). The present study was undertaken to investigate the ultrastructural changes associated with bovine parainfluenza 3 virus infection, and to determine whether the morphogenesis of this virus is similar to that of other parainfluenza viruses, or of measles virus.

METHODS

Virus. The SF-4 strain (Reisinger et al. 1959) of bovine parainfluenza 3 virus was obtained from the American Type Culture Collection. On receipt, it had been passaged twice in bovine kidney cell cultures and three times in bovine embryo cell cultures. In this laboratory, it was maintained in LLC-MK2 cells. The virus pool used in this study had an infectivity of 10^7.5 TCD 50/ml.

Cell cultures and media. The LLC-MK2 line of rhesus monkey kidney cells, obtained from the American Type Culture Collection, was propagated in synthetic medium HB-597 (Connaught Medical Research Laboratories); this medium is a modification of synthetic medium 199 (Morgan, Morton & Parker 1950) supplemented with 2% heat-inactivated foetal calf serum. Antibiotics were added to a final concentration of 250 units of penicillin/ml. and 100 µg. of streptomycin/ml.

Light microscope studies. Monolayer cell cultures grown on cover-slips in Leighton tubes were inoculated with SF-4 virus at a multiplicity of 5, 0.5 and 0.05 TCD 50/cell. Virus was allowed to adsorb to washed, drained cultures for 1 hr at room temperature. Maintenance medium was then added, and the inoculated cultures, and controls, were incubated at 37°C. Coverslip cultures were fixed in a mixture of methanol (100 ml.) formalin (5 ml.) and acetic acid (1 ml.) at 24, 48, 72 and 96 hr after inoculation, and were subsequently stained with haematoxylin and eosin.

Electron microscopical studies. Monolayer cell cultures grown in 4-oz Brockway bottles were inoculated with SF-4 virus at a multiplicity of 0.7 TCD 50/cell. Virus was allowed to adsorb to washed, drained cultures for 1 hr at room temperature. Maintenance medium was then added and the inoculated cultures, and controls, were incubated at 37°C. Cultures were harvested and fixed for electron microscopy at intervals between 1 and 5 days after inoculation. Cells were fixed first in 5% glutaraldehyde, then in 1% osmium tetroxide, dehydrated in alcohols, and embedded in a mixture of Epon and Araldite. Thin sections were stained with uranyl acetate and lead citrate, and were examined in a Philips EM 200 at 60 kv.

For the examination of the structure of SF-4 virus in suspension, infected cultures were frozen and thawed once, and the resulting lysate negatively stained with 2% potassium phosphotungstate at pH 6.5.
RESULTS

Morphology of negatively stained virus

Negatively stained Sf-4 virus particles appeared as roughly spherical particles with an overall diameter of 280 to 580 nm., within which could usually be seen a mass of helical nucleocapsids (Fig. 1, 2). In intact and in ruptured virus particles the nucleocapsids (diameter 17 to 19 nm.) had the characteristic 'herringbone' pattern seen with other paramyxoviruses (Waterson & Hurrell, 1962). The virus envelope was about 12 nm. thick with a fringe 10 to 12 nm. long.

Cytopathology in coverslip cultures stained with haematoxylin and eosin

The most striking pathological feature seen in infected cultures stained with haematoxylin and eosin was the presence of eosinophilic inclusions in the nucleus and cytoplasm. At 24 hr after inoculation, small multiple inclusions, each surrounded by a clear zone, were evident in the cytoplasm of at least 90% of the cells infected with an input multiplicity of 5 (Fig. 3). Small, faintly eosinophilic inclusions were also occasionally seen in cell nuclei at 24 hr. Nuclear inclusions were clear at 48 hr, when they appeared in almost every nucleus (Fig. 4). The incidence and size of nuclear and cytoplasmic inclusions increased with time.

Although occasional small syncytia were found in control cells, the occurrence of these multinucleated cells was considerably greater in infected cultures (Fig. 3, 4). At 24 hr after inoculation, there were 5 to 6 syncytia/field of 200 infected cells, compared with 1 syncytium/field of control cells. A normal incidence of cells in mitosis was seen in infected cultures at 24 hr, although many of these contained eosinophilic inclusions (Fig. 3). Mitotic figures were no longer seen at 48 hr in infected cultures, although they were still evident in controls.

Similar pathological changes were observed in cultures inoculated with diluted virus, although they appeared one to two days later.

Cytopathology and morphogenesis in electron micrographs

In LLC-MK2 cells inoculated with an input multiplicity of 0.7, the first sign of progeny virus development at 24 hr was the appearance of small filamentous cytoplasmic inclusions, usually located in a perinuclear position (Fig. 5). Inclusion material in the perinuclear region tended to be more tightly packed than that located in other parts of the cytoplasm. At 48 hr, cytoplasmic inclusions were larger and more numerous. The diameter of individual filaments within the inclusions measured 17 nm. Other changes in the cytoplasm included an increased number of polyribosomes, swollen mitochondria and displacement of cytoplasmic organelles by filamentous inclusions.

Nuclear inclusions were first seen at 48 hr, in the form of small patches of closely packed thin filaments, identical in appearance to those in the cytoplasmic inclusions (Fig. 6, 7). At high magnification, certain oblique sections of the filaments appeared to follow a helical configuration.

The appearance of nuclear inclusions was concurrent with the first sign of virus maturation by budding at the plasma membrane. In isolated areas the plasma membrane appeared thickened, the outer aspect being covered with a fringe (Fig. 8). Along the inner surface of the membrane lay a row of tubules cut in cross-section. Adjacent to these areas and outside the cell were roughly spherical particles completely surrounded by a similar fringed membrane.

Another type of particle was found in Sf-4-infected cells, within dilated portions of rough endoplasmic reticulum (Fig. 9). These particles were of various diameters (180 to 500 nm.). They consisted of a limiting membrane, similar to rough endoplasmic reticulum, with a
Fig. 1, 2. Electron micrographs of SF-4 virus particles negatively stained with phosphotungstic acid. The virus particle in Fig. 1 is partially ruptured, displaying free nucleocapsids.

Fig. 3, 4. Light micrographs of LLC-MK2 cell cultures infected with SF-4 virus. (Fig. 3) Early stage of infection showing numerous cytoplasmic inclusions, small syncytia, and one infected cell undergoing mitosis (arrow). (Fig. 4) Late stage of infection showing both nuclear and cytoplasmic inclusions in every cell.
Fig. 5. Section through adjoining cells from SPF-4-infected cultures showing large filamentous cytoplasmic inclusions (arrows).

Fig. 6. Cell showing early nuclear inclusion (arrow) and larger cytoplasmic inclusions (CI).
heterogenous and usually granular interior. Such particles occurred singly and in clusters of up to 40, always close to cytoplasmic inclusions. Clusters of particles were also found within the perinuclear space between the two layers of the nuclear membrane (Fig. 10). This type of particle was never observed in cells showing budding at the plasma membrane.

Fig. 7. Section through nucleus of LLC-MK2 cell infected with SF-4 virus, showing filamentous nuclear inclusion (NI).

Fig. 8. Budding of SF-4 virus particles at plasma membrane. Nucleocapsid tubules cut in cross-section lie along inside of the fringed envelope (arrow).

DISCUSSION

The structure of bovine parainfluenza 3 virus (strain SF-4) appears to be indistinguishable from that of other paramyxoviruses. Negatively stained particles ranged in size from 280 to 580 nm. and contained a nucleocapsid 17 to 19 nm. in diameter. These dimensions compare favourably with those reported for the same virus by Kahn et al. (1969) who recorded a range of 80 to 540 nm. and a nucleocapsid diameter of 17 nm. The cytoplasmic aggregates of nucleocapsid-like filaments seen in SF-4-infected cells are typical of the paramyxovirus group as a whole, and have been reported for bovine parainfluenza 3 virus (Reczko & Bögel, 1962; Ané, 1967). However, similar inclusions in the nucleus have not previously been described for this virus or for any other parainfluenza virus.

It has recently been confirmed that the aggregation of nucleocapsids in both the nucleus
Fig. 9. Particles contained within dilated portion of endoplasmic reticulum (arrow), beside a cytoplasmic inclusion (CI).

Fig. 10. Particles within dilated nuclear membrane (arrow), beside cytoplasmic inclusions (CI). Note swollen mitochondria.
and cytoplasm is a characteristic cytological change induced by measles virus (Nakai & Imagawa, 1969; Nakai, Shand & Howatson, 1969), another member of the paramyxovirus group. Furthermore, it has been concluded that these aggregates correspond to the eosinophilic inclusions shown by light microscopy in the nucleus and cytoplasm of measles virus-infected cells. Our conclusion for SF-4 virus is similar, although we failed to provide direct evidence based on correlated studies by light and electron microscope. It is interesting to note that, although measles virus and related viruses (distemper, rinderpest) are morphologically indistinguishable from the parainfluenza viruses, the two subgroups apparently share no common antigens.

The significance of the endoplasmic reticulum type of budding is unknown, and at present we are unable to determine whether or not it is directly related to virus maturation. It has not previously been described for a paramyxovirus infection in vitro, but is very similar to a type of budding reported by Herndon & Rubenstein (1968) in brain tissue from patients with subacute sclerosing panencephalitis, a disease in which measles virus has been strongly implicated (Chen et al. 1969; Horta-Barbosa, Fuccillo & Sever, 1969; Katz, Oyanagi & Koprowski, 1969).

In conclusion, on the basis of light and electron microscopical observations, it appears that the morphogenesis of bovine parainfluenza 3 virus more closely resembles that of the serologically unrelated measles virus than that for the serologically related parainfluenza viruses.

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REFERENCES


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