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SHORT COMMUNICATION

CONSISTENT APPEARANCE OF MICROTUBULES IN CELLS PRODUCTIVELY INFECTED WITH VARIOUS STRAINS OF TYPE 2 HERPES SIMPLEX VIRUS[1]

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Electron microscopic examination showed microtubular structures in FL cells infected with all 18 strains of HSV-2 examined, but not in cells infected with 9 strains of HSV-1. These structures were also detected in other cultured cells (Vero and Earle's L cells) infected with HSV-2, and also in vivo in cells, such as neuronal cells of the spinal ganglia and liver cells, of one-day-old suckling mice (DDD strain) infected with this type of virus. Thus the microtubules were consistently detected in cells productively infected with HSV-2. No other herpesviruses so far examined produced microtubular structures such as those observed in HSV-2 infected cells.

Murphy et al. (1967) first reported filamentous structures in the nucleus of neuronal cells of mouse brain tissues infected with HSV. Subsequently, Couch and Nahmias (1969) observed the appearance of these structures, referred to as microtubules by these investigators, on infection by a particular serological type of HSV and found microtubules in the nucleus and cytoplasm of cells infected with HSV-2, but not in cells infected with HSV-1. Similar observations were made by Schwartz and Roizman (1969) and also by us (Nii and

1 Parts of this work were presented at the Annual Meeting of the Society of Japanese Virologists in Tokyo on November 5, 1973, and at the Symposium on Herpesviruses, held by the Czechoslovak Society for Microbiology of the Czechoslovak Academy of Sciences at Liblice Castle, Czechoslovakia on June 7, 1976.

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Katsume, 1971).

For the past ten years we have been making electron microscopic studies on cells infected in vivo and in vitro with various strains of two serological types of HSV to confirm whether the appearance of microtubules is related to HSV-2 infection. The present communication describes results of our electron microscopic observations related to this problem.

First we examined whether microtubules always appeared in FL cells infected with HSV-2. Eighteen strains of HSV-2, including both freshly isolated and laboratory strains, and 9 strains of HSV-1 were used. The serological types of the freshly isolated strains were determined by Dr. Yoshino, Tokyo University, and by one of the authors (S.N.). FL cell monolayers were infected with HSV-1 at an MOI of 1–10/cell and with HSV-2 at an MOI of 0.05–1/cell, and after adsorption for 1 h, the inocula were replaced by maintenance medium. Infected cells were harvested when CPE became extensive in the monolayers. Samples for electron microscopy were prepared as described previously (Nii and Yasuda, 1975a) and thin sections were examined in a Hitachi 11B or 12A electron microscope.

The microtubules were seen as filamentous or lattice structures depending on how they were sectioned (Couch and Nahmias, 1969; Ochi and Moriwaki, 1975; Oda and Mori, 1976). Occasionally filamentous structures resembling those of HSV-2 infected cells were found in normal FL cells, but lattice structures never were. Therefore, to avoid confusion, we tried to detect both filamentous and lattice structures in sections of cells infected with any given viral material. We could detect these structures in cells infected with all 18 strains of HSV-2 tested, but not in cells infected with

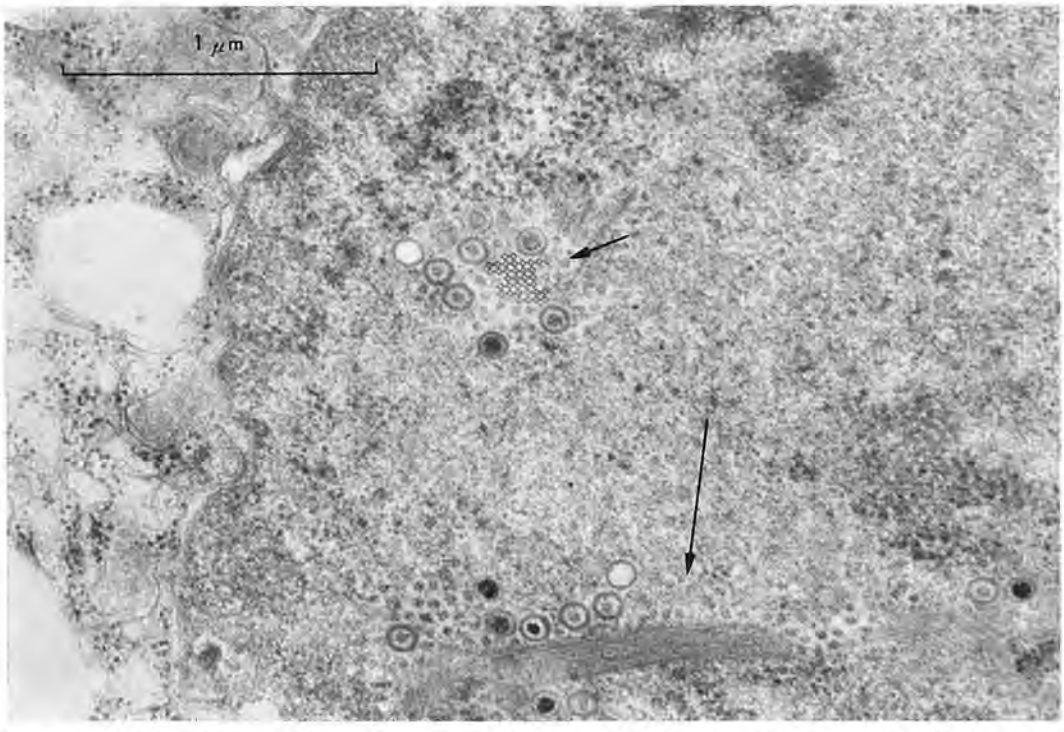

FIGURE 1. A lattice (short arrow) and filamentous structures (long arrow) in the nucleus of a liver cell of a mouse infected with the Ogiwara strain of HSV-2. Intranuclear virus particles of various shapes are also seen.

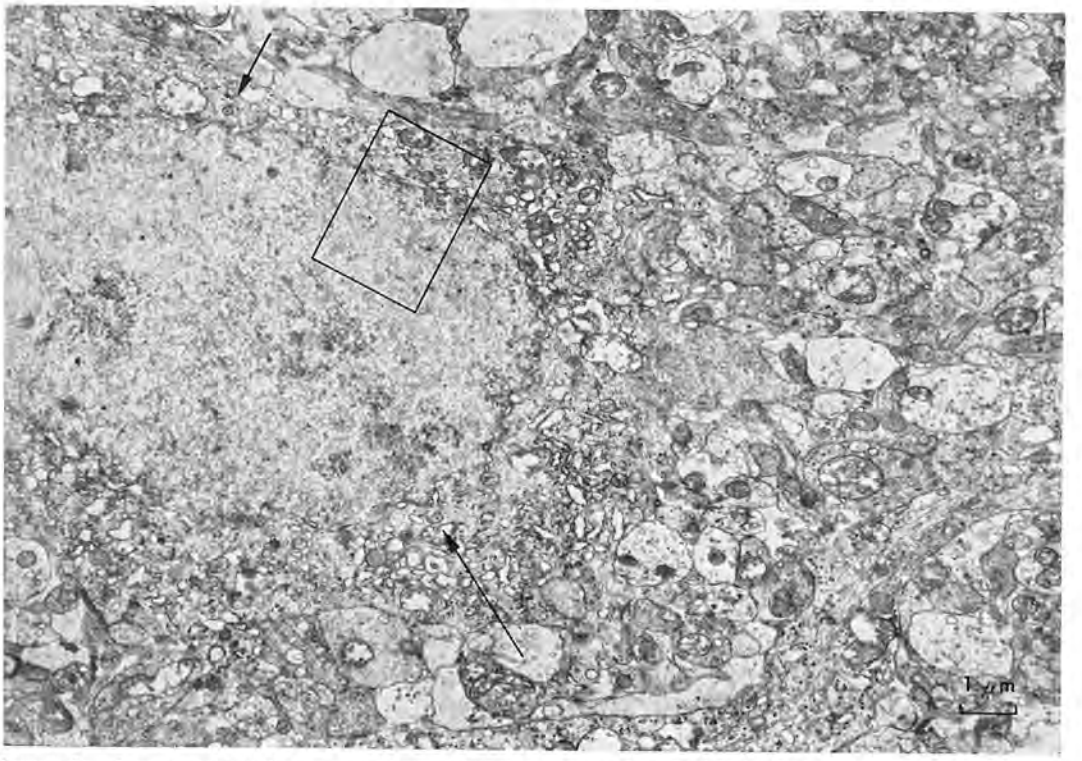

FIGURE 2. A neuronal cell from a spinal ganglion of a mouse infected with the Ogiwara strain of HSV-2. An enveloped particle (short arrow) and a particle in the process of envelopment (long arrow) are seen in the cytoplasm. The framed part of the nucleus is shown at higher magnification in Fig. 3.

9 strains of HSV-1. These findings demonstrate a close association between the appearance of microtubules of characteristic morphology and HSV-2 infection.

Next we examined cells infected with two other cultured cells, Vero and Earle's L cell lines. Both lines were infected with the UW 268 strain, used as a prototype strain of HSV-2, at an MOI of about 1 and then processed in the same way as FL cells. After infection, the characteristic microtubules were detected in both cell lines, suggesting that the structures appeared in all kinds of cells that permitted productive growth of HSV-2.

We also examined cells infected with this virus in vivo. One-day-old suckling mice of the inbred DDD strain were inoculated intraperitoneally with 0.002 ml of a viral sample of the Ogiwara strain having a titer of 5×10^3 PFU. Half the mice were sacrificed 3 days later and the rest 4 days later, and their liver, adrenals, spinal cord, spinal ganglia, skin, lungs and intestine were excised and examined by electron microscopy. So far we have found microtubules in infected cells of the liver and spinal ganglia. In the nucleus of the liver cell shown in Fig. 1, both lattice (short arrow) and filamentous (long arrow) structures are seen with adjacent virus particles and 30–35 nm particles. Figure 2 shows a neuronal cell of a spinal ganglion. The framed area of the nucleus and cytoplasm are shown at higher magnification in Fig. 3, where filamentous structures (arrows) and several intranuclear particles can be seen. Thus microtubules can also be seen in cells infected in

vivo with HSV-2.

For comparison, we examined cultured cells infected with six other herpesviruses; i.e., varicella-zoster virus, human cytomegalovirus, EB virus (EBV), Marek's disease virus (MDV), a herpesvirus of turkeys and herpesvirus cuniculi (HC). Cell suspensions in which a high percentage of the cells contained EBV particles were obtained by cultivation of P3HR-1 cells for 2 weeks at 33 C (Hinuma et al., 1967; Nagoya and Hinuma, 1972), while cells each infected with the other herpesviruses were collected when CPE was extensive. On electron microscopic observation, however, no microtubular structures were detectable such as those seen in HSV-2 infected cells. We did observe two kinds of tubular structures in HC infected cells (Nii and Yasuda, 1975b) and one kind of tubular or rod-shaped structure in MDV infected cells, but these were quite different in morphology from the microtubules of HSV-2 infected cells. Rather similar microtubules to those of HSV-2 infected cells were found in the cytoplasm of P3HR-1 cells, but they were not the same (Fig. 4): when closely packed, the microtubules in P3HR-1 cells occasionally had a hexagonal arrangement, i.e. each microtubule had six adjacent ones (Fig. 5), whereas in HSV-2 infected cells microtubules usually had four adjacent ones.

These electron microscopic observations showed that microtubules with a characteristic morphology consistently appeared in cultured cells infected with various strains of HSV-2 and that their appearance was not dependent on the kind of host cells in vivo or in vitro. Aurelian and Strandberg (1974) observed lamellae as well as microtubules in HEp-2 cells

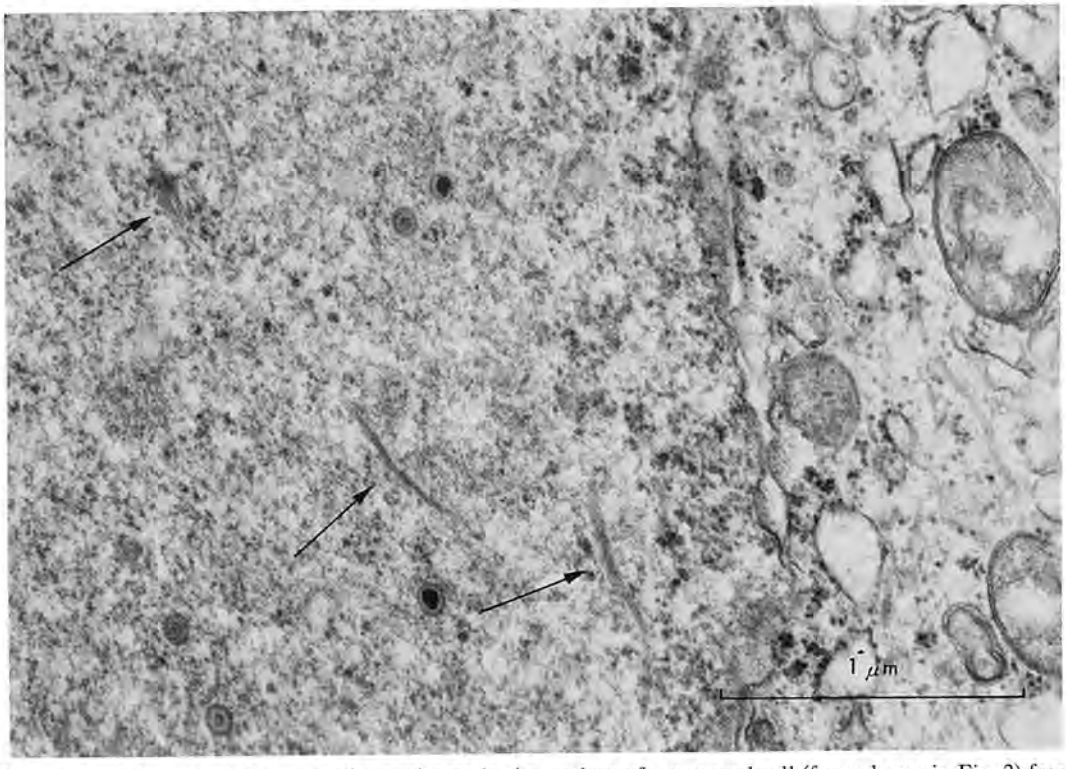

FIGURE 3. Filamentous structures (arrows) seen in the nucleus of a neuronal cell (framed area in Fig. 2) from the spinal ganglion of a mouse infected with HSV-2. Several intranuclear virus particles are also seen.

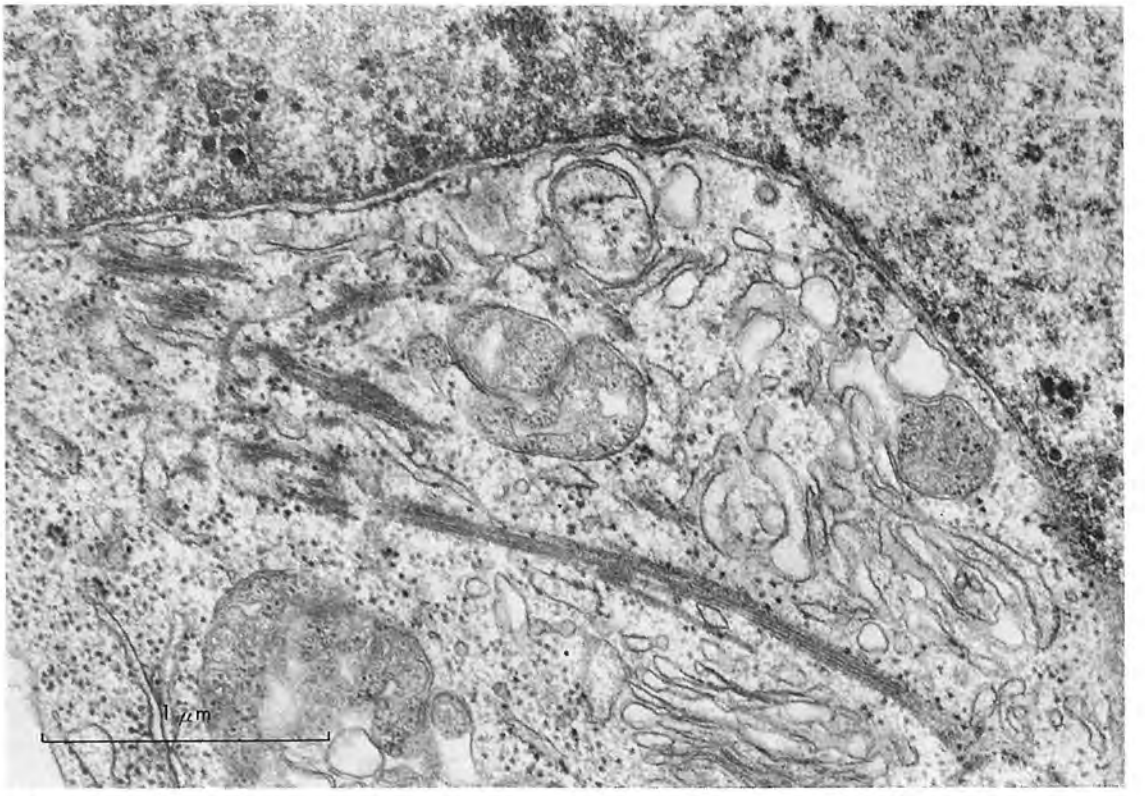

FIGURE 4. Filamentous structures (microtubules) seen in the cytoplasm of a P3HR-1 cell.

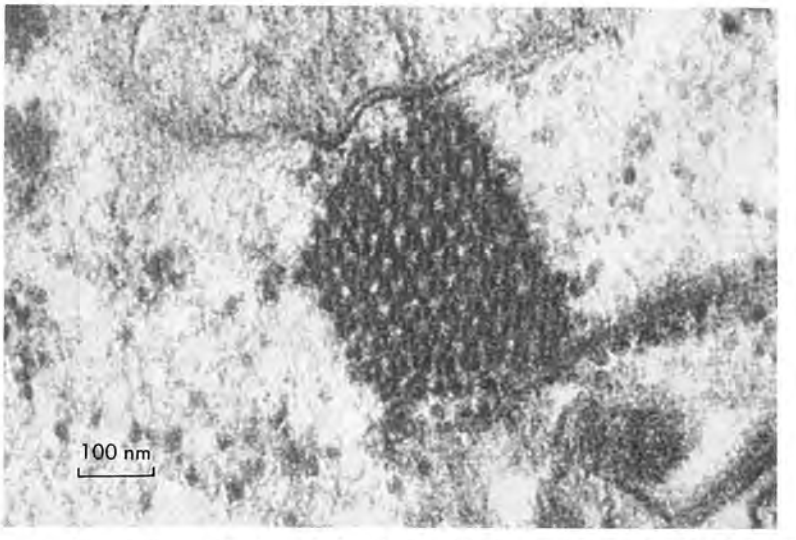

FIGURE 5. Hexagonal arrangement of microtubules observed in the cytoplasm of a P3HR-1 cell.

infected with one strain of HSV-2 isolated from cervical tumor cells. However, this is the only report of lamellae on HSV-2 infection and it is not contradictory to the close association between the appearance of microtubules and infection with this virus.

Based on the present studies with eight herpesviruses and also on numerous electron microscopic studies on various herpesviruses reported by many other investigators, we conclude that the microtubular structures first reported by Murphy et al. (1967) are characteristic of HSV-2 infection. The microtubules seen in the cytoplasm of P3HR-1 cells, although similar, are somewhat different from those of HSV-2 infected cells. The crystalline or lattice-like arrays of dense material found in the nuclei of cells infected with herpesvirus ateles (King et al., 1972) were also different from the microtubules.

Our extensive surveys with various virus strains and host cells indicate that these microtubules with a characteristic morphology are a useful biological marker of HSV-2 infection. In fact, when these microtubules are detected in cultured cells infected with a freshly isolated strain of some human herpesvirus or in tissues taken from lesions of patients, it can be concluded that the relevant viral agent is HSV-2. Thus, detection of these microtubules is significant from both pathological and virological points of view.

So far we have not encountered a mutant or recombinant virus deficient in production of the microtubules but with the antigenicity of HSV-2, or conversely a virus capable of producing them but with the antigenicity of HSV-1. But such virus strains might be produced artificially in vitro, and if so, the microtubules would be a useful tool for genetical studies on HSV.

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