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## INTRACISTERNAL VIRUS-LIKE PARTICLES IN BRAIN OF A MULTIPLE SCLEROSIS PATIENT

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### SUMMARY

Doughnut-shaped particles, 55–65 nm in diameter, were revealed by electron microscopy in the cisterns of the rough endoplasmic reticulum of cells from an active lesion in autopsied brain tissue from a multiple sclerosis patient. The morphology of the particles closely resembled that of coronaviruses.

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### INTRODUCTION

Until now, a possible viral etiology in multiple sclerosis (MS) has been postulated on the basis of the seemingly abnormal immune response to measles virus among MS patients (e.g. Adams and Imagawa 1964; Norrby, Link and Olsson 1974), and the isolation of a parainfluenza type 1 virus from 2 cases of MS (Ter Meulen, Koprowski, Iwasaki, Kackell and Muller 1972). The observation of paramyxovirus-like intranuclear filaments in brain lesions of MS patients (Prineas 1972) has received much attention, but skepticism about the viral nature of these structures has been expressed because: (1) they looked quite different from paramyxoviruses (Dubois-Dalcq, Schumacher and Sever 1973); (2) they were found in a variety of diseases other than MS (Shaw and Sumi 1975), and (3) there was no immunocytological evidence of their viral origin. So far there have been no reports of complete virus structures in MS brain tissue.

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This communication describes the presence of intracisternal particles resembling coronaviruses in perivascular inflammatory cells in a lesion from brain tissue taken at autopsy from an MS patient.

#### MATERIALS AND METHODS

##### *A case report*

The patient had developed episodic unsteadiness of gait and intention tremor of the upper extremities when she was 20 years of age. About 2 years later, she developed a spastic paraparesis and urinary incontinence and on examination, was found to have nystagmus on lateral gaze, ptosis on the left, intention tremor of the upper extremities and neck, dysmetria, pronounced paraparesis and impairment of vibratory and position sense in both feet. These neurological symptoms followed the classic relapsing course of clinical MS with progressive worsening, until the plateau stage at age 28, when she was confined to bed. She died of a pulmonary infection at age 33.

Permission was granted for autopsy only of the brain; macroscopic and routine histological examination 4 hr after death revealed typical MS plaques of various ages around the lateral ventricles, on the floor of the fourth ventricle and in the cerebral white matter. There were no evidences of meningoencephalitis, although occasional areas in or around the plaques showed perivascular inflammatory cells. No neoplastic lesions were observed.

##### *Electron microscopy*

Tissues were removed at autopsy from 5 plaques and nearby white matter, fixed in phosphate buffered 3.5% glutaraldehyde, post-fixed in phosphate-buffered 1% osmium tetroxide, and embedded in epoxy resin. Thick sections stained with toluidine blue were examined in a light microscope, and thin sections stained with uranyl acetate and lead citrate were examined in a Hitachi HU-11 electron microscope.

#### RESULTS

Light-microscopic examinations of toluidine blue-stained, epon sections of all 5 plaques revealed complete loss of myelin and severe gliosis. Two of the plaques showed no perivascular cuffing; in 3 plaques thin perivascular cuffs, consisting mainly of lipid-laden macrophages and plasma cells, were seen. These "chronic type" cuffs (Tanaka, Iwasaki and Koprowski 1975) were not observed outside the plaques. At the margin of 1 plaque located in the paraventricular white matter of the cerebrum, thick perivascular cuffs, consisting of many lymphocytes and a few lipid-laden macrophages were seen. This "acute type" of cuff (Tanaka et al. 1975) was seen around several vessels associated with a thin rim of perivascular demyelination. In these inflammatory lesions were found large mononuclear cells with elongated, and peripherally located nuclei and abundant basophilic cytoplasm (Fig. 1, inset).

Under electron microscope, the cisterns of hypertrophied and tremendously expanded rough endoplasmic reticulum (ER) of large mononuclear cells easily distinguishable from other types of inflammatory cells, were seen filled with many particles (Fig. 1). The individual particles were doughnut-shaped, having outer diameters of 55-65 nm and inner diameters of 25-35 nm, with electron lucent centers. The shell of the particles appeared granular; most lacked the distinct double-membrane structure, and the outer surface of the shell was irregularly studded with amorphous materials (Figs. 2 and 3). No budding process and no clear connection between the particles and the membrane of the ER was observed, although the ER membrane

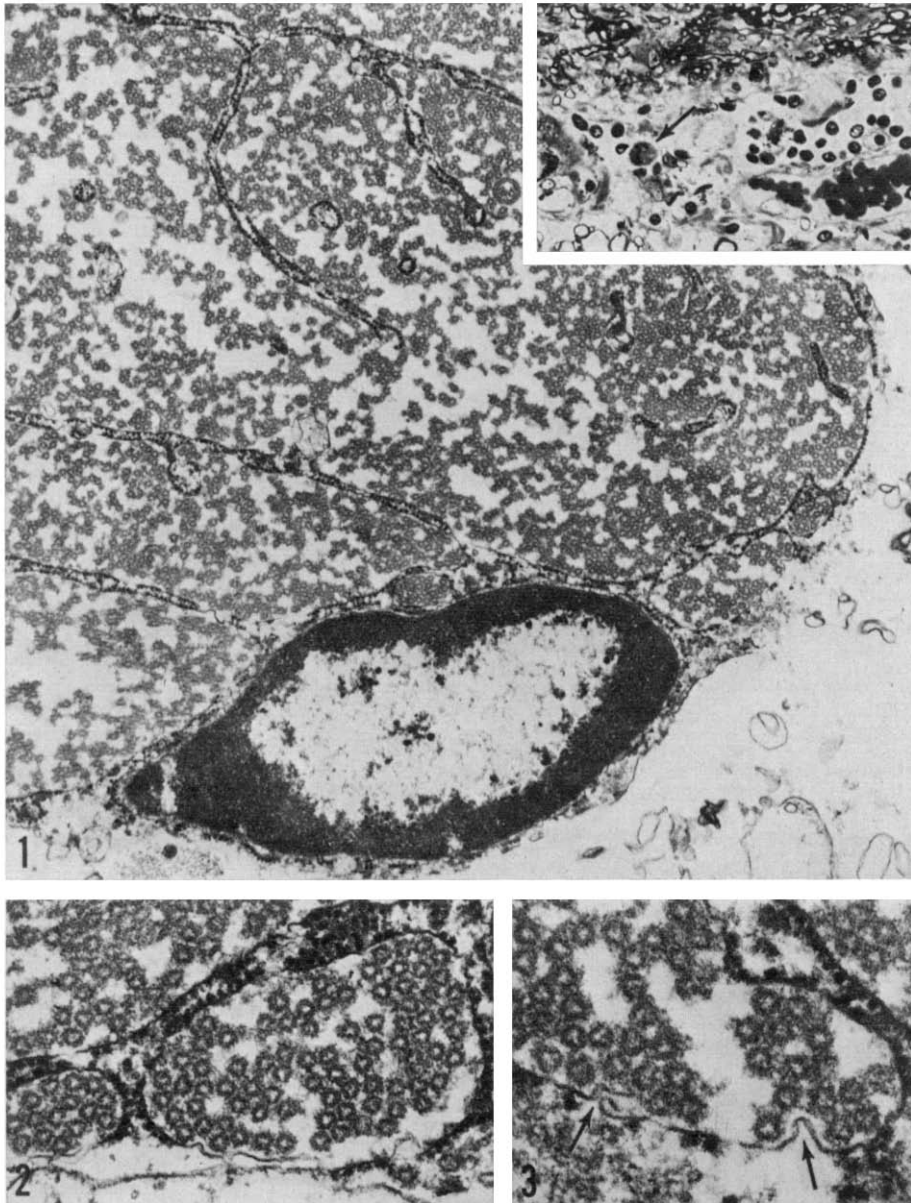


Fig. 1. Electron micrograph of an unidentified perivascular infiltrate. Numerous particles are seen in the extremely expanded cistern of rough ER.  $\times 16,000$ . Inset: Light micrograph of a perivascular cuff showing an unusual large cell with osmiophilic cytoplasm (arrow). Epon-embedded and toluidine blue-stained,  $\times 200$ .

Fig. 2. Higher magnification of the particles.  $\times 53,000$ .

Fig. 3. The membrane of ER protrudes into the cistern (arrows).  $\times 77,000$ .

protruded into the cistern on rare occasions (Fig. 3, arrows). The particles were found only in the cistern of the rough ER and nowhere else in the cells. No extracellular particles were observed. The particles were found in only 1 of the 8 blocks prepared from the plaque. It must be noted, however, that acute types of cuffs were detected in only 2 of the 8 blocks.

Three lines of brain cell cultures were established from this patient. Electron microscopy of the cultured cells, however, over an observation period of 9 months failed to reveal structures identical to the particles seen in the MS brain tissue.

#### DISCUSSION

From an ultrastructural point of view, the particles described here are unlike any known cellular constituents; they most closely resemble coronaviruses. Although strains of coronaviruses vary widely in morphology, they are usually circular, 80–100 nm in diameter (if the characteristic “fringes” of 20 nm projections are included) with electron lucent centers, and bud and collect in the cisterns of the ER (Almeida, Berry, Cunningham, Hamre, Hofstad, Mallucci, McIntosh and Tyrrell 1968; Lampert, Sims and Kniazeff 1973). Among many coronaviruses, the morphology of the JHM strain of mouse hepatitis virus, whose diameters measure about 80 nm, appears to resemble most closely that of the particles observed in this MS brain except that the fringes of the mouse hepatitis virus are distinct and petal-shaped, and the process of virus budding is common (Lampert et al. 1973). The particles described here also resemble the intracisternal type A particles that have been found in a variety of malignant and normal tissues of animals, except that the shell of such type A particles has two distinct membranes, the inner one usually a bit thicker than the outer one (Dalton, De Harven, Dmochowski, Feldman, Haguenu, Harris, Howatson, Moore, Pitelka, Smith, Uzman and Zeigel 1966).

Another possibility is that the intercisternal particles might represent a non-specific change of ER associated with an inflammatory reaction or demyelination. Intracisternal “tubuloreticular structures” have been reported in a variety of viral and degenerative diseases and even in normal subjects (e.g. Baringer and Swoveland 1972; Brunning and Parkin 1975). Intracisternal “toroidal particles” have also been reported in brain from cases of meningoencephalitis of unknown etiology (Powell, Braude and Lampert 1975) and of normal pressure hydrocephalus with no evidence of inflammation (Suzuki 1974). The tubuloreticular structures, however, were distinctly different from the particles seen in this case, having reticular profiles, diameters of 25–30 nm and only superficially resembling the nucleocapsids of paramyxoviruses. Toroidal particles, 90–100 nm in diameter and with clear double-circular profiles, are unlike any known viruses or cellular constituents. To the best of our knowledge, particles similar to those seen in this MS brain have never been seen in human tissues and certainly not in human brain tissue.

Assuming that the particles seen in this MS brain are coronavirus, the finding is especially interesting in view of the reports that the JHM strain of mouse hepatitis virus can cause demyelination in the mouse after virus-induced destruction of oligo-

dendrocytes (Cheever, Daniels, Pappenheimer and Bailey 1949; Lampert et al. 1973). However, in the absence of immunological evidence implicating coronavirus in the etiology of MS and since the possibility that the patient might have had a viral disease superimposed upon MS cannot be dismissed, the significance of the presence of the particles in MS brain must be interpreted with caution.

In the past 3 years, we have subjected brain tissues from 12 histologically and clinically verified cases of MS to meticulous electron-microscopic survey for the presence of viral structures; this is the only case in which this kind of structure was observed. The difficulty in assessing the pathognomonic significance of this observation in the disease lies mainly in the apparent insensitiveness of electron microscopy as a tool in the search for viral structures. For instance, electron microscopy was able to detect perivascular cuffs in only 6 of the 12 cases in which routine light microscopy had revealed the presence of perivascular cuffs (Tanaka et al. 1975). Despite this obvious disadvantage, electron microscopy is still the only tool for the search of unknown agents in diseased tissue. It is hoped that this report will stimulate further ultrastructural study of MS brain tissue in the search for viral structures.

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