Ultrastructure of Herpes simplex Virus Infection of the Nervous System of Mice

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Summary. The nervous system and small intestine of mice infected with herpes simplex virus were examined by electron microscopy from the viewpoint of virus-host interaction.

The host cells examined included the neuron, astrocyte, oligodendrocyte, and Schwann cell. The susceptibility of the latter was not less than that of the neuron. The endothelial cell, perineurial fibrocyte and smooth muscle cell were also host cells. Replication of herpes virus in the nervous system was proven to be identical to that occurring in vitro; initial reproduction of nucleocapsids in the nucleus and subsequent maturation at the nuclear membrane with envelope formation, followed by discharge into the cytoplasmic reticular cavities and finally release from the host cell. Inconsistency in the distribution of virus particles and viral antigen was chiefly concerned with the host cell nucleus and the glial cytoplasm.

Herpes virions, though few, were identified in the axons of peripheral nerves, and in the periaxonal space of myelinated fibres in the brain and the nerve ganglia. Virions were present in tiny vesicles in the perikarya or as naked particles. In the distal parts of peripheral nerve, there was marked dissociation in the amount of virions between Schwann cells and the axon. The significance of the endoneural space and the axon in the neural spread of infection is discussed briefly.

Key words: Herpes simplex Virus — Ultrastructure — Viral Infection — Virus-Host Interaction — Nervous System

Introduction

Reports on the ultrastructural aspects of herpes simplex virus infection of the nervous system have been made by Swanson et al. (1966), Severin and White (1968), Rabin et al. (1968), Baringer and Griffith (1970), Kristensson (1970), and Norris (1972). Many are concerned with the neural spread of the virus, and have certain limitations regarding the complete range of events which take place during infection.

Whether there is any host-dependent feature of virus replication specific for the nervous system and the host cell range were our chief interests in electron microscopy.

The suspected discrepancy between the distribution of virus antigen, positive by immunofluorescent staining which may depend upon preparation of antiserum, and the location of virus particles in nervous tissues should be clarified. The present paper complements our previous studies using immunohistochemical methods (Yamamoto et al., 1965, 1968).

The nature of neural spread is one of the most important problems regarding herpetic infection of the nervous system. Fluorescent and electron microscopic
studies appeared to favour a mediating role by Schwann cells. Regarding axonal transmission of the infective agent as suggested by Kristensson et al. (1971), this question is still debated. The present report is a contribution to the further interpretation of the latter problem in the relation to the mobility of herpes simplex virions in a variety of the nerve constituents.

**Material and Methods**

The HF strain of herpes simplex virus at the 25 to 36th passage through the brain of suckling mice was used.

Fifty two of the DD strain of Swiss Albino mice aged 7 days were inoculated intraperitoneally with about $10^3$ TCID$_{50}$ of the virus.

General weakness and/or marked paralysis of the hind limbs usually developed 3 days after inoculation. The mice were sacrificed by exsanguination at 1 day intervals from 1 to 4 days after inoculation. Small portions of the brain, spinal cord, dorsal root and coeliac ganglia, coeliac plexus, sympathetic trunk, sciatic nerve, and small intestine, were fixed in 2% osmium tetroxide in veronal acetate buffer at pH 7.4. After dehydration with alcohol they were embedded in epoxyresin. Thick sections stained with toluidine blue were prepared for selecting fields for electron microscopy. Sections cut on the Porter-Blum microtome were stained with lead citrate and examined in a Hitachi-7S electron microscope. The presence of infection was simultaneously checked by the fluorescent antibody method.

**Results**

A. **Central Nervous System**

1. **Morphological Alteration**

Ultrastructural changes were recognized 3 days after inoculation. Pontobulbar and spinal lesions were most severe and extensive, while affection of the cerebral hemispheres and cerebellum was limited and inconstant. Similar observations were made by fluorescent staining.

The initiation of herpes infection was indicated by a definite change in the cell nuclei and by mobilization of phagocytes. In toluidine blue preparations the chromatin of the nerve or glia cell nuclei surrounded a central pale area. As the lesions advanced, the tissue disruption due to an irregularly dilated extracellular space and swollen astrocyte processes appeared. Various numbers of leucocytes and phagocytes infiltrated the necrotic parenchyma and intermingled with fragments of cells and their processes. The myelinated nerve fibres appeared to be much more resistant.

**Nerve cells** infected with herpes virus often showed partial break down of the plasma membrane, swelling or vacuolar degeneration of mitochondria and dilatation of the endoplasmic reticulum. Another striking feature was the change in the nucleus (Fig. 1) which presented deep or shallow, irregular undulations of the whole membrane, or the nucleus was destroyed resulting in a mixture of nucleoplasm and cytoplasm. Large clumps of chromatin accumulated near the nuclear membrane but often filled almost the entire nucleus.

**Oligodendrocytes** showed changes similar but less prominent than those in the neurons namely undulation or disruption of the nuclear and cytoplasmic membranes and aggregation of chromatin. Involvement of the **astrocytes** was readily observed in lesions which developed at the margin of the brain and spinal cord.