Morphological Changes in Productive and Abortive Infection by Feline Herpesvirus

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Received for publication 26 January 1970

Feline herpesvirus produces characteristic morphological alterations in feline kidney cells. Nucleocapsid particles are formed in infected nuclei and are enveloped as they pass through the modified inner nuclear membrane. Aggregates of dense granular material and filamentous structures also regularly appear in infected nuclei. Infection of human embryonic lung cells by feline herpesvirus results in the appearance of intranuclear inclusion bodies, aggregates of dense granular material, and bundles of parallel filaments but no nucleocapsid particles.

Feline herpesvirus (FHV) replicates in feline kidney (FK) cells but not in human embryonic lung (HEL) cells. The virus attaches to but does not penetrate human cells. When the cells with attached virus are subsequently fused with Sendai virus, completely inactivated by beta-propiolactone, FHV causes characteristic cytopathic effects 24 to 48 hr after inoculation, but no infectious virus can be recovered from the infected cells (11). Thus, the natural barrier to penetration by FHV can be overcome by fusion of resistant HEL cells. The complete absence of infectious virus in infected human cells indicates the presence of one or more additional defects in the virus-cell relationship that are independent of the barrier to penetration.

The present study was undertaken to determine the effect of these defects on the morphogenesis of feline herpesvirus infection by comparing the late ultrastructural changes seen in productive FHV infection of FK cells and abortive FHV infection of HEL cells.

MATERIALS AND METHODS

Viruses. FHV, isolate number 1217, was generously supplied by Abraham Karpas (3). Pools of virus were prepared by harvesting infected primary FK cells and medium after the development of extensive cytopathic effect. The virus suspension was frozen and thawed three times, clarified by slow centrifugation, and stored at -60 C. Infectivity titers of these preparations varied from 10 to 7.5 TID50/0.1 ml. Sendai virus was prepared and inactivated with beta-propiolactone and stored as described by Neff and Enders (6). No residual infectious virus was detected.

Cells. To prepare FK cells, kidneys from immature cats were trypsinized and grown in Eagle’s medium with 10% fetal calf serum. HEL cells were prepared from the 10th to 15th subcultures of a cell line derived from human embryonic lung (11).

Inoculation and fusion of cells. Monolayer cultures of cells in 8-oz (236 ml) prescription bottles were inoculated with FHV suspended in 10 ml of Eagle’s medium at an approximate multiplicity of 100 infectious units per cell and with 1 ml of inactivated Sendai virus in appropriate concentration to result in cell fusion. Cultures were incubated for 6 hr at 36 C. Inoculated cultures were then washed three times with 10 ml of Hanks solution and incubated in Eagle’s medium with 10% fetal calf serum. Infected cultures were examined 48 hr after the simultaneous inoculation of FHV and inactivated Sendai virus. Parallel cultures of uninoculated cells or cells inoculated with FHV or Sendai virus alone were used as controls.

Light microscopy. Cultures were fixed in Bouin, fluid, embedded in collodion, and stained with hematoxylin and eosin.

Electron microscopy. Monolayer cultures were fixed in situ with 2% cold cacodylate-buffered glutaraldehyde for 30 to 60 min. The cells were scraped off glass with a policeman, washed in a buffered sucrose solution, and postfixed with 1% osmium tetroxide. They were then dehydrated in graded alcohols and embedded in epoxy resin. Thin sections were stained sequentially with uranylacetate and lead citrate and examined in an RCA electron microscope (EMU 3G).

RESULTS

Infection of FK cells with FHV. Infection of FK cells with FHV resulted in complete cytopathic effect in 48 hr as determined by light microscopy. Giant cells formed and frequently contained 10 to 20 nuclei with characteristic inclusion bodies (Fig. 1A). Electron microscopy of infected FK cells revealed ultrastructural changes in all cells. Infected nuclei contained numerous randomly
dispersed particles with a single electron-opaque shell. Most of the capsids contained a central core (Fig. 2). Granular material and filamentous structures also frequently appeared in the nucleoplasm without any definite relationship with capsids or nucleocapsids. The dense regular granules measured approximately 30 nm and occurred in small aggregates in many areas of the nucleus (Fig. 3 and 4). Fine filamentous structures in parallel arrangement were usually found in isolated bundles (Fig. 3 and 4) but rarely filled larger areas of the nucleus (Fig. 5). The nuclear membranes often became markedly convoluted (Fig. 6) and were thickened in areas adjacent to nucleocapsids (Fig. 5). Particles of electron-opaque shells were present between the inner and outer layers of the nuclear membrane (Fig. 6) and in cytoplasmic compartments (Fig. 7). In some sections, the cytoplasmic compartments formed linear channels which were continuous with the cytoplasmic membrane at the cell surface (Fig. 8).

The inoculation of inactivated Sendai virus simultaneously with FHV did not alter any of these morphological changes.

**Infection of HEL cells with FHV.** Simultaneous inoculation of HEL cells with FHV and inactivated Sendai virus resulted in extensive fusion of cells and the formation of intranuclear inclusion bodies characteristic of FHV infection in approximately one-fourth of the nuclei of polykaryons examined by light microscopy (Fig. 1B). One-hundred nuclei from infected HEL cultures were examined by electron microscopy. No intact nucleocapsid particles were seen in any of the nuclei of polykaryons which often demonstrated other changes typical of FHV infection in FK cells. Aggregates of dense regular granules were present in 27% of the nuclei (Fig. 9). Both the size and appearance of these aggregates closely resembled those seen in FK cells. Well-defined bundles of filamentous structures were seen in approximately one-half of the nuclei which contained granular aggregates (Fig. 10). No changes in the nuclear or cytoplasmic membranes resembling those in FK cells were seen. No particles with membranes were detected in the cytoplasm.

HEL cells inoculated with FHV alone were not morphologically altered. HEL cells exposed only to Sendai virus formed polykaryons but failed to demonstrate any of the changes described above.

**DISCUSSION**

The late ultrastructural changes in productive infection of FK cells by FHV have been described here for the first time. These alterations are quite similar to changes induced by other members of the herpesvirus group in the cells of a variety of species (1, 2, 4, 5, 8, 12). The morphological absence of viral particles in abortive infection of HEL cells correlates well with the previously described failure to recover any infectious virus from these cells (11). The additional absence of alterations of the nuclear membranes of infected HEL cells suggests that there are two or more distinct defects in abortive FHV infection.

Aggregates of dense regular granules (4, 8, 9) and filamentous structures (1, 2, 5, 12) have been described in the nuclei of cells infected with various representatives of the herpesvirus group. The similarity of these changes in cells of different species suggests that they are specific alterations induced by the virus. The function of these structures remains unknown. There is no convincing evidence to suggest that they are structural components of the virion. The presence of these structures without formation of virus particles in abortive FHV infection indicates either that they are not structural subunits or that other factors are required for the assembly of subunits into particles.

The morphological defects in FHV replication in HEL cells are more extensive than those described in other abortive herpesvirus infections. Spring et al. (10) reported a failure of envelopment of nucleocapsids in abortive infection of dog kidney cells by herpes simplex particles. Nii et al. (7) suggested that hydroxyurea-treated human amnion cells elaborated herpes simplex particles devoid of deoxyribonucleic acid. These particles failed to develop "normally dense cores" and were not enveloped at the nuclear membrane. Chitwood and Bracken (1) showed that addition of p-fluorophenylalanine to human amnion cells infected with herpes simplex virus resulted in marked accumulation of fibrillar and granular structures in addition to viral particles. Siminoff and Menefee (9) reported that bromodeoxyuridine inhibits but does not completely prevent the formation of intranuclear capsids.

These studies indicate that a variety of cell-virus interactions may occur in herpesvirus infections under different conditions. The observations described here suggest that early and marked defects occur in abortive infection of HEL cells by FHV.

**ACKNOWLEDGMENTS**

This investigation was supported by Public Health Service research grants AI-01992-13-VR from the National Institute of Allergy and Infectious Diseases and HE 6370 from the National Heart Institute, and by the American Cancer Society postdoctoral fellowship PF 415.
FIG. 1. (A) Culture of FK cells inoculated with FHV. A single giant cell contains many nuclei with characteristic inclusion bodies and condensed chromatin. Hematoxylin and eosin stain. ×400. (B) Culture of HEL cells inoculated with FHV and inactivated Sendai virus. Some of the nuclei of the polykaryon contain intranuclear inclusions and condensed chromatin similar to the changes seen in FK cells. Hematoxylin and eosin stain. ×400.

FIG. 2. FK cell with intranuclear empty capsids and nucleocapsids and with intracytoplasmic-membraned virus particles. ×20,000.
Fig. 3. FK cell with intranuclear aggregates of dense regular granules and parallel bundles of fibrillar structures. Scattered nucleocapsids are also present. $\times 20,000$.

Fig. 4. (A) FK cell with an aggregate of dense regular granules and scattered capsids in the nucleus. $\times 40,000$. (B) FK cell with a bundle of parallel filamentous structures in the nucleoplasm. $\times 50,000$.

Fig. 5. FK cell with FHV nucleocapsids adjacent to macular thickenings in the altered inner nuclear membrane. Note the extensive fibrillar change in the nucleoplasm. The fibrillar structures are seen in cross and longitudinal section. $\times 30,000$. 

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FIG. 6. FK cell with an irregular nucleus and complex budding and folding of the nuclear membranes to form intracytoplasmic protrusions. Virions are present between the inner and outer layers of the nuclear membrane. ×35,000.

FIG. 7. FK cell with virions in compartments in the cytoplasm. ×55,000.

FIG. 8. FK cell with an elongated intracytoplasmic compartment containing virions. The lining of this channel is continuous with the cytoplasmic membrane. ×35,000.
FIG. 9. HEL culture inoculated simultaneously with FHV and inactivated Sendai virus. The cell is a polykaryon in which three nuclei share a single outer nuclear membrane. Aggregates of dense regular granules are present in the altered nucleoplasm. \( \times 30,000 \).

FIG. 10. HEL culture simultaneously inoculated with FHV and inactivated Sendai virus. Distinct parallel bundles of filamental structures are present in the nucleus. \( \times 40,000 \).
LITERATURE CITED


