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ULTRASTRUCTURAL STUDIES ON THE REPLICATION OF HERPES SIMPLEX VIRUS IN PK AND XTC-2 CELLS

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Summary. — Ultrastructural changes showed the following characteristics of restricted replication of herpes simplex virus 1 (HSV 1) strains MA and HSZP in PK and XTC-2 cells: 1) minimal cytopathic changes in PK cells as compared to more pronounced alterations in XTC-2 cells; 2) formation of single nucleocapsids or their absence in the nuclei of PK cells infected with the HSZP strain; 3) lack of budding and envelopment and absence of reduplication of the nuclear membrane; 4) persistence of partially uncoated virions within the endocytic vacuoles in the cytoplasm of PK cells; and 5) formation of dense inclusion bodies in addition to the presence of defective virions in the cytoplasm of XTC-2 cells and vacuolation of their cytoplasmic membranes. The replication of HSV 1 in PK and XTC-2 cells seemed to be blocked at both early and late stages of virus replication. At low multiplicity of infection, no virus particles were formed.

Key words: herpes simplex virus; PK and XTC-2 cells; restrictive replication; electron microscopy

Introduction

The replication of herpes simplex virus type 1 (HSV 1) is restricted in PK (pig kidney) and XTC-2 (*Xenopus laevis* frog) cells. The limited synthesis of the intracellular and extracellular infectious virus in these cells is dose-dependent. No viral antigen was demonstrated by immunofluorescence in the nuclei of HSV 1-infected PK cells. In contrast to XTC-2 cells, which contained viral antigen in their nuclei and cytoplasm, in PK cells the antigen was seen only in the cytoplasm in close vicinity of the nuclear membrane (Szántó *et al.*, 1980). Certain morphological changes in the course of productive virus replication are shared by HSV 1-infected cells (Morgan *et al.*, 1959; Nii *et al.*, 1968; Schwartz and Roizman, 1969), while others may differ in various cell lines (Schlehofer *et al.*, 1979). Impaired maturation of HSV 1 and HSV 2 virions was described in Ama-1 cells, an l-amanitin resistant subline of CHO cells. In these cells, typical cytopathic changes develop; the synthesis of viral protein, glycoprotein and DNA occur even earlier than in the highly permissive HEp-2 cells, but later on the synthesis of viral macromolecules ceases in parallel with the inhibited maturation of virions (Campadelli-Fiume *et al.*, 1980).

The restricted replication of HSV1 in PK and XTC-2 cells, especially the absence of viral antigen in the nuclei of PK cells, led us to examine the ultrastructure of these cells in the course of the replication cycle.

Materials and Methods

Viruses and cells. The strains MA and HSZP of HSV 1 were passaged in rabbit lung (ZP) cells (Szántó *et al.*, 1972). Porcine kidney (PK) and *Xenopus laevis* frog (XTC-2) cells were used as described (Szántó *et al.*, 1980).

Infection of cells. PK cells were infected with either virus strain (MA and HSZP) at a multiplicity of 12.5 PFU/cell. XTC-2 cells were inoculated with 2.8 PFU of the HSZP strain or 9.1 PFU of the MA strain. After 60 min adsorption, the residual virus was removed by 3 washings with phosphate buffered saline (PBS) and cells were incubated at 36° C for 24, 48 72 hr in medium Epl (Szántó *et al.*, 1980).

Electron microscopy. Infected and control PK and XTC-2 cells were examined at intervals. The medium was removed, cells were scraped off by a rubber policeman, centrifuged for 10 min at 2000 rev/min and fixed in 2.5% glutaraldehyde in 0.2 mol/l sodium cacodylate buffer, pH 7.2 at 4° C. After short washing in the same buffer, the cells were post-fixeed in 1% OsO_4 in the same buffer for 60 min at room temperature. Fixed cells were dehydrated through an acetone series and embedded into Araldite CY 212 (Serva, Heidelberg). Ultrathin sections cut on LKB Ultratome III were stained with a 2% aqueous solution of uranyl acetate and leasd citrate according to Venable and Coggeshall (1965) and examined in a Philips EM 300 microscope at 80 kV.

Results

Ultrastructure of PK cells infected with HSV 1

In cells infected with the MA strain of HSV 1, only very few nucleocapsids were seen in the nuclei by 24 hr post infection (p. i.). The diameter of the nucleocapsids was 75 nm and they contained each a 35-nm core of a low electron density (Fig. 1). The nuclei of infected cells showed very slight chromatin margination; the high chromatin condensation at the nuclear margin, typical of productive infection, was not observed. The nucleolus showed stepwise segregation of its fibrilar components. Fine granular aggregates derived from the nucleolar material were found in the vicinity of the nucleolus. Neither reduplication of the nuclear membrane, nor envelopment of the nucleocapsids — inevitable for the egress of virus particles through the nuclear membrane — were observed. The cytoplasm revealed numerous vacuolar structures containing enveloped HSV particles. These had dense or occasionally ribbon-shaped cores and revealed an electron-dense shell filling up the space between the nucleocapsid and the envelope. Less frequently particles showing different stages of uncoating or naked nucleocapsids were seen in the cytoplasmic vacuoles. In addition to HSV particles, the membrane-bound vacuoles contained structures resembling residual cores of the disrupted virus particles of low electron density (Fig. 2).

The changes in MA-infected PK cells at 48 hr p. i. were similar to those just described. The nuclei contained only single nucleocapsids and no envelopment was observed. The cytoplasm, furthermore, revealed membrane-bound round vacuoles which contained HSV particles at different stages of disruption. The cytoplasm in the vicinity of such vacuoles contained a thick network of fine fibrils 7.5 nm in diameter, representing the cellular cytoskeleton. The ultrastructure of the MA-infected PK cells at 72 hr p. i. was nearly the same as at previous intervals: single nucleocapsids occurred in some nuclei but no envelopment at the nuclear membrane was seen. The cytoplasmic membrane-bound vacuoles contained disrupted HSV particles. Enveloped particles with electron-dense cores and containing dense material between the nucleocapsid and its envelope were attached to the surface membrane. At the cell surface also naked nucleocapsids with low density cores could be seen. The fibrilary structures of the cytoskeleton were oriented to the areas of attachment of enveloped particles at the surface membrane (Fig. 3).

PK cells infected with the HSZP strain revealed more pronounced ultrastructural changes in their nuclei. At 48-72 hr p. i. the nuclei were round, showing no reduplication of their membrane. The chromatin was condensed into a tiny band at the nuclear margin. The nucleoli showed "segregation" of their components, while the granular components prevailed and the fibrilar material was tightly condensed into ring-shaped structures or small clusters. The nucleoplasm contained several dense granular inclusions usually present in the early stage of productive virus replication. Nucleocapsids were not seen even by 72 hr p. i. (Fig. 4). The membrane-bound huge vacuoles found in the cytoplasm contained a considerable amount of disrupted HSV particles — either naked nucleocapsids or cores — but no enveloped HSV particles. The larger vacuoles consisted of smaller ones; the latter had an incomplete surface membrane. In the vicinity of vacuoles, channels of the slightly widened endoplasmic reticulum were seen (Fig. 5). The PK cells infected with the HSZP strain showed no signs of nucleocapsid envelopment and maturation at any of the intervals examined.

Ultrastructure of XTC-2 cells infected with HSV 1

XTC-2 cells could be examined only at 24 hr p. i., because the pronounced cytopathic changes at later intervals led to their detachment from the glass. The ultrastructure of the MA-infected XTC-2 cells resembled that of the productively infected cells. There occurred polykaryocytes with irregular or lobular nuclei, the nuclear chromatin was widely condensed at the margin, the nucleoplasm contained small clusters of fine granular material and in the highly electron-dense nucleoli the fibrilar component prevailed (Fig. 6). These extensive changes in the fine structure of XTC-2 cells — in other cells usually associated with productive replication of HSV — were not accompanied by formation of virus particles. The nuclei only rarely contained scarce naked nucleocapsids 75 nm in diameter and low density cores 35 nm in diameter. The nuclear membrane formed various infoldings, the space between its two layers was widened but no envelopment was found. The cytoplasm of XTC-2 cells was not filled with large vacuoles like those containing enveloped or partially disrupted particles in the HSV-infected PK cells. In contrary, the cytoplasm of HSV-infected XTC-2 cells contained dense inclusions, numerous empty membrane-bound vacuoles and widened cisternae in which naked nucleocapsids with electron-dense cores were seen in addition to enveloped particles revealing cores of a different degree of density (Fig. 7).

Pronounced changes were found in the intercellular space: numerous protrusions and lobulation of the surface membrane led to the formation of vacuolar gaps. The vacuolized areas of the surface membrane only rarely contained viral particles or naked nucleocapsids (Fig. 8).

XTC-2 cells infected with the HSZP strain revealed changes similar to those in cells infected with the MA strain. However, the former differed from the latter by less pronounced cytopathic changes and by a lower number of nucleocapsids in the nuclei. The extracellular space formed numerous vacuoles derived from the surface membranes of infected cells. In these, enveloped particles with dense cores or naked capsids were found (Fig. 9).

Discussion

As described by Szántó *et al.* (1980), replication of HSV 1 in PK and XTC-2 cells is very limited, the latter cells being more permissive for the virus. Alternatively, the replication of the MA strain is less limited than the replication of the HSZP strain. As demonstrated by electron microscopy, HSV particles are adsorbed to both PK and XTC-2 cells, are able to penetrate into endocytic vacuoles and cause certain ultrastructural changes in the infected cells although the yields of infectious virus are low in comparison to permissive cells.

Nucleocapsids were formed in the nuclei of PK cells infected with the MA strain but no typical changes associated with the release of mature virion into the cytoplasm, i. e. no envelopment and budding, did occur. The finding of enveloped virions within cytoplasmic vacuoles is, therefore, of special interest. These vacuoles contained virions showing different degrees of uncoating and disruption. The deenvelopment of virions in PK cells seemed to be impaired: the particles thus persisted within endocytic vacuoles. Probably only a few virions could release their DNA into the nucleus and were able to induce the synthesis of the nucleocapsid material. In addition, our findings suggest that the particles occurring in the extracellular space represent detached residual rather than newly formed virions. The nuclei of PK cells infected with the HSZP strain showed certain alterations associated with the infection, but they remained free of nucleocapsids even at 72 hr p. i. Their cytoplasm showed huge vacuoles containing viral particles at different stages of disruption.

The intracellular titres of HSV produced in PK cells at a high input multiplicity were relatively low (Szántó *et al.*, 1980). The low yield of infectious virus can be explained by formation of naked nucleocapsids as well as by the presence of particles which escaped disruption in cytoplasmic phagosomes. Possibly PK cells may not possess enzymes necessary for the disintegration of the penetrated HSV particles, which persist for up to 12 hr within phagocytic vacuoles showing different stages of disruption. Ama-1 cells infected with HSV 1 and HSV 2 also formed only a few nucleocapsids; they revealed neither budding and envelopment of virions nor reduplication of the nuclear membrane.

In XTC-2 cells infected with the MA strain prominent cytopathic changes developed relatively quickly. The nuclei only rarely contained nucleocapsids. Neither budding nor envelopment took place at the nuclear membrane. In contrary to PK cells, the cytoplasm of XTC-2 cells revealed no phagocytic vacuoles that would contain viral particles. Thus, the release of the viral DNA from virions seemed to be not impaired. The cytoplasm, however, revealed dense bodies and defective HSV particles. The origin of dense bodies could not be determined from ultrastructural observations. Numerous dense inclusion bodies were seen in the cytoplasm of rabbit embryonal lung cells infected with herpesviruses isolated from wild-living rodents in the course of a productive replication cycle (Čiampor *et al.*, 1981). The widespread formation of vacuoles from the cytoplasmic membranes neither was related to the occurrence of new nucleocapsids nor did these membranes participate in virus envelopment. XTC-2 cells infected with the HSZP strain contained even less nucleocapsids in their nuclei than the MA strain-infected cells. Summing up, both PK and XTC-2 cells infected with two different HSV 1 strains showed the following features: 1) low-grade cytopathic changes, less pronounced in PK than in XTC-2 cells; 2) formation of single nucleocapsids in the nuclei and even absence of any capsids in HSZP-infected PK cells; 3) the lack of budding, envelopment and virion release in addition to the absence of the reduplication of nuclear membranes; and 4) the persistence of engulfed virions within pinocytic vacuoles in the cytoplasm of PK cells and the formation of dense inclusions in addition to the presence of defective virions in the cytoplasm of XTC-2 cells.

Olshevsky and Becker (1970) and Tankersley (1964) showed that arginine deprivation inhibits migration of viral structural proteins to the nuclei of infected cells. Arginine deprivation induces changes similar to those described in Ama-1 cells (Campadelli-Fiume *et al.*, 1980) and PK and XTC-2 cells. The common features are the limited maturation of virions and the blockade of budding and envelopment. In the majority of nonpermissive cell systems described (Lowry *et al.* 1971; Docherty *et al.*, 1972; Adler *et al.*, 1978) the virus replication was restricted due to inhibition of the early stage of proteosynthesis, so that there was either no or limited viral DNA synthesis. The replication of the virus in nonpermissive PK and XTC-2 cells seemed to be blocked at two different steps: first, at the stage of the release of viral genetic material from penetrated virions, and second at the stage of virus envelopment and maturation. In addition, there were differences between the strains MA and HSZP in the extent of their limited replication. The morphological analysis of HSV replication in the two cell lines offers a guide for futher biochemical investigations on the nature of their nonpermissiveness.

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Explanation of Electron Micrographs (Plates VIII-XII):

Fig. 1. Nucleus of a PK cell 24 hr p. i. with the MA strain containing naked nucleocapsids. × 72 600.

Fig. 2. Endocytic vacuole in the cytoplasm of a PK cell 24 hr p. i. with the MA strain containing virions at different stages of disintegration. × 75 600.

Fig. 3. Defective virions on the surface membrane of a PK cell 72 hr p. i. with the MA strain. × 66 000.

Fig. 4. Nucleus of a PK cell 72 hr p. i. with the HSZP strain containing finely granular dense inclusions. × 20 000.

Fig. 5. Endocytic vacuoles in PK cells 48 hr p. i. with the HSZP strain containing virions at different stages of disintegration. × 66 000.

Fig. 6. Polykaryocyte in an XTC-2 cell 24 hr p. i. with the MA strain showing typical chromatin margination. × 7 260.

Fig. 7. Cytoplasm of XTC-2 cells 24 hr p. i. with the MA strain revealing dense inclusion bodies and incomplete virions. × 66 000.

Fig. 8. Vacuolized surface membranes of XTC-2 cells 24 hr p. i. with the MA strain. × 20 000.

Fig. 9. Intercellular space in XTC-2 cell culture 24 hr p. i. with the HSZP strain. Particles at different stages of disintegration. × 66 000.