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Virus-like particles in MDCK cells persistently infected with Borna disease virus

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Abstract

A line of Madin Darby canine kidney (MDCK) cells persistently infected with Borna disease virus was examined by electron microscopy. Thin sections revealed the presence of intracytoplasmic virus-like particles ranging from 50–100 nm in diameter. Nuclei of the infected cells exhibited accumulation of electron-dense granular structures 15–18 nm in diameter. The intracytoplasmic particles were roughly spherical with a limiting membrane, suggesting the presence of a lipid-containing envelope. The internal structure consisted of strand-like material which in some cases was condensed underneath the envelope. The possible relationship of these particles to Borna disease virions is discussed.

Key words: Madin Darby canine kidney cell; Borna disease

Borna disease virus (BDV) is the etiological agent of a neurological disease manifested by profound behavioral abnormalities, accumulation of disease-specific antigens on limbic system neurons and, frequently, the presence of inflammatory cell infiltrates (Lipkin et al., 1992; Ludwig et al., 1988; Richt et al., 1992). Naturally occurring infections with BDV have been confirmed in horses, sheep, cats and ostriches (Ludwig et al., 1988; Lundgren et al., 1993; Malkinson et al., 1993), and the disease can be experimentally transmitted to a wide range of animal species from birds and rodents to non-human primates. There is a variable period of incubation, as well as diverse pathological manifestations depending on the species, immune status and age of the host, as well as the particular virus strain used for

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the infection (Ludwig et al., 1988; Richt et al., 1992). Furthermore, serological data support an association of BDV with specific psychiatric disorders, providing further impetus for the study of this neurotropic infectious agent (Amsterdam et al., 1985; Bode et al., 1992, 1988, 1994; Fu et al., 1993; Rott et al., 1991; VandeWoude et al., 1990).

Although BDV remains unclassified, the recent cloning and sequencing of the BDV genome (Cubitt et al., 1994) has revealed a genomic organization similar to that of other members of the Mononegavirales order characterized by having a negative non-segmented single-stranded (NNS) RNA genome. However, it has also been shown that in contrast to the other known NNS-RNA animal viruses, BDV replication and transcription occur in the nuclei of infected cells (Cubitt and de la Torre, 1994). BDV antigens are detected in both the cytoplasm and the nuclei of infected cells, and intranuclear antigens are found in association with Joest-Degen inclusion bodies (Ludwig et al., 1988). However, little information has been obtained on the structural properties of the virus particle or the process of virus assembly in infected cells. Evidence has been obtained that the virus particle possesses a lipid-containing envelope (Ludwig et al., 1988).

BDV is highly neurotropic, with cells and tissues of non-neuronal origin exhibiting low susceptibility to BDV infection. However, by cocultivation with BDV-infected brain cells, non-neuronal cells become persistently infected with BDV, and persistently infected MDCK cell lines have been established (Ludwig et al., 1988; Herzog and Rott, 1980). Because this cell line has been employed extensively for electron microscopic studies of the maturation of enveloped viruses (e.g., Rodriguez-Boulan and Sabatini, 1978; Roth et al., 1979; Rindler et al., 1984), it was of interest to examine thin sections of BDV-infected cells in order to determine the possible presence of particles that could represent BDV virions.

MBV cells were derived by establishing a BDV persistent infection in the canine kidney epithelial cell line MDCK (ATCC CCL 24). MDCK MBV cells were grown in Dulbecco's modified Eagle's medium containing penicillin, streptomycin, 1% glutamine and 10% heat-activated fetal bovine serum (FBS). BDV stock used for the establishment of MBV cell lines was prepared as described (Cubitt and de la Torre, 1994). Infection of MDCK cells was conducted as described (Cubitt and de la Torre, 1994) using 0.5 focus forming units (FFU) per cell. The samples studied included 6 MDCK samples taken over a 3-month period. All infected samples were taken from a persistently infected cell line between passages 20 and 25. Three blocks were embedded from each cell passage studied and sectioned for microscopy.

For electron microscopy, confluent monolayers of MDCK and MBV cells were washed twice with PBS and fixed with 1% glutaraldehyde (Grade 1, Sigma 5882) in PBS, then washed with PBS, post-fixed with OsO_4, dehydrated in a graded ethanol series and embedded in an epoxy resin mixture. Tannic acid enhanced staining was employed on all samples. Thin sections were stained with uranyl acetate and lead citrate, and examined in a Philips 410 electron microscope. The MBV cells used for these studies corresponded to passages 20 to 25 after the establishment of the persistent infections.

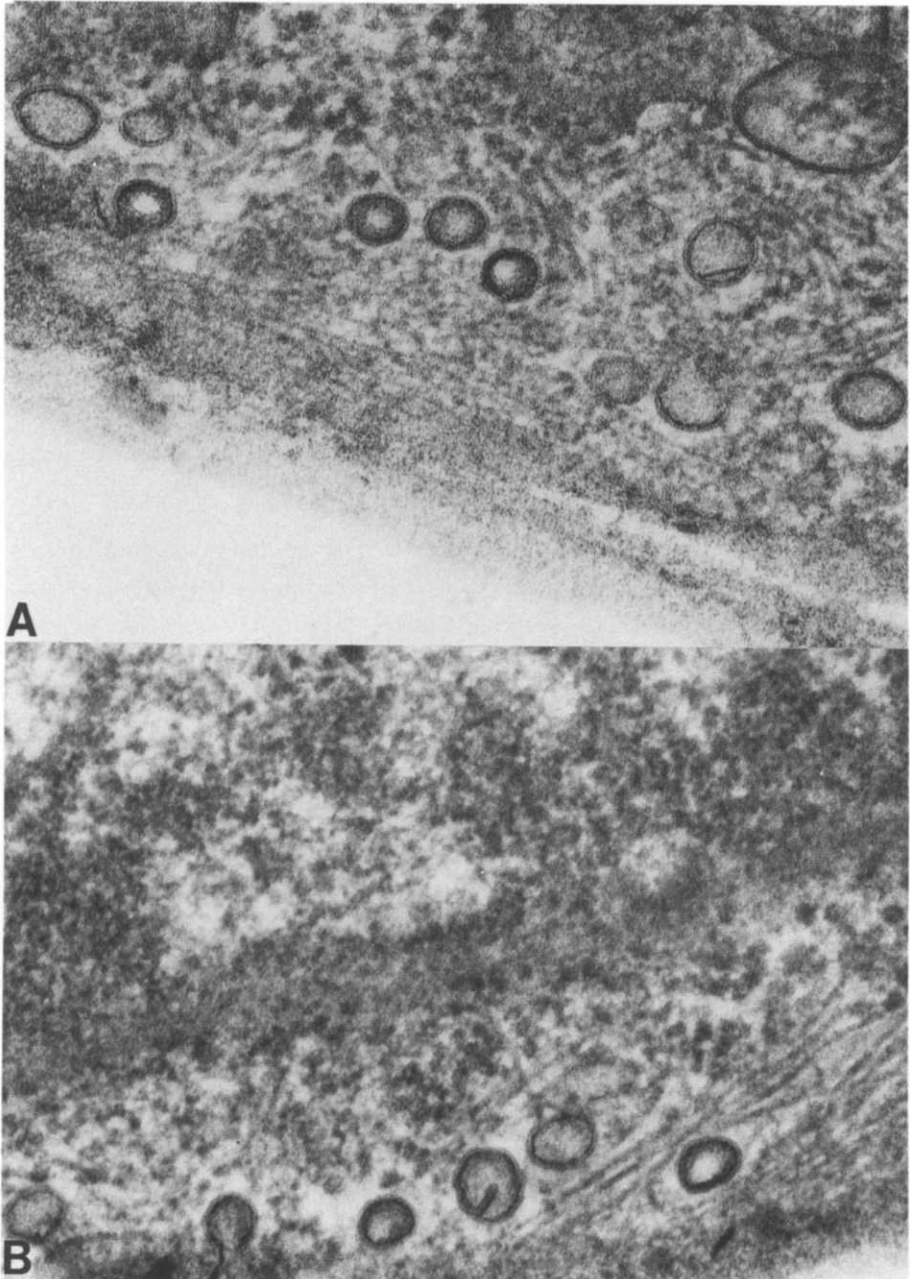

Fig. 1. Thin sections of MDCK cells persistently infected with BDV. A: Intracytoplasmic virus-like particles 50–100 nm in diameter in proximity to the cell surface. B: Similar particles, some of which are in proximity to microtubule-like structures. N, nucleus. Magnification: ×110,000.

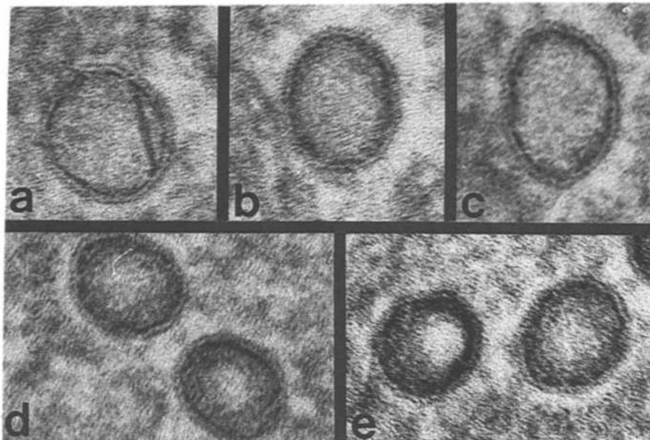

Fig. 2. Selected virus-like particles at higher magnification. Some of the particles (a,b,c) have a fine strand-like internal structure dispersed homogeneously within the outer envelope. Other particles (d,e) show condensed, strand-like material closely associated with the outer envelope. Magnification: ×210,000.

As shown in Fig. 1, a striking feature of BDV-infected MDCK cells was the presence of enveloped particles varying from about 50–100 nm in diameter. The particles were found in groups free in the cytoplasm, close to the plasma membrane. Most of the particles were roughly spherical, although some were oval-shaped. At higher magnification (Fig. 2), the particles can be seen to have a limiting outer membrane with an electron dense inner leaflet. Some particles show a relatively homogeneous internal structure which sometimes has a strand-like appearance, whereas in other particles the internal components are apparently condensed in close apposition to the outer membrane leaving an electron-lucent central region. The particles were found in proximity to the basolateral plasma membrane, but showed no association with cisternae of the endoplasmic reticulum, the Golgi complex, or other intracytoplasmic membranes. The assembly process or site at which the particles are assembled was not observed. Some particles (e.g., one particle in Fig. 1A) appeared to have an incompletely formed envelope consisting of a crescent-like structure.

It has recently been documented that replication and transcription of BDV occur in the nuclei of infected cells (Cubitt and de la Torre, 1994). Immunofluorescence studies have also revealed granular fluorescence throughout the nuclei of BDV-infected cells, whereas the plasma membrane was not reported to be stained by specific antisera (Ludwig et al., 1993). Within the nuclei of BDV-infected cells, accumulations of electron-dense granules about 15–18 nm in diameter were observed (Fig. 3). Although the sizes of the individual granules could not be clearly differentiated from similar granules seen in uninfected cells, the finding of large clusters of such granules was characteristic of BDV-infected cells, and was not seen in uninfected cells.

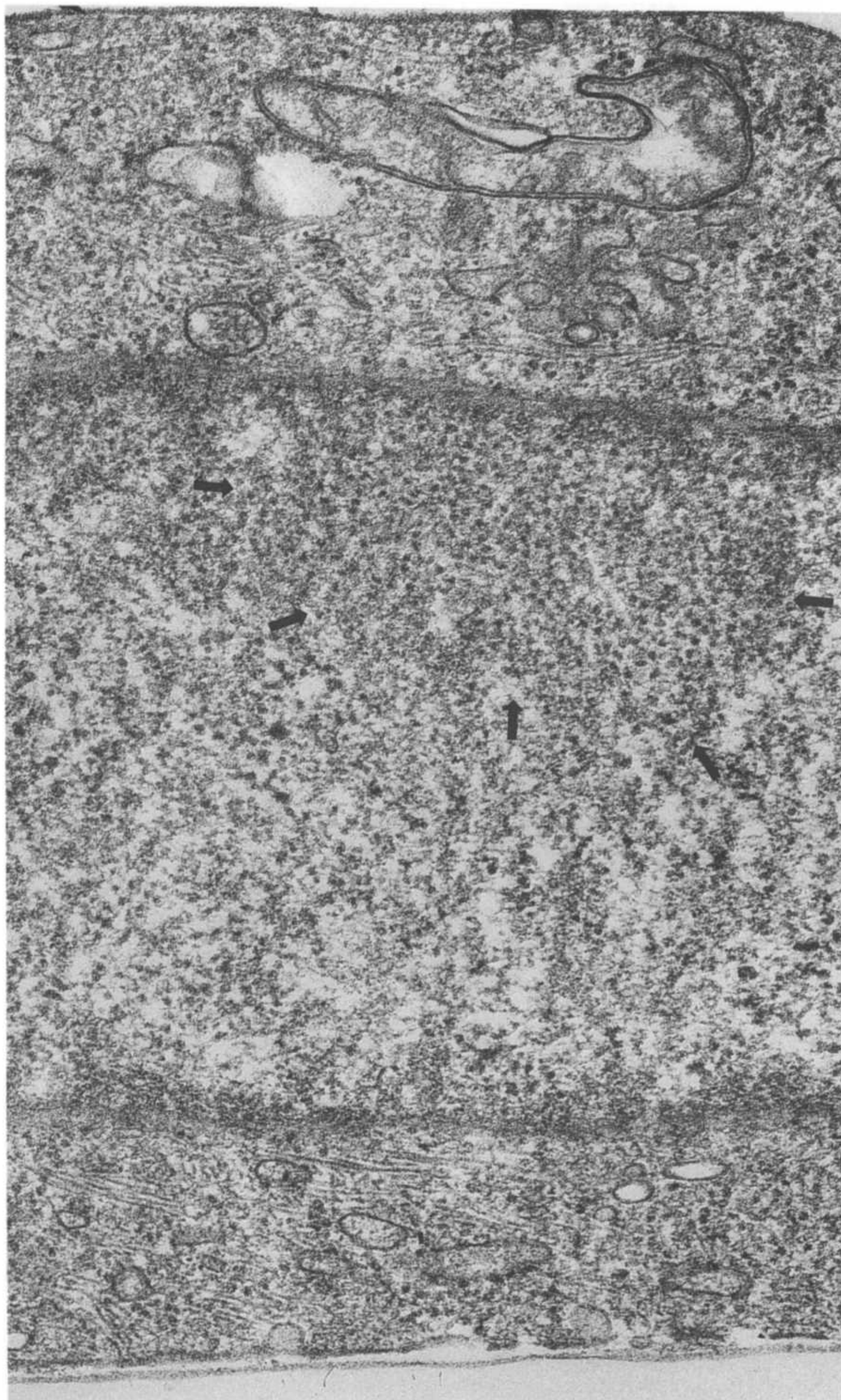

Fig. 3. Accumulation of electron-dense granules (arrows) in the nucleus of a persistently-infected MDCK cell. Magnification: ×13,580.

In previous studies of negatively stained preparations of virus-like particles released from BDV-infected GMK cells, spherical particles 50–60 nm in diameter were reported which exhibited an outer layer of spike-like projections (Ludwig and Becht, 1977). The particles observed in the present study may be somewhat similar, although the size range was broader and a significant fraction of particles was observed with a larger size. Because virus release occurs at very low levels in the persistently-infected MDCK cells, it has not been possible to compare the morphology of the sectioned particles with negatively stained preparations of released particles. Further, since only a low level of infectious virus is obtained in this system, the possibility exists that at least some of the virus-like particles observed could represent non-infectious, defective particles.

Many enveloped viruses express virus-specific antigens at the plasma membrane and assemble virions by budding at the cell surface. Enveloped viruses of other families, such as bunyaviruses or coronaviruses, are assembled by budding into cisternae of the endoplasmic reticulum or the Golgi complex (Pettersson, 1991). In all of the above instances, the outer surfaces of the viral envelopes are covered with a layer of virus-coded glycoproteins which are synthesized as transmembrane proteins in the endoplasmic reticulum. The glycosylated external domain is found in the lumen of the ER and/or other membranes of the exocytic pathway, and/or on the outer surface of the plasma membranes. In contrast to such assembly processes, the envelopes of the particles observed in BDV-infected cells are in direct contact with the cytoplasmic matrix, a topology which would not be expected to be compatible with the presence of a layer of external surface glycoproteins. Thus, it is surprising to find particles with the morphology of enveloped virions within the cytosol, and their assembly process remains uncertain. It has, however, been reported that rabies virus assembly can sometimes occur free in the cytoplasm (Hummeler and Tomassini, 1973). It is possible that the particles we observed represent an unusual type of endocytic vesicle; however, vesicles of similar appearance have not been observed in uninfected MDCK cells. We did not observe release of particles into the medium, which is consistent with the finding that little or no infectious virus is released into culture medium from the persistently infected MDCK cells. If the enveloped particles observed in the present study represent BDV virions, their presence within the cytoplasm suggests an unusual assembly process which may not involve expression of viral antigens in the plasma membrane. It will be of interest to obtain further information on the structural components of BDV particles and to compare their assembly process with the other members of the order Mononegavirales. Such studies will presumably require a cell culture system which produces higher yields of infectious virus particles.

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