Electron Microscopy of CRFK Cells Infected with Canine Parvovirus

Tsutomu HIRASAWA*, Toshiharu HAYASHI†, and Shin-ichiro KONISHI

Department of Veterinary Microbiology, and †Department of Veterinary Pathology, Faculty of Agriculture, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan

(Received 6 October 1986/Accepted 20 May 1987)

ABSTRACT. Electron microscopic observation was carried out on the CRFK cells inoculated with canine parvovirus. The cells were synchronized by thymidine before inoculation. The earliest findings were chromatin margination and nucleolar deformation. Virus particles about 20 nm in diameter were first seen, forming clumps in the nucleus 18 hr postinoculation (p. i.). Numerous virus particles were observed in the nucleus 48 hr p. i. They usually formed large aggregates, and were occasionally scattered within the nucleus. The host cells showed marked nuclear deformation and cytoplasmic vacuolation. Virus particles were also found in the degenerated cytoplasmic matrix 48 and 72 hr p. i., forming aggregates, sometimes being associated with membranous structures. These findings suggested that the virions were composed within the nucleus of CRFK cells, and then moved into the cytoplasm.

KEY WORDS: canine parvovirus, CRFK cell, electron microscopy.

We previously reported the growth of canine parvovirus (CPV) on CRFK cells, and morphology and immunohistology of infected cells. CPV grew well in the freshly seeded or synchronized CRFK cells, and viral antigen filled the nucleus with production of intranuclear inclusions and then spread into the cytoplasm [13]. In both enteric and myocardial forms of natural CPV infections, electron microscopy revealed that most virus particles were present within the nucleus [5, 7, 9, 12, 23, 27, 29] while some in the cytoplasm [5, 7, 29]. In vitro, however, viral morphogenesis has not been fully studied except for Paradiso et al. [21] who demonstrated the presence of virions in the nucleus. The present paper is to describe the ultrastructural morphology of CPV-infected CRFK cells.

MATERIALS AND METHODS

Virus and cells: CPV, Cp 49 strain, was propagated and titrated as previously described [2]. CRFK cells were synchronized at early S phase by treating with 3 mM thymidine [13], and the stock virus fluid was inoculated at a multiplicity of infection of 1 TCID₅₀/cell. After absorption at 37°C for 1 hr, the culture supernatant was removed, and cells were washed twice with serum-free medium. After adding the growth medium [2], the cultures were incubated at 37°C.

Electron microscopy: Cells were harvested by a rubber policeman at 12, 18, 24, 48 and 72 hr postinoculation (p. i.). Both synchronized and asynchronous non-infected cells were served as controls. After centrifuge at 1,000 rpm for 10 min, cell pellets were fixed with 2.5% glutaraldehyde in phosphate-buffered saline (PBS) at 4°C for 2.5 hr and postfixed with 1% osmium tetroxide in PBS at 4°C for 2 hr. After dehydration in a graded series of ethanol and propylene oxide, the cells were embedded in Spurr resin. Ultrathin sections were made and stained with uranyl acetate and lead citrate, and they were observed under a JEM-100S (JEOL) electron microscope at...
80 kV or a HU-12 (Hitachi) at 75 kV.

RESULTS

As shown in Fig. 1, CRFK cells normally had an oval nucleus with a large nucleolus at the center. Binuclear or multinucleated cells were rare. Heterochromatin was located along the nuclear membrane, and euchromatin was scattered throughout the nucleus, being associated with granular substance. There was no significant difference in ultrastructural features between synchronized and asynchronous cells except for a small number of cytoplasmic vacuoles in the formers.

At 12 hr p. i., the nucleus was somewhat swollen showing chromatin margination (Fig. 2) and containing a segmented or amorphous nucleolus with irregular-shaped margin (Fig. 3). The nucleolus was often

Fig. 1. Non-inoculated CRFK cell treated with thymidine, with a nucleolus at the center of the nucleus (N). ×7,800.

Fig. 2. Chromatin margination in an infected CRFK cell, 12 hr p. i., ×20,000.

Fig. 3. Virus clump (large arrow) in the nucleus with a segmented nucleolus (arrows), 18 hr p. i., ×21,000.
Fig. 4. Intranuclear virus aggregate with unclear outline, 48 hr p. i., ×33,000.

located near the nuclear membrane or occasionally attached to the marginated chromatin.

At 18 hr p. i., virus particles about 20 nm in diameter were detected in clumps within the swollen and polymorphic nucleus (Fig. 3). The viral clumps sometimes connected with marginated chromatins. The number of virion-containing cells increased at 24 hr p. i., and their nucleus became polymorphic.

At 48 hr p. i., virus particles were numerous in the nucleus, forming large aggregates (Fig. 4). Occasionally some isolated virions were seen in the nucleus. At more advanced stage of infection, nuclear deformation was prominent, showing unclear or partially disrupted nuclear membrane. The cytoplasm of the host cells had many vacuoles of various size, presumably expanded endoplasmic reticula, and few organellae remained.

At 48 and 72 hr p. i. virus particles were also detected in the cytoplasm of most severely infected cells. Compact virus aggregates were also seen (Figs. 5 and 6), and free
virions were distributed in the cytoplasmic matrix, sometimes forming small clumps or being attached to membranous structures (Fig. 7).

DISCUSSION

The nuclear swelling, chromatin margination and nucleolar deformation appearing early stage of infection were described with in vitro culture infected with feline panleukopenia virus (FPLV) [17, 19] antigenically close to CPV [2, 8, 20].

Although the viral growth was suggested to occur as early as 12 hr p. i. in our previous report [13], virions were detected within the nucleus of host cells 18 hr p. i. but not at 12 hr p. i. CPV [21] and FPLV [17, 19] were described to appear first in the nucleus without mentioning the exact time of their appearance. The appearance of mature parvovirus virions in infected cells seems quite different among investigators [1, 4, 22, 24-26] probably due to very small virions which might be difficult to detect on the
preparations, except for virus particles in clumps.

Although only small-sized aggregates or clumps of viruses were shown in the nucleus of some CPV-infected cells [5, 29], large-sized aggregates of viruses appeared within the nucleus at 48 hr p. i. in the present study. Yasoshima et al. [29] demonstrated a crystalline-arrangement of CPV particles associated with lattice-like structure within the nucleus of infected epithelial cells of Lieberkühn’s crypts, and similar findings were obtained in natural FPLV infections [6, 18], while no such structure was revealed by other electron microscopic studies on CPV [5, 7, 9, 12, 21, 23, 27] and FPLV [17, 19, 28]. The crystalline formation was detected only in a few cases of other parvovirus infections [1, 3, 4, 10, 11, 14–16, 22, 24–26].

At later stage of infection, 48 and 72 hr p. i., virus particles were numerous in the cytoplasm as shown in natural CPV infections [5, 29] as well as in FPLV infections [6, 17, 18], and they were readily distinguishable, supporting our previous immunohistological observations [13]. For the releasing of virus from the nucleus into cytoplasm of infected cells, there might be two possibilities, either via nuclear pores or after destruction of the nuclear membrane. Attachment of the virus particles to the outer nuclear membrane (Fig. 7) as well as intense virus-specific fluorescence at the nuclear membrane [13] might support the former. The latter might be supported by morphological similarity between virus aggregates within the nucleus (Figs. 3 and 4) and those in the cytoplasm of infected cells (Figs. 5 and 6).

ACKNOWLEDGMENTS. The CRFK cells were obtained from Dr. J. F. Weaver, staff research associate, of Naval Bioscience Laboratory, and were produced with support from the National Cancer Institute, Biological Carcinogenesis Branch, Division of Cancer Cause and Prevention under the auspices of the Office of Naval Research and Regents of the University of California.

REFERENCES