Morphology and Morphogenesis of a New Paramyxovirus (PMV 107)

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SUMMARY

The morphology of the virions and nucleocapsids of paramyxovirus 107 (PMV 107) and the replication of the virus were investigated by electron microscopy. The virions and nucleocapsids exhibited the same structural properties as other paramyxoviruses. Nucleocapsids were found in the nucleus and cytoplasm of infected bovine embryonic lung (BEL) cell cultures. A similar situation has been described for the morbilliviruses measles, SSPE, distemper and rinderpest. Alignment of nucleocapsids beneath the plasma membrane and budding of PMV 107 in the productive BEL cell infections were also similar to the morbillivirus-infected cells. In a line of monkey cells (CV1) persistently infected with PMV 107 only cytoplasmic nucleocapsids could be demonstrated. On the basis of its morphology and morphogenesis it is suggested that PMV 107 should be classified as a paramyxovirus. Since nucleocapsids could also be found in the nucleus of infected BEL cells the morphogenesis of PMV 107 closely resembles that of viruses of the morbillivirus group.

INTRODUCTION

During investigations on the aetiology of sporadic bovine encephalomyelitides Bachmann et al. (1975) isolated a paramyxovirus-like agent (PMV 107) which was recovered only after co-cultivation of brain cells from the diseased animal with other bovine cells and permanent cell lines of simian origin. PMV 107 replicated in cell cultures with formation of syncytia and inclusion bodies in the cytoplasm and nucleus.

PMV 107 antigen in infected cells reacted with seven out of fifteen sera from patients with subacute sclerosing panencephalitis (SSPE) in the indirect fluorescent antibody test. Titres of these sera against PMV 107, however, were significantly lower than against SSPE virus. Subsequently it was shown in hybridization experiments with measles virus and SSPE virus 18S mRNA that this isolate is genetically related to measles, SSPE and distemper viruses (Hall & Ter Meulen, 1976). Like some SSPE virus strains, infectious PMV 107 could not be detected in cell free supernatants from infected CV1 and bovine kidney or brain cell cultures. However, infectious virus was recently produced by growing the agent in bovine embryonic lung cells.

This report describes a study of the morphology and morphogenesis of PMV 107 in order to characterize the agent further.
Methods

Cell cultures. The continuous cell lines CV₁ (monkey kidney; ATCC, CCL 70), Vero (monkey kidney; ATCC, CCL 81) and BHK-21/13 (baby hamster kidney; ATCC, CCL 10) were grown as monolayers in minimal essential medium with Earle's basal salt solution (EMEM), which was supplemented with 5% calf serum (FCS; Flow Laboratories, Bonn, FRG). Bovine embryonic lung cell cultures (BEL) were prepared from 3 to 5-month-old calf foetuses by trypsinization of lungs at 33 °C according to standard methods. Growth medium consisted of EMEM and 5% FCS.

Virus. All studies were made with PMV 107 (Bachmann et al. 1975), which had been passaged 11 times in persistently infected CV₁ cells (PMV 107-CV₁ cells). The infections of BHK and BEL cell cultures were carried out by co-cultivation of one part of trypsinized PMV 107-CV₁ cells with two parts of trypsinized BHK or BEL cells. After infection had been initiated in BHK and BEL cell cultures it was carried on in these cells also by co-cultivation, since a better infection was obtained by this method. Usually, a defined c.p.e. developed within 2 to 5 days after infection which was characterized by formation of syncytia and subsequent cell lysis in 24 h. CV₁ cells persistently infected with PMV 107 were subcultured at 3 to 5 day intervals. According to the amount of degeneration observed in these cultures, fresh uninfected CV₁ cells were added to this system every 3 to 6 weeks.

For comparison, measles virus strain Edmonston B (kindly supplied by Dr M. R. Hilleman, Westpoint, Pa. U.S.A.) was grown in Vero cells, which were infected at a multiplicity of 0.005 p.f.u./cell for 1 h at 37 °C before EMEM containing 2% FCS was added.

Virus replication was demonstrated by the indirect fluorescent antibody technique using (1) a PMV 107 hyperimmune serum prepared by inoculating rabbits intracutaneously with gradient purified PMV 107 mixed with 50% Freund's adjuvant, and giving an i.m. booster inoculation four weeks later, and an FITC-conjugated goat anti-rabbit globulin (Biomérieux, Marcy-Etoile, France) and (2) serum from an SSPE patient together with an FITC-conjugated rabbit anti-human globulin.

Purification of virions and nucleocapsids. One litre of tissue culture fluid from PMV 107-infected BEL cell cultures was harvested when about 70% of cells showed c.p.e. The fluid was clarified by centrifuging at 2200 g and virus was pelleted in a Beckman Spinco L 65-2 at 35000 rev/min for 90 min using a Ti 42 rotor. The pellet was resuspended in 3 ml of NTE (0.01 M-tris-HCl, pH 7.4, 0.1 M-NaCl; 0.001 M-EDTA) by sonication (Branson sonifier S75; Branson, Danbury, Conn., U.S.A.) and centrifuged again at 2200 g for 10 min. The virus was layered on to a gradient of 20 to 55% (w/w) sucrose in NTE buffer and centrifuged in an SW 40 rotor at 30000 rev/min for 90 min. Bands containing virus were collected and dialysed overnight against NTE buffer and then layered on a linear 25 to 60% (w/w) sucrose gradient in NTE buffer. Centrifugation was carried out in an SW 40 rotor for 6 h. There was one visible band which was collected, dialysed against NTE and examined by electron microscopy.

For the preparation of nucleocapsid, PMV 107-infected BHK cells and measles virus-infected Vero cells were treated with 0.2 μg/ml actinomycin D (Serva, Heidelberg, W. Germany) after 10 to 36 h p.i. Thirty min after addition of actinomycin D the cells were labelled with 5-³H-uridine (Serva, Heidelberg, W. Germany) at a concentration of 2 μCi/ml. Following further incubation for 24 to 30 h, cells were harvested by scraping them into the medium with a rubber policeman. The cells were sedimented at 4 °C and 400 g for 5 min, washed once in PBS, resuspended in a few ml of distilled water, allowed to swell at 4 °C for 10 min and then disrupted in a Dounce homogenizer. Separation of cell fragments and nuclei was carried out by centrifuging the suspension at 7000 g for 10 min at 4 °C.
resulting supernate was pipetted directly on to a CsCl (Merck, Darmstadt, W. Germany) gradient (0.5 ml 40%, w/w, CsCl; 2 ml 30%, w/w, CsCl; 2 ml 25%, w/w, CsCl) and centrifuged at 40,000 rev/min in a SW 41 rotor for 170 min at 4 °C. After fractionation of the gradients, radioactivity was measured by adding 50 μl of each fraction to 10 ml of Bray’s solution. Peak fractions of the radioactive nucleocapsids were dialysed overnight against NTE buffer.

Electron microscopy. Drops of gradient fractions containing virion and nucleocapsid were spread over Formvar and carbon-coated copper grids. The specimens were stained with 2% potassium phosphotungstic acid (pH 7.0).
Fig. 3. Thin section of a PMV infected BEL cell 72 h after infection. Large aggregation of rigid strands in cytoplasm which are different from typical nucleocapsids. P = plasmofilaments; C = cytoplasm.

For ultrahistology, bottles of PMV 107-infected BEL and CV1 cultures were harvested 24, 48, 72 and 96 h after seeding. After decanting the medium, cells were scraped off with a rubber policeman, washed in PBS and sedimented at 600 g for 5 min. The pellet was fixed in 1% glutaraldehyde for 5 min and 2% osmium tetroxide for 30 min. After dehydration in alcohol the cell pellet was cut into 1 to 2 mm pieces and embedded in gelatine capsules with Durcupan (Fluka, Buchs, Switzerland). After polymerization at 60 °C over a period of 2 to 3 days, ultrathin sections were prepared with a LKB ultrrotome and stained with uranyl acetate and lead citrate (Reynolds, 1963) on copper grids. Electron micrographs were taken with a Siemens Elmiskop 1.

RESULTS

Virion and nucleocapsid morphology

Although there is great variation in shape, particles were roughly spherical. Their diameters varied from about 120 to 350 nm with a few smaller and a few larger particles. Intact virions have an outer envelope, about 8 to 9 nm thick, which bears short projections (approx. 14 nm in length) on its outer surface. In partially disrupted particles some internal structures are seen. The internal material is coiled and consists of rod-shaped nucleocapsids (Fig. 1).

Electron micrographs of negatively stained PMV 107 nucleocapsids isolated from infected cells showed tightly coiled and rigid rods, about 17 nm in diam. The great heterogeneity in length indicates that most of the nucleocapsids are fragments (Fig. 2). They exhibit a typical
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Fig. 4. Thin section of a PMV 107-infected BEL cell 96 h p.i. Note the oval areas in the nucleus consisting of coiled nucleocapsids surrounded by fibrillar material. N = nucleocapsids; K = nucleus; C = cytoplasm.

'herring bone' structure and their surfaces were serrated and had an average periodicity of 5 nm. Pieces of nucleocapsids exhibiting an end-on orientation were spherical in section and had a hollow round centre. Their inside diam. measured between 5 and 6 nm. Similar data were obtained with measles virus nucleocapsids.

Fine structure of PMV 107-infected cell cultures

BEL cells

The first structural changes were found in PMV 107-infected BEL cells between 72 and 96 h p.i. They consisted of large masses of electron dense, tubular material in the cytoplasm. The diameter of these tubules measured approx. 17 to 18 nm. They were coiled and could be detected in different parts of the cytoplasm (Fig. 6 and 7). In addition large accumulations of 'rough', rigid and elongated strands with a parallel orientation, measuring 17 to 18 nm in diam. (Fig. 3) occurred in the cytoplasm. They are different from the nucleocapsids shown in Fig. 6 and 7. Their function and role in the morphogenesis are not known.

At the same time interval after infection, oval areas which were surrounded by fibrillar
material were detected in the nucleus of the cells. These oval areas consisted of coiled nucleocapsids with a 'smooth' surface and an outer diam. of about 15 nm (Fig. 4). In some of these structures the surrounding fibrils were missing (Fig. 5). Nuclear chromatin was marginated at the nuclear membrane (Fig. 4). The aggregates of tubules in the nucleus and cytoplasm apparently corresponded to inclusion bodies seen with the light microscope.

Alignment of nucleocapsids and budding processes were observed about 96 h p.i. Fig. 6 shows electron dense alterations in the plasma membrane with aligned nucleocapsids beneath. In addition there are areas with coiled nucleocapsids near the membrane. Apparently, particles are released by budding from these altered cellular membranes (Fig. 7 and 8).

**CV₁ cells**

In CV₁ cells in which PMV 107 undergoes a persistent, non-productive infection, cytoplasmic nucleocapsids were either coiled or stretched out and exhibited a ‘smooth’ surface. Similar to PMV 107 in BEL cells, rigid filaments, which are different from nucleocapsids, were seen in CV₁ infected cells. Although small nuclear inclusion bodies were seen in some of these cells, areas with tubules could not be detected in the nucleus. Contrary to earlier
observations (Bachmann et al. 1975), there was no visible alignment and budding in these cells.

**DISCUSSION**

The morphological appearance of PMV 107 indicates that the agent is a paramyxovirus. Virion structure resembles that of other paramyxoviruses (Horne & Waterson, 1960; Plowright et al. 1962; Norrby et al. 1963; Waterson, 1965). This conclusion is supported by the morphological findings with isolated PMV 107 nucleocapsids. Waters & Bussell (1974) compared negatively stained nucleocapsids of measles virus, canine distemper virus, and SV-5 under the electron microscope, and found essentially no morphological differences between these virus species. Data on size and structure of PMV 107 nucleocapsids are in agreement with these observations.

The demonstration of nucleocapsid-like structures in the nucleus of PMV 107-infected BEL cells coincides with results obtained with measles virus (Nakai & Imagawa, 1969), SSPE virus (Martinez et al. 1974), rinderpest virus and distemper virus (Tajima et al. 1971) infected cells. The structure of these inclusion-like areas in the nucleus can clearly be differentiated from para-influenza-virus-3 nuclear inclusion bodies (Reczko & Bögel, 1962) and from 'nuclear bodies' described by Bouteille et al. (1965) and Dupuy & Bouteille (1972) that contain granular material.
PMV 107 nucleocapsid-like structures were never distributed over the whole nucleus, as observed in distemper virus infected cells by Koestner & Long (1970). Nucleocapsids, however, are not always found in the nuclei of distemper, measles or rinderpest virus-infected cells. Cornwell et al. (1971) postulate that virus strains differ in this property. From the data obtained with PMV 107 it is more likely that differences in the occurrence of nuclear nucleocapsids are connected with the particular cell type used. In PMV 107-infected BEL cells, nuclear nucleocapsids were a regular finding, but they could not be demonstrated in persistently infected CV₁ cells. Similar observations were made with cells persistently infected with measles virus (Heneen et al. 1973; Menna et al. 1975).
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The smaller size of nuclear filaments (15 nm) in PMV 107-infected cells in comparison to cytoplasmic filaments (17 nm) agrees with data known for measles and SSPE viruses (Nakai et al. 1969), whereas nuclear filaments in distemper and rinderpest virus infected cells have the same diameter as cytoplasmic nucleocapsids (Tajima et al. 1971).

Cytoplasmic nucleocapsids in PMV 107-infected cells are similar to those of other paramyxoviruses. The same applies to alignment and budding in productive infections (Nakai et al. 1969; Dubois-Dalcq et al. 1974); these could not be demonstrated in the PMV 107–CV1 cell system. The arrays of long and rigid strands that appeared in the cytoplasm of BEL and CV1 cells infected with PMV 107 do not resemble nucleocapsids, and have not been described before in paramyxovirus-infected cells. Although there is an association with PMV 107 infection in vitro, their function can only be speculated on. This is in disagreement with earlier, preliminary observations (Bachmann et al. 1975) where alignment and extracellular, intact but non-infectious particles were demonstrated. The reason for this is unknown; however, data obtained by the freeze-etching technique with PMV 107-infected cells show no budding processes in CV1 cells, in contrast to BEL cells (M. Dubois-Dalcq, personal communication).

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REFERENCES


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