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Cytopathological effects of *Suid herpesvirus 1* (strain A_2) in cell line DEC 99

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Cytopathological effects of the *Suid herpesvirus 1,* strain A_2 (SuHV-1/A_2) were studied in cell cultures from permanent cell line DEC 99. Rounding of the cell, formation of syncytia with different number of nuclei and destruction of the cell layer were the main cytopathological changes detected. Double staining of the cells with acridine orange and ethidium bromide revealed many apoptotic cells at different cell dead levels. Condensation and fragmentation of the chromatin as well as a great number of full and empty virus particles were observed by means of electron microscopy. It was concluded that the data concerning virus morphogenesis are insufficient. Therefore further investigations in this field have to be carried out to demonstrate the interactions between DEC 99 cell line and SuHV-1/A_2.

Key words: apoptosis, Aujeszky's disease, DEC 99, *Suid herpesvirus 1,* syncytia.

Introduction

Aujeszky's disease is an important viral disease in swine with high economic impact. The causative agent *Suid herpervirus 1* (SuHV-1) is a member of family *Herpesvirirdae,* subfamily *Alphaherpesvirinae,* genus *Varicellovirus* [5]. Like the other alphaherpesviruses SuHV-1 produces distinct cytopathic effects in susceptible cell cultures including rounding of cells, destruction of the cell layer and in some cases formation of syncytia [3]. Numerous types of cell lines or primary cell cultures are susceptible for propagation of SuHV-1 [7], including chick embryo cells [10]. However permanent cell lines from mammalian origin have been predominantly used for studying the characteristics of the virus. Therefore the aim of the present investigations was to study the cytopathological effects of the SuHV-1, strain A_2 in cell cultures from permanent duck cell line DEC 99.

Materials and Methods

Cell cultures. Cell cultures (CCs) from the permanent cell line DEC 99 were cultivated in a CO_2 incubator (5% CO_2, 37,5°C and 95% humidity) in a combination of Medi-

um 199 and Iskove's modification of Dulbecco's medium (IMDM), containing 25mM HEPES buffer (Sigma), supplemented with 10% fetal bovine serum (FBS) (Sigma) and antibiotics in usual concentrations.

Virus stock. Virulent strain A_2 of *Suid herpesvirus 1* (SuHV-1/A_2) was used, kindly supplied by the Department of Virology at the National Diagnostic & Research Veterinary Institute, Sofia, Bulgaria.

Cell infection with SuHV-1/A_2. DEC 99 cells were exposed to SuHV-1/A_2 ($10^{5.3}$ $TCID_{50}$/ml) after the confluence of cell monolayers reached 50%. The virus adsorption was performed for 40 min in tissue culture flasks and plates and after that the maintenance medium was added. Experimental controls including uninfected monolayers were used in all studies.

Double vital staining of DEC 99 cells with acridine orange (AO) and ethidium bromide (EB). In this study, DEC 99 cells uninoculated and inoculated with the SuHV-1/A_2 (third passage on DEC 99 cells, $10^{5.3}$ $TCID_{50}$/ml) were grown in 24-well plates on specially prepared glass lamellas (20×20 mm). At 6, 12, 24, 36, 48, 60 and 72 h p.i. lamellas with DEC 99 cells were harvested, washed in PBS and double-stained by mounting with cell monolayers down on microscope slides using a drop of a double vital staining solution. The solution was prepared by adding 100 μL of 1 mg/mL EB (Sigma) and 100 μL of 1 mg/mL AO (Sigma) to 10 mL of PBS. The investigations were performed on fluorescent microscope Leika DM 500B (Wetzlar, Germany) equipped with the FITC combination of filters.

Electron microscopy (EM). For transmission EM (TEM) infected cells with third passage on DEC 99 cells SuHV-1/A_2 (48 h p.i.) were fixed overnight at 4°C in 2.5% glutaraldehyde, postfixed for 2 h in 1% osmium tetraoxide, dehydrated in graded alcohol and embedded as pellet in Durcupan (Fluka). Yellow-gold ultrathin sections (70–100 nm thick) were prepared and mounted on 400 mesh copper grids and stained with saturated solution of uranyl acetate followed by lead citrate according to the standard technique. All electron microscopy examinations were carried out on an electron microscope JEOL 1200 EX at an accelerating voltage of 80 kV and an instrumental magnification of 2,000–75,000×. Electron micrographs were taken on Kodak EM Film 4489, 6½ x 9cm. The negatives were scanned directly on a HP Scanjet 4890 scanner at 600 dpi resolution using the "SCAN FILM" option.

Results

Double vital staining cytological assay. Normal, uninfected DEC 99 monolayers were composed of closely packed elongated or polygonal morphologically heterogeneous fibroblast like cells **(Fig.1, a)**. The nuclei exhibited dull-green fluorescence and contained 1-7 well-defined nucleoli. The cytoplasm was with dull-green fluorescence and contained large perinuclear accumulations of lysosomal granules. At the 6th h p.i. many cells were shrunken and rounded with enriched number of lysosomes or with lysosomal fusions **(Fig. 1, b)**. After 12 hours of infection the cells were rearranged and formed grape-like clusters where a lot of cells were with late apoptotic nuclei and the cells at periphery were with severe plasma membrane blebbing **(Fig. 1, c)**. At the 24th hour they were cell aggregates composed of intact dead cells **(Fig. 1, d)** and syncytia connected with cytoplasmic tails **(Fig. 1, e)**. At the 36th and 48th hours many nuclei were with dissolved nucleoli. The cytoplasm of many of these cells was stained homogeneously red due to the destruction of the lysosomes **(Fig. 1, f)**. Single viable cells and numerous syncytia were found on the 60th-72nd h p.i. Many syncytia were with decreased number or missing lysosomes **(Fig. 1, g)**. In some cells nuclei exhibited bright fine granular

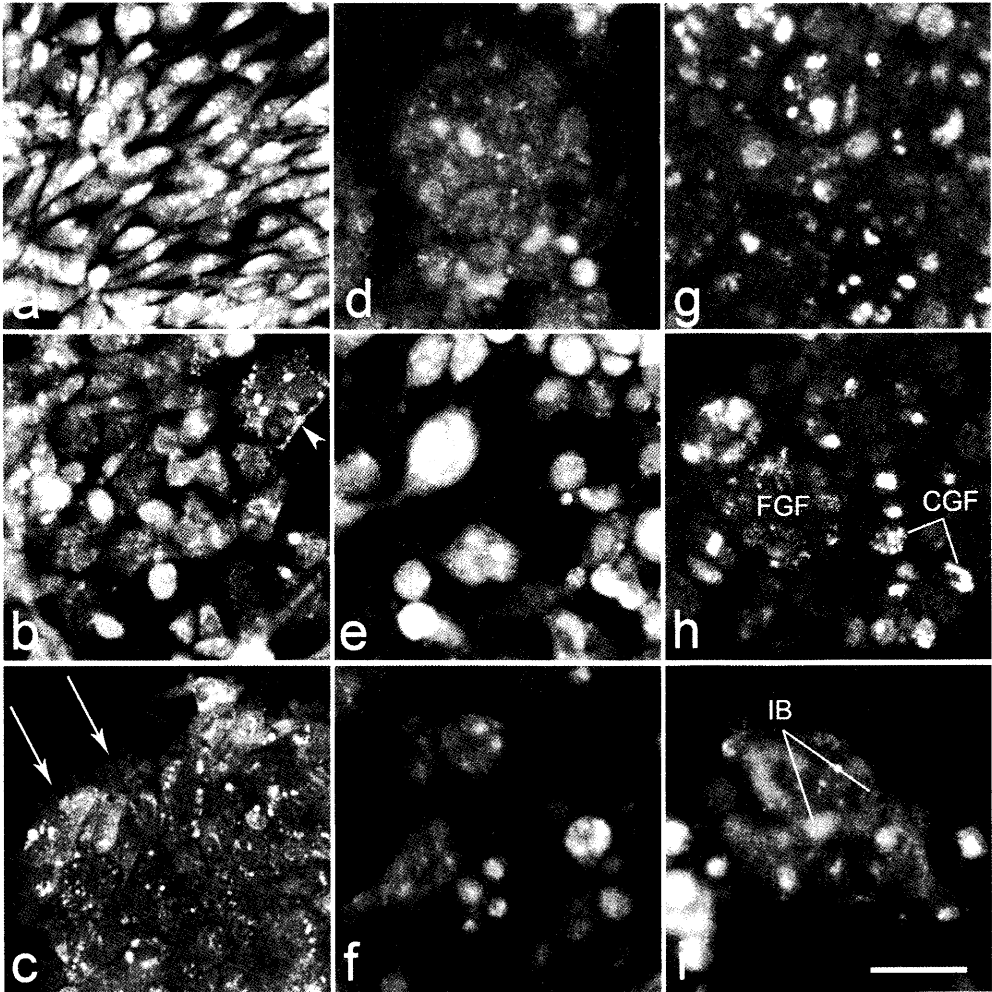

Fig. 1. Cell cultures from cell line DEC 99 infected with SuHV-1/A_2. Normal, uninfected DEC 99 at the 24th h p.i. (a). Shrunken and rounded cells with enriched number of lysosomes or with lysosomal fusions generating largest lysosomal granules (arrowhead) at 6th h p.i. (b). Cells with late apoptotic nuclei and cells with plasma membrane blebbing (arrows) in grape-like clusters at the 12th h p.i. (c). Cell aggregates composted of dead cells (d) and syncytia with cytoplasmic tails between (e) at the 24th h p.i. Cells with dissolved nucleoli and homogeneously red stained cytoplasm at the 36th h p.i. (f). At the 60-72 hour p.i. (g – i): Syncytia with lytic nucleoli and decreased number or missing lysosomes (g); Cells with bright fine granular yellow-green (FGF) or coarse granular (CGF) nuclear fluorescence (h) and nuclei with rearrangement of the chromatin in reticular pattern thus forming different intranuclear inclusion bodies (IB) (i). Vital acridine orange-ethidium bromide staining. Bar = 50 μm.

fluorescence, while other ones were with coarse granular fluorescence **(Fig. 1, h)**. There were also nuclei with rearrangement of the chromatin in reticular pattern thus forming different intranuclear inclusion bodies **(Fig. 1, i)**.

Electron microscopy. The ultrastructural features included condensation or margination of chromatin as well as dense irregular or granular aggregates in the nuclei.

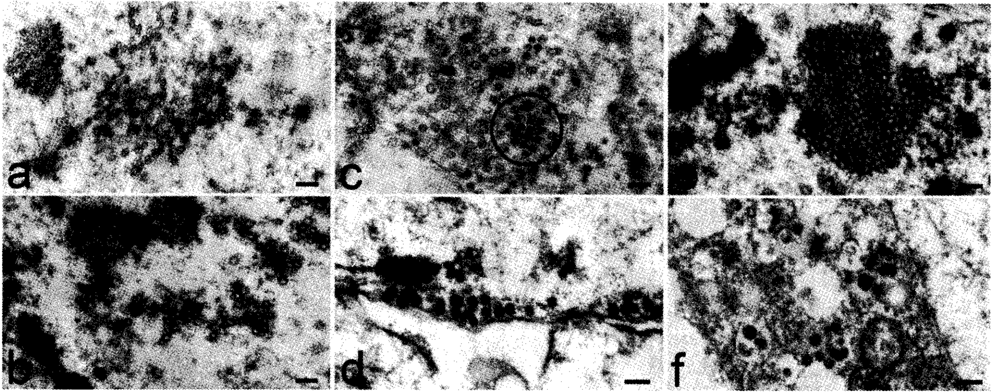

Fig. 2. Electron micrographs of ultrathin sections from DEC 99 cell cultures infected with SuHV-1/A_2 at the 48th p.i. SuHV-1/A_2 virions with different shape and different amount of electron-dense material in the nuclei (a – e). Predominantly naked empty (type A particles) capsids (b). Predominantly naked capsids with electron lucent cores (type B particles). The circle enclose naked capsids with electron dense (type C particles) cores (c). Completely assembled virions near the inner nuclear membrane (d). Viral particles arranged in paracrystalline arrays in the nucleus of an infected cell (e). Cytoplasmic viral aggregates enclosed in vacuoles (f). Uranyl acetate-lead citrate staining. Bars = 200 nm.

The observed SuHV-1/A_2 particles were naked empty capsids (type A particles) **(Fig. 2, a)** or capsids enclosing electron lucent (type B particles) **(Fig. 2, b)** or electron dense (type C particles) cores **(Fig. 2, c)**. The completely assembled virions were usually found located near the inner nuclear membrane or enclosed between inner and outer nuclear membranes **(Fig. 2, d)** Viral particles in various stages of assembly were also frequently found within the cytoplasm. They were associated with smooth membranous structures or accumulated in different in size and shapes aggregates. In addition, paracrystals composed of viral capsids enclosing a variety of electron dense material were frequently observed in the nuclei of DEC 99 cells **(Fig. 2, e)**. Some cytoplasmic viral aggregates were enclosed in irregularly shaped vacuoles **(Fig. 2, f)**.

Discussion

The results of the present studies are in agreement with those of other authors studying the SuHV-1 in primary or permanent cell lines from different origin [4, 9, 10]. By means of double staining of the DEC 99 cells with AO/EB have been revealed typical cytopathological changes characteristic for alphaherpesviruses [3, 10] including detachment, shrinking and rounding of the cells and formation of syncitia. The AO/EB technique revealed also enrichment of the number of lysosomes or lysosomal fusions at the 6th h p.i. and destruction of lysosomes after the 36th h p.i., but the interaction between SuHV-1 and lysosomes remain unclear. It is necessary to note here that the used technique have not been widely used in virological studies. It was developed to establish life and death cells on the basis of the cell membrane integrity and specific intercalation of the used fluorophores with the cell DNA [2]. However as it could be concluded from the present study and earlier [8] the AO/EB (or AO and propidium iodide) staining could be very useful for studying the cytopathological changes induced by viruses in cell cultures as it has been for SuHV-1/A_2 in DEC 99 cells and *Caprine herpesvirus 1* in MDBK cells.

According to the current knowledge A-, B-, and C-type capsids are usual findings during the herpesvirus assembly [1] and results in agreement with that were obtained in the present study. On the other hand herpesvirus virions with morphologically similar shape and structure were simultaneously established within the nucleus and the cytoplasm. This finding could be due to the DEC 99 cell culture properties which have been developed from a duck embryonic cell origin [6] but the data are insufficient. It is considerably different from that described by other scientists who studied the morphogenesis of the SuHV-1 using cell culture lines from mammalian origin [4]. However several successful passages of SuHV-1/A_2 performed in DEC 99 cells (unpublished data) undoubtedly indicate that despite the differences in the morphogenesis an infectious virus progeny is produced. Finally we are well aware of the problems inherent the difficulty to establish a dynamic process from the limited static electron micrographs and we are convinced that further investigations in this field have to be carried out to demonstrate more detailed peculiarities of the SuHV-1/A_2 propagation within DEC 99 cell line.

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