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Filamentous Structures of Type 2 *Herpesvirus hominis* Infection of the Chorioallantoic Membrane

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In view of recent recognition of the existence of two *Herpesvirus hominis* (HVH) types with antigenic and biological differences, an electron microscopic study was undertaken of pocks produced on the choriallantoic membrane of embryonated eggs after infection with type 1 and type 2 HVH strains. Besides the typical morphological features of herpesvirus infection noted by several investigators, it was observed that type 2 HVH also produced microtubules measuring approximately 19 nm in both nucleus and cytoplasm. Although the nature of these filamentous structures is still unclear, consideration is given in this paper to the possibility that they may represent viral structural subunits, aberrant forms or neoantigens.

Two types of *Herpesvirus hominis* (HVH) with different antigenic and biological properties have recently been recognized (7, 20, 23, 29). Type 1 HVH is primarily isolated from nongenital sites and is transmitted nongenitally, whereas most type 2 HVH strains are recovered from genital sites and have a genital transmission (venereal or mother-to-newborn). More recently, the deoxyribonucleic acid (DNA) content of the two HVH types has also been found to be different (9).

One biological difference observed (21) was that the size of pocks and histological changes on the chorioallantoic membrane (CAM) differed according to the infecting HVH type. Thus, type 1 HVH strains produced small (<0.5-mm diameter) pocks and caused primarily an involvement of the ectodermal layer, but type 2 HVH strains produced larger pocks (>0.5-mm diameter) and involved subectodermal layers of the CAM as well. It was of interest to determine whether any differences could be demonstrated at the ultrastructural level in CAM pocks produced after infection with type 1 and type 2 HVH strains.

This report presents observations on the presence of filamentous structures (microtubules) in the nucleus and cytoplasm of type 2 HVH-infected CAM cells which could not be found in type 1 HVH-infected cells. The possible nature of these structures is discussed.

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MATERIALS AND METHODS

Virus. The source and passage history of strain "McIntyre-VR3," prototype of HVH type 1 in our laboratory, and strain "MS," prototype of HVH type 2, have been described previously (7).

Egg inoculation. Embryonated eggs, 10 to 12 days old, were inoculated with 0.1 ml of various dilutions of each virus type on the CAM using the false air sac technique (21). Eggs were incubated at 37 to 35 C for 3 days.

Electron microscopy. Membranes containing 20 to 50 pocks were first fixed in situ with cold 3% glutaraldehyde in phosphate buffer (*p*H 7.3) for 1 to 2 min. Pock-containing membranes were then excised and fixed for 2 hr in cold buffered glutaraldehyde. After washing the membranes with three 10-min changes of cold phosphate buffer, the tissues were postfixed for an additional hour with 1% phosphate-buffered osmium tetroxide. Several pocks were carefully dissected with a dissecting microscope, leaving only a thin border of intact membrane around the pock. Individual pocks were then dehydrated through a graded series of alcohol and carried through three changes of propylene oxide, after which they were embedded in Araldite 502 (27). Silver sections were made with a Porter-Blum MT-2 ultramicrotome, then mounted on uncoated copper grids, stained with lead citrate (13), and viewed with either a Zeiss 9A or Hitachi HS-8 electron microscope.

RESULTS

The typical morphological features of HVH infection reported by several investigators (6, 16, 17, 31) in CAM or tissue culture cells were observed in CAM cells infected with both of the

type 1 or type 2 HVH strains used. These features included the margination of chromatin, the 100-nm particles with single-limiting membranes in the nucleus, and the enveloped viral particles in the cytoplasm (Fig. 1). Also corresponding to observations of other investigators (6, 17, 32) were the marked thickening and reduplication of the nuclear membranes, with virus occasionally found within vesicles close to the nuclear membrane (Fig. 2).

The most striking findings were the filaments (microtubules) in cells infected with the type 2 HVH strain, which were not observed in type 1 HVH-infected cells. The microtubules appeared in longitudinal section as parallel filaments about 15 nm apart (Fig. 3). In transverse section the microtubules appeared in lattice formation; the individual round units were 19 to 22 nm in diameter with a center-to-center distance of 28 nm (Fig. 2). Occasionally, both types of configurations could be found in the same cell. Also noted in some cells was the proximity of virus particles to the microtubules, whereas in other cells (Fig. 2) the microtubules appeared to be well separated from the viral particles. In rare cases microtubules were encountered in the cytoplasm. Nuclear filaments were found in less than 10% of the type 2 HVH-infected cells. The cells containing filaments appeared to be localized in groups. At these local foci about 90% of the infected cells contained filaments.

DISCUSSION

Similar filamentous structures as those reported in this paper have been observed in independent studies on mouse brain infection with the "Rex" HVH strain by Murphy, Harrison, and Whitfield (19). These structures, however, were observed only in the nucleus by these investigators. Of particular interest is that the "Rex" strain, isolated from vesicles on the hand, was later subjected to serological typing and found to belong to HVH type 2. Thus, in two different hosts and with two different HVH type 2 strains, simi-

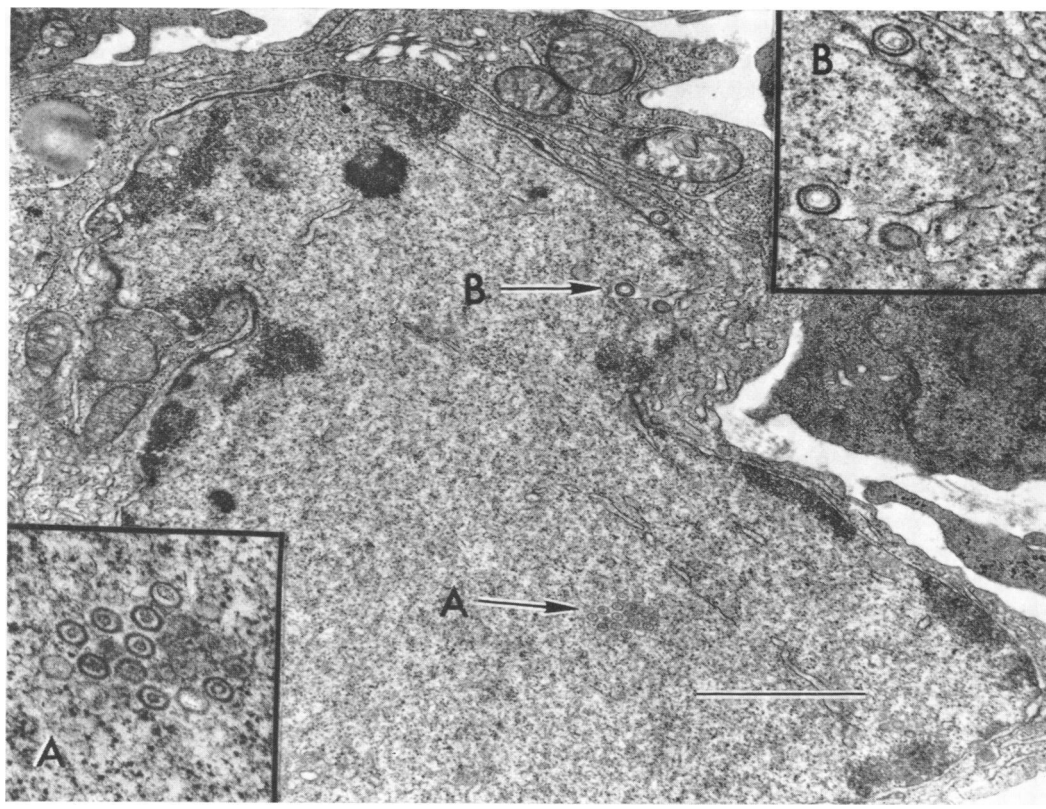

FIG. 1. *CAM cell infected with HVH type 1 (McIntyre-VR3) virus. Note the absence of filaments (× 4,500). The inset of area A in the nucleus shows a cluster of nuclear particles with only one membrane around the inner viral capsid (×45,000). Inset of area B shows the cytoplasmic virus particles having two enveloping membranes (×33,000). The scale in the lower right-hand corner represents 5μm.*

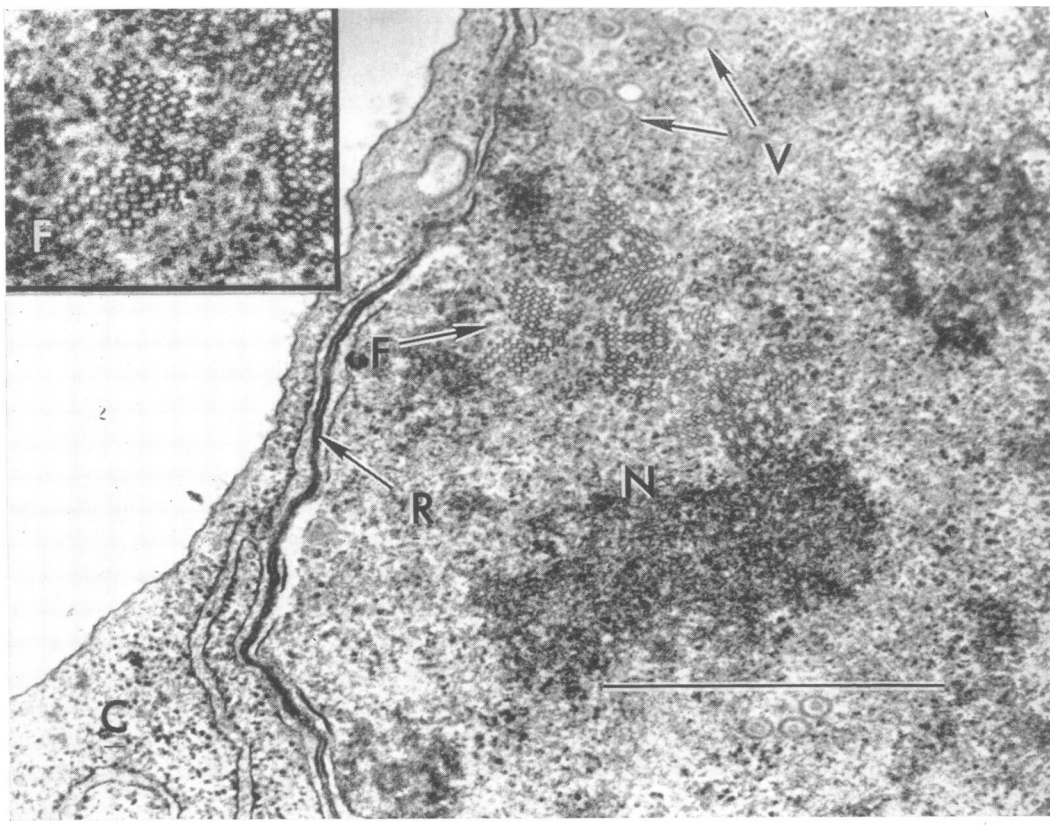

FIG. 2. *CAM cell infected with HVH type 2 (MS) strain of virus. The nuclear region (N) contains both viral particles measuring 100 nm (V) and cross sections of the nuclear filaments (F). The filaments have a cross-sectional diameter of about 19 nm. Also, reduplication (R) of the nuclear envelope is clearly evident. A portion of the cytoplasm is also seen (C). Main picture × 45,000; inset × 81,000. Line in lower right-hand corner equals 1 μm.*

lar filamentous structures have been observed. Other investigators (6, 16, 17, 31) have not observed these structures using nongenital HVH strains, which have almost invariably been found to belong to type 1 HVH (7, 20, 29). In particular, filaments have not been observed by Siegert and Falke (31) with the same Lennette strain (type 1 HVH) as was used in this study. The filaments Morgan et al. described (18), found with the "JM" strain of HVH (type unknown) and the filaments and other forms observed by Chitwood and Bracken (4) in a strain of HVH growing in cells treated with *p*-fluorophenylalanine, do not resemble the microtubular structures found by Murphy et al. (19) and those described in this communication.

Findings of filaments in the cytoplasm would suggest that these may be protein in nature. Roizman (*in press*), in reviewing the biosynthesis of the whole virus, has proposed that protein, first synthesized in the cytoplasm, is then transported into the cell nucleus to coat the DNA nucleoid. Our findings of the occasional close proximity of filaments to the virus, also observed by Murphy et al. (19), would lend some support to this possibility. Lunger (14) has also suggested that the filaments observed in the cytoplasm of Lucké frog renal adenocarcinoma cells may represent viral capsid material.

Whether filamentous structures observed with a variety of other animal viruses are actually morphologically identical or have a similar mechanism of formation is difficult to ascertain. Filamentous structures have been noted with several poxviruses (18, 24, 30), Shope fibroma virus (2), mouse cytomegalovirus (28), vesicular stomatitis virus (25), rabies virus (15), polyoma and Killam rat viruses (5, 10), and porcine and human adenoviruses (3, 12, 18). Filamentous structures have also been observed with several other herpesviruses or herpesvirus-like agents: equine abortion virus (1, 26), avian infectious laryngotracheitis virus (33), and Lucké frog renal adenocarcinoma agent (8, 14, 32, 34).

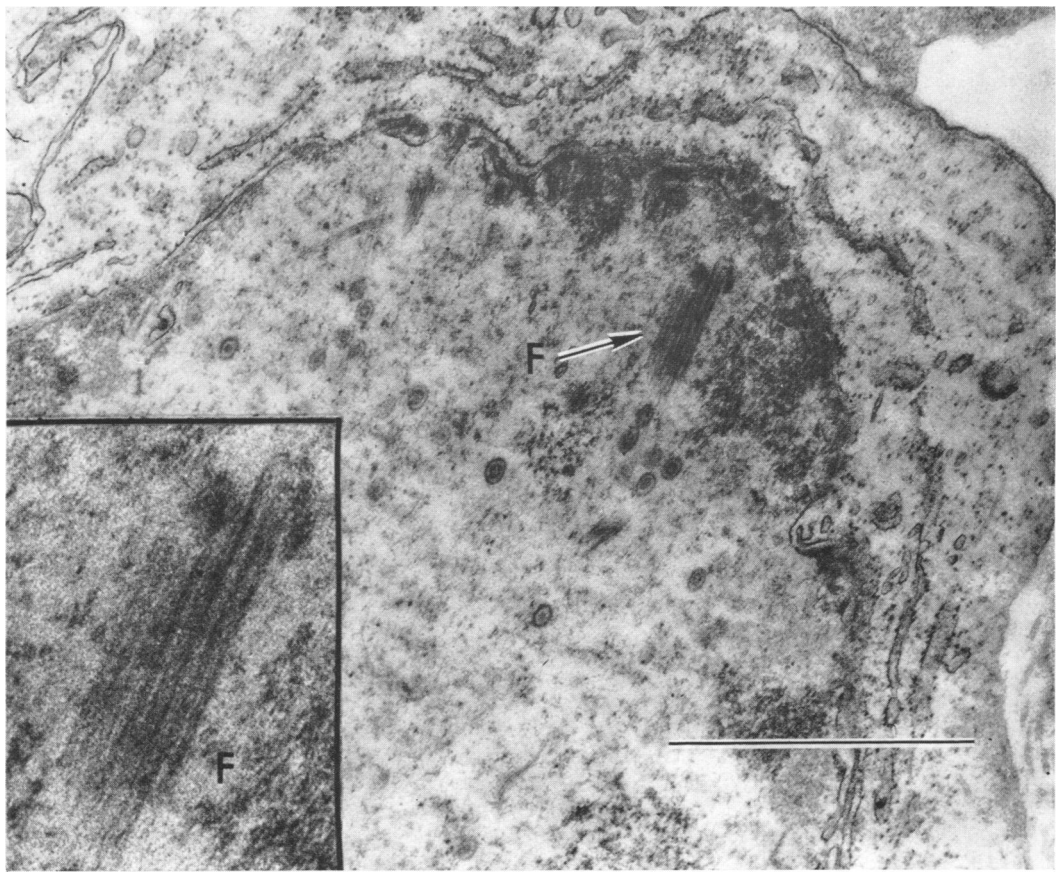

FIG. 3. *CAM cell infected with type 2 HVH (MS strain). Note the longitudinal sections of the nuclear filaments (F). Many viral particles are also present. Main picture × 40,000; inset × 110,400. Index in lower right-hand corner equals 1 μm.*

Of possible relevance to ongoing studies attempting to establish an etiological association between type 2 HVH and cervical neoplasia (11, 22) are two recent reports on the association of tubular or filamentous structures with cancer. Stackpole and Mizell (32) concluded from their studies with frog renal adenocarcinoma virus that the tubular structures they observed represented aberrant herpes-type virus-related structures which are regularly produced during virus replication within tumor cells. Kalnins et al. (12) found that ferritin-labeled T antibodies reacted specifically with filamentous structures in nuclei of hamster cells infected in vitro with tumorigenic adenovirus 12 and in the cytoplasm of adenovirus-induced neoplastic cells from Syrian hamsters. These authors suggested that such filamentous structures could be considered as "viral footprints" which in neoplastic cells may serve as indicators of viral etiology.

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