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ELECTRON-MICROSCOPIC APPEARANCE OF THE DA VIRUS, A DEMYELINATING MURINE VIRUS

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SUMMARY

The DA virus is a neurotropic murine virus which can induce acute encephalomyelitis in suckling mice and a chronic myelopathy in weanlings. The agent has been attenuated by serial passage in baby hamster kidney (BHK-21) cells. When attenuated virus is inoculated in 8-week-old C3HeJ mice a myelopathy of delayed onset with prominent demyelination of lateral and anterior columns occurs. The DA virus is believed to be related to the Theiler murine encephalomyelitis (TME) viruses because of the similar clinical and pathological conditions which it causes, and because neutralization tests indicate shared antigens between it and GD7, a TME virus. This paper reports electron-microscopic studies of BHK-21 cells infected with DA virus. The cells were prepared 24 and 48 hr after inoculation. Cytopathic effects were observed and infected cells contained plaques consisting of numerous 25 nm virus particles in crystalline array. The virions were exclusively intracytoplasmic and were morphologically indistinguishable from human poliomyelitis virus. These observations appear to establish DA as a picorna virus, related to the TME virus group. The chronic myelopathy caused by DA may prove relevant to chronic demyelinating myelopathies in man, such as multiple sclerosis, and also to amyotrophic lateral sclerosis.

INTRODUCTION

Theiler's murine encephalomyelitis viruses (TMEV) comprise a group of infec-

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tious agents which have common antigenic properties and can cause poliomyelitis in mice. The first of these viruses, Theiler’s original virus (TO), was isolated from a weanling uninoculated mouse which had developed flaccid paralysis of the hind limbs (Theiler 1937). The disease could be reproduced by injecting infected CNS material into the brains of healthy mice. TO virus is a neurotropic agent capable of destroying neurons in the brain and anterior horn cells of the spinal cord. Although TO virus can be isolated from the intestines of almost all weanling mice less than 6 weeks old, it is rarely found in mouse CNS and occasional CNS infection can, like human poliomyelitis, be described as a “biological accident” (Calisher and Rowe 1966).

The DA virus was recovered in 1948 from a mouse in the Harvard colony which had shown spontaneous paralysis over a period of 2 months (Daniels, Pappenheimer and Richardson 1952). The agent was propagated by serial passage in mouse brain and induced an acute encephalomyelitis in suckling mice. Daniels et al. (1952) also observed a more chronic myelopathy which developed in weanling animals. The virus size was determined to be less than 100 nm by ultra-filtration techniques and DA virus was assumed to be a member of the picorna viruses of lower animals, a group which includes TMEV (Daniels et al. 1952). The DA virus was classified as a picorna virus on the basis of its clinical behavior and the pathologic lesions it caused. Recently, antigenic relationships have been demonstrated between DA and GDVII (Lehrich, Arnason and Hochberg 1976), a picorna virus which is a member of the TMEV group (Sturman and Tamm 1966). The DA virus was classified as a picorna virus on the basis of its clinical behavior and the pathologic lesions it caused. Recently, antigenic relationships have been demonstrated between DA and GDVII (Lehrich, Arnason and Hochberg 1976), a picorna virus which is a member of the TMEV group (Sturman and Tamm 1966). While serologic studies and its biologic activity supported the original claim that DA virus is a picorna virus, virus particles have not been visualized ultrastructurally. Failure to see them in electron microscopic examination of white matter undergoing demyelination was attributed to a very low content of infectious agent in the CNS of affected mice (Dal Canto and Lipton 1975). However, the DA virus has been tissue culture adapted (Lehrich et al. 1976) and the purpose of this paper is to show the ultrastructural appearance of DA virus grown in BHK-21 hamster fibroblasts.

MATERIALS AND METHODS

Viruses

The DA virus, in mouse brain suspension (fifth passage, prepared in 1952) was kindly supplied by Mrs Joan B. Daniels (Commonwealth of Massachusetts Virus Laboratories). The virus was adapted to BHK-21 hamster fibroblasts by means of serial passage. Cells were disrupted by three cycles of freezing and thawing on the fourteenth day after inoculation of virus material, and the tissue homogenates were then inoculated on to monolayers of BHK-21 cells. Cytopathic effects first appeared on the second passage in BHK-21 cells. Third passage virus was used to inoculate BHK-21 cell monolayers for electron-microscopic study.

Electron microscopy

Specimens were harvested for electron microscopy at 24 and 48 hr after inoculation with DA virus. Uninoculated BHK-21 fibroblasts were used as controls. The cells...
were removed from the surface of the tissue culture vessel by trypsinization and were recovered from the tissue culture medium by centrifugation in an International centrifuge operating at 150 × g. The cell pellets were allowed to fix in 2.5% phosphate-buffered glutaraldehyde for 4 hr at 4 °C. The glutaraldehyde was then decanted and the pellets retained in phosphate buffer prior to processing for electron microscopy. The cell pellets were postfixed for 45 min in Dalton’s chrome-osmium solution, dehydrated in a graded alcohol series, infiltrated with a propylene-oxide Epon mixture and embedded in Epon. One micron sections cut from Epon blocks were stained with methylene blue and examined by light microscopy. Ultrathin sections were cut from selected blocks, stained with uranyl acetate and lead citrate, and examined on a Siemens Elmiskop 101 operating at 80 kV. Viral size was determined from electron micrographs using the method described by Hatta (1976).

RESULTS

Light microscopy

Cytopathic effects were evident 24–48 hr after inoculation of BHK-21 cells. Light-microscopic examination of 1 μm plastic sections showed that many of the cells became rounded and that nucleoli became more prominent than in control BHK-21 cells. Many degenerating cells were seen at 24 hr but destruction of cells was much more prevalent in the samples taken at 48 hr.

Fig 1 BHK-21 fibroblast from tissue culture infected 24 hr previously. Numerous membranous and vesicular structures are present in the centrosphere region and the nucleus is displaced to the periphery × 9000.
Fig 2  Intracytoplasmic viral crystal composed of 24 nm electron-dense spherical particles in orthogonal array  40,000
Electron microscopy

Pathologic alterations in the infected tissue cultures were present both in cells which showed viral replication and in other cells without evident viral particles. Cytoplasmic changes consisted of proliferation of membranous structures and vesicles in the centrosphere region of the cell (Fig 1), dilated endoplasmic reticulum and numerous cytoplasmic filaments. Nuclear shape was often irregular, with margination of chromatin to the periphery and very prominent nucleoli. Viruses were identified in...
sections from every block examined, both in specimens collected 24 hr after inoculation and in those taken at 48 hr. Viruses were more frequently identified and degenerative changes more pronounced in the latter group of cells. Virus particles aggregated to form intracytoplasmic crystals which were generally large (Fig. 2) and exclusively intracytoplasmic. The viral crystals were composed of orthogonal arrays of spherical electron-dense particles, the average diameter of which was 25 nm. In many instances organelles such as mitochondria and other membrane-bounded structures were encompassed by the virus crystal (Fig. 3). Individual virions were separated from each other by a 2-4 nm electron-lucent space. Virus particles were present in apparently viable cells and also in degenerating cells which had markedly swollen endoplasmic reticulum and ruptured nuclear membranes and lacked recognizable mitochondria. In sections from tissue culture preparations harvested 48 hr after inoculation most of the cells had undergone necrosis, but viral crystals were prominent. Within these crystals individual virus particles showed varying electron density and viral arrays were irregular (Fig. 4), as distinct from the homogeneously electron-dense viral units in crystals from cells processed 24 hr after inoculation.

DISCUSSION

Electron-microscopic studies of tissue culture cells inoculated with DA virus show that the agent is a 25 nm spherical electron-dense particle morphologically re-
sembhng human polio virus as well as certain murine picorna viruses. The particles are arranged orthogonally in the cytoplasm forming crystalline lattices (Fig 2) and can thus be readily distinguished from other 25 nm electron-dense structures such as polyribosomes and glycogen granules. The International Committee on Enteroviruses (Melnick et al. 1963) has ruled that picorna virus size ranges from 15 to 30 nm. The estimated diameter of poliovirus type I cultured in HeLa cells is 26–28 nm (Dales, Eggers, Tamm and Palade 1965). Other enteroviruses which lie within the 15–30 nm size range are FA virus, also a member of the TMEV group, encephalomyocarditis (EMC) virus, mengovirus, and Coxsackie virus. These agents grow within the cytoplasmic matrix forming orthogonal crystalline arrays as does human poliomyelitis virus (Dales et al. 1965).

The changes associated with DA virus growth in BHK-21 cells included marked proliferation of smooth walled vesicles in the centrosphere region of the cytoplasm, intracytoplasmic filamentous accumulation, flattening and displacement of the nuclei and nucleolar changes. In the cells containing viruses the particles showed hexagonal packing, and viral crystals frequently formed around mitochondria and other cytoplasmic organelles. Each of these changes has been observed during growth of EMC virus and mengovirus in tissue cultured L cells. Both EMC and mengovirus are picorna viruses which measure 27–28 nm (Dales and Franklin 1962) and which cause encephalomyelitis in mice. Similar cytopathic changes have been observed in tissue cultured mouse liver cells infected with murine hepatitis virus (MHV) (David-Ferreira and Manaker 1965), a member of the larger coronavirus group.

In DA virus-infected cells harvested 48 hr after inoculation, viral particles showed varying electron density and the crystalline arrays became irregular. This change was described as “melting” by Mattern and Daniel (1965), who observed it in HeLa cell cultures infected with human poliovirus.

Dal Canto and Lipton (1975) have described an electron-microscopic study of the sequence of events leading to demyelination in DA virus-infected mice, but the virus was not seen in the infected central nervous system tissue. They attributed this to the low titers of virus within infected spinal cord (maximum titers never exceeded $10^5\text{LD}_{90}/g$ tissue). It is also conceivable that the virus may exert a cytocidal effect without new virus production. In studies of mengovirus, a picorna virus which shares some biologic properties with TMEV (Andrewes and Perreira 1972) tissue cultured macrophages were killed without virus production, whereas infected murine fibroblasts produced large quantities of virus (Buck, Granger, Taylor and Holland 1967). It is possible that the DA virus, like the mengovirus, has varying tropisms for cells of different tissues.

The long persistence of infective DA virus in mouse CNS (Lipton 1975, Lehrich et al. 1976) raises the possibility that demyelination may be due to a cytocidal effect of the DA virus on the oligodendrocyte. Virus replication within oligodendrocytes has been described in JHM virus encephalomyelitis (Lampert, Sims and Kniazeff 1973) and abnormalities of the myelin-plasma membrane connections of infected oligodendrocytes have been illustrated (Powell and Lampert 1975). Lipton and Dal Canto (1976) present evidence suggesting an immune basis for the late appearing demyelinat-
ing lesions in DA virus infected mice. They report that immunosuppression prevents the onset of demyelination. In their view local persistence of antigen for a long period of time may be a critical factor in inducing an immune reaction. Striking inflammatory changes have been seen in mouse spinal cords 9 months after DA virus inoculation (Salomon 1976) and further ultrastructural studies are required to determine whether the delayed lesions in white matter are due solely to an immune mechanism or to a direct cytocidal effect.

Tissue culture adapted DA virus is a slow virus capable of causing a chronic demyelinating myelopathy. Its study may assist investigation of such human diseases as multiple sclerosis, amyotrophic lateral sclerosis (ALS) and the poliomyelitis-ALS syndrome.

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