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CASE REPORT

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# Papovavirus detection by electron microscopy in the brain of an elderly patient without overt progressive multifocal leukoencephalopathy

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**Abstract** Virions resembling papovavirus were demonstrated in glial cells in the brain of an aged patient without overt progressive multifocal leukoencephalopathy. The patient was not in a severely immunocompromised state. On histological examination, only a few tiny incomplete necrotic foci were found in the subcortical area. These foci were widely dispersed. Rare, swollen oligodendroglial cells and astrocytes in which papovavirus capsid protein (VP-1) was demonstrated immunohistochemically were present around the foci. The two typical types of virus particles i.e. 35 to 40 nm round particles and elongated particles, were observed in the nuclei of the swollen glial cells. The latter were in the minority. Distinct crystals were also found in the nuclei. The centre-to-centre distance of the particles in the crystals, about 40 nm, and the electron-opaque spots of the round-shaped virions and of the elongated particles, were indicative of structural subunits of papovavirus capsids. This case provides further evidence that papovavirus, possibly JC virus, may be reactivated in the brains of aged patients who are not in an immunocompromised state.

**Key words** Papovavirus · Progressive multifocal leukoencephalopathy · Electron microscopy
Elderly patient

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## Introduction

The notion of progressive multifocal leukoencephalopathy (PML) as an atypical viral infection in patients with compromised immunity was proposed by Richardson [14]. ZuRheim and Chou [18] provided strong electron microscopic evidence for the viral infection hypothesis which showed that abnormal oligodendrocytes in and around the lesions contained numerous papovavirus-like particles. Since the isolation and designation of the virus as JC virus by Padgett et al. [11], JC virus has been known to be the cause of PML. We recently demonstrated JC viral DNA and protein by in situ hybridization and immunostaining in the brains of apparently immunocompetent aged patients who did not have PML [8]. This finding was confirmed using the polymerase chain reaction method both by us [9] and several other groups [3, 13, 16]. In the present study, one of the brains in which JC viral DNA was detected most strongly was examined by electron microscopy. In order to select the locations of the cells where the virions may be detected, we detected virions resembling papovavirus, possibly JC virus, in three overt PML cases prior to this study.

## Case report

A 75-year-old woman was admitted to Tokyo Metropolitan Geriatric Hospital in June 1980 for left hemiparesis and dysarthria. A CT scan revealed a small, low-density area in the right cerebrum. The hemiparesis and dysarthria disappeared within 1 month. The patient was able to go about her life normally with the exception of calculation. In April 1981 her left hemiparesis and dysarthria recurred, and a CT scan and arteriography revealed occlusion of the ascending branch of the right middle cerebral artery. Her blood pressure was 130–150/70–80. Shoulder-hand syndrome developed on the left side in May. Prednisolone, 10 mg/day, was prescribed for one month resulting in the disappearance of this syndrome and prednisolone was stopped. The patient was transferred to a nursing home, and her mental impairment remained at the same level as before. After discharge, she was bedridden and became unable to speak more than a few words. From June 1985, she needed assistance in eating. Beginning in July 1988 she suffered repeated bouts of pneumonia and died on 5 September 1988.

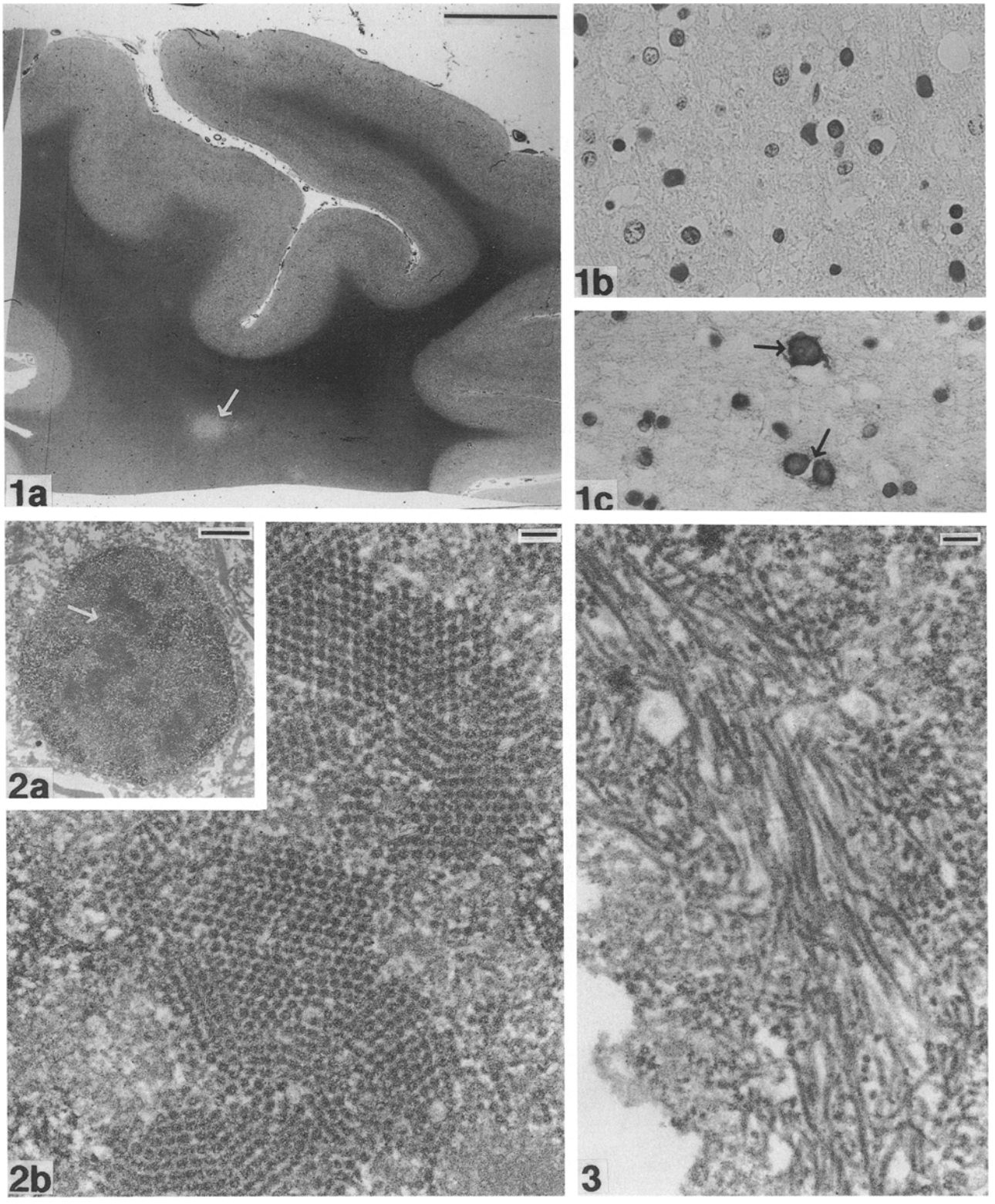


**Fig. 1** **a** The largest focal demyelinated lesion, where the sections were made for electron microscopy. (× 3.8). **b** Histological findings in the same area. Swollen oligodendroglial cells and large astrocytes are observed (H & E; × 400). **c** Immunohistochemical staining for VP-1. *Arrowheads* point to positive cells, which contain many virions. (× 400)

**Fig. 2** **a** A glial nucleus with a complex composite of crystals. (× 8000). **b** A portion of the nucleus in **a**. Electron-lucent centres are present in some. (× 60000)

**Fig. 3** A portion of a glial nucleus packed with elongated particles. (× 60000)

Autopsy was performed 10 h after death at Tokyo Metropolitan Geriatric Hospital. Significant findings outside the central nervous system consisted of severe organizing and organized pneumonia associated with scattered foci of aspiration in the left lung. Viscous sputum was found to have obstructed the glottis resulting in apnoea and death.

The brain weighed 1100 g and exhibited marked atrophy of the right hemisphere. Coronal sections through both cerebral hemispheres revealed a large, old infarction in the territory of the right middle cerebral artery. This large vascular lesion was confirmed to have resulted from an arteriosclerotic stenosis of a branch of the right middle cerebral artery. Severe sclerosis was found in the cerebral arteries, but no lesions resembling a demyelinating process could be detected in the white matter macroscopically. Routine histological examination was carried out on paraffin sections of the cerebral, cerebellar and brain stem areas. Away from these old vascular lesions, a few tiny foci of incomplete necrosis were found in the subcortical white matter of the parietal and frontal lobes. The margins of the necrotic area were obscure. The largest lesion was about 2 mm in diameter (Fig. 1a). There were some areas where the number of glial cells was decreased without necrosis. These foci were widely dispersed and non-confluent. Rare, swollen oligodendroglial cells and a few, fairly large astrocytes were observed mainly at the edge of the minute incomplete necrotic area (Fig. 1b). Some of these glial cells had altered nuclei, with distinct margination of chromatin along the nuclear membrane. Others featured bizarre nuclei. Lymphocytes, macrophages and plasma cells were not increased.

Paraffin-embedded 4 μm-thick sections from the subcortical white matter of the parietal lobe were stained with toluidine blue or immunohistochemically and examined under the light-microscope. Immunohistochemistry was performed on deparaffinized specimens by the avidin DH-biotinylated horse-radish peroxidase technique using polyclonal antibodies raised against either the common papovavirus capsid antigen or glial fibrillary acidic protein (GFAP), as described previously [9]. Areas where the large cells with large nuclei or the histochemically VP-1 positive cells were clustered, were embedded in Epon after fixation in 1% cacodylate-buffered osmium tetroxide for 30 min at 4° C and dehydration in graded ethanol rinses. Two thin (60–100 nm), small (1× 1 mm) sections were cut from the edge of the largest lesion with diamond knives (Fig. 1a), and a small section was also cut from the area where the number of glial cells was reduced and the large glial cells were scattered without necrosis. These sections were stained with uranyl acetate and lead citrate, and examined using an Hitachi H-600 transmission electron microscope. All cells (294 cells) except those in the peripheral region were examined in one section taken from the largest lesion. There were conspicuous post-mortem changes, with poor preservation of cell membranes and cytoplasmic organelles. Identification of individual cells was almost impossible. The cells were identified as glial cells by comparing toluidine blue and immunohistochemically stained sections. The size of virion-bearing nuclei, 4.5–9.5 μm (mean: 6.3 μm) was larger than that of the virion-negative nuclei, 3.0–6.3 μm (mean: 4.0 μm). Cells strongly positive for VP-1 at the periphery of the nuclei (Fig. 1c), contained many virions. Numerous randomly distributed, round, 35 to 40 nm particles were observed in the nuclei of 22% of the glial cells. Orderly intranuclear arrays of particles or distinct crystals were found in 65% of the virion-bearing nuclei (Fig. 2). The centre-to-centre distance between the particles in the crystals was roughly 40 nm. The shape of the individual particles in the crystals was mostly hexogonal. Elongated virions, referred to as "filamentous" or "tubular forms", were found in 29% of the virion-bearing nuclei (Fig. 3). Their length was variable, and their width was about one half to two-thirds that of the round virions. Electron-lucent centres were seen in some of both types of virions. The viral envelopes were not seen. Two-thirds of these heavily virion-bearing cells were found near the margin of the necrotic area. The features of the other section from the largest lesion were almost the same as described above. In the third section, no crystals or tubular forms were found in the cells.

## Discussion

We reported previously that JC viral DNA and papovavirus capsid protein were present in the brains of some elderly patients who did not exhibit any recognizable PML or underlying immunosuppressed states [8]. To confirm the presence of this virus on an ultrastructural level, we conducted electron microscopic studies in the present report. The present case (case 7 in the previous report) is that in which JC viral DNA was most strongly detected. The patient was not in an immunosuppressed state, although she had a history of treatment with prednisone, 10 mg/day, for one month, 7 years before her death. At post-mortem examination, a few tiny incomplete necrotic foci were found together with rare swollen oligodendroglia and a few fairly large astrocytes. Since no findings characteristic of PML, such as large multifocal lesions, confluent lesions, or giant transformed astrocytes were present, this case was not consistent with classical PML. Admitting this inconsistency, a few possibilities might be suggested for its cause. The first is that these findings represent the initial changes in PML. The features in our case resemble the early lesion described by ZuRhein [19] except that there were more cells with crystalized virions in our case. The second speculation is that the patients PML was in remission. A few cases of transient and well-documented sustained remission of PML have been reported [1, 7, 12, 15]. Typical PML lesions are not detected in the brain in remission. The multifocal nature of the process is evident, however, in the presence of demyelination in various stages. This was not true of our own case, because only a few tiny, incomplete necrotic areas were detected in this brain.

Some of the glial cells in these areas were positive for papovavirus capsid protein immunohistochemically. Electron microscopy revealed the presence of virions in these cells. The virions were characterized as follows: Two kinds of virions, rounded and elongated, were present with the former predominating. The round particles were 35 to 40 nm in diameter and most of the individual particles in the crystals were hexogonal in form. Electron-lucent centres were seen in some and the centre to centre distance between the particles in the crystals was about 40 nm. These characteristics were nearly the same as those described by ZuRhein [19] in PML: the two principal types of virus particles encountered, round and elongated, were seen most clearly in less dense nuclei. In support of these criteria, it should be noted that, with the exception of bovine papillomavirus, elongated and rounded forms of virions were only found together in nuclei infected by papovaviruses [2, 19]. Moreover, papovaviruses are unique in size among those viruses which infect humans persistently. The viruses reported to be the cause of the demyelination are: alpha virus, 60–70 nm in diameter; corona virus, 70–120 nm in diameter; herpes virus, 100–110 nm in diameter; paramyxovirus, 150–300 nm; papovavirus, 30–55 nm; picornavirus, 20–30 nm; retrovirus, 80–130 nm and rhabdovirus 80× 70–85 nm [4]. Papova virus and picorna virus are the two viruses

that do not bear envelope proteins. Based on these features, the virions demonstrated in this case were probably papovaviruses, which include polyomavirus and papillomavirus. Papilloma virus particle size is 52 to 55 nm in diameter [6] while the polyomaviruses in the brains of PML patients have been reported to be between 28 and 45 nm in diameter [19]. As the size of the particles in our own case was between 35 nm and 40 nm, the virions were probably polyomaviruses, specifically JC viruses [10].

Gibson et al. [5] reported that in one patient with neuro-Behçet syndrome without PML, JC viral DNA was found to be weakly positive using molecular hybridization, however, they were unable to find any virus particles. The present case is the first in which papova virions were detected in the brain of a non-immunocompromised aged patient without overt PML. The morphological evidence per se is not sufficient to designate it as the lesion responsible for her clinical condition, because the demyelinated foci in the white matter were extremely small. Nevertheless, papovavirus replication may have occurred in the brain of this patient. Since JC virus is of low pathogenicity and requires impairment of cell-mediated host defences to allow a progressive nervous system infection [17], the disease may be static in this case.

A few tiny demyelinating lesions may be overlooked at routine autopsies. If such lesions were noticed and examined electron microscopically, papova virions might be demonstrated in the brains of aged patients more frequently than expected.

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## References

1. Bauer WR, Turel AP, Johnson KP (1973) Progressive multifocal leukoencephalopathy and cytarabine. Remission with treatment. JAMA 226: 174–176
2. Bernhard W, Febvre HL, Cramer R (1959) Miseen vidence au microscope lectronique d'un virus dans des cellules infect 'ees in vitro par l'agent du polyome. C R Acad SCI [III] 249: 483–485
3. Elsner C, Dorries K (1992) Evidence of human polyomavirus BK and JC infection in normal brain tissue. Virology 191: 72–80
4. Fazakerley JK, Buchmeier MJ (1993) Pathogenesis of virus-induced demyelination. In: Advances in virus research, Maramorosch (ed) vol 42. Academic Press, pp 249–324
5. Gibson PE, Gardner SD, Field AM (1986) Use of a molecular probe for detecting JCV DNA directly in human brain material. J Med Virol 18: 87–95
6. Gissmann L, Pfister H, zur Hausen H (1977) Human papillomavirus (HPV). Characterization of four different isolates. Virology 76: 569–580
7. Kepes JJ, Chon SM, Price LW (1975) Progressive multifocal leukoencephalopathy with 10-year survival in a patient with non-tropical sprue. Neurology 25: 1006–1012
8. Mori M, Kurata H, Tajima M, Shimada H (1991) JC virus detection by in situ hybridization in brain tissue from elderly patients. Ann Neurol 29: 428–432
9. Mori M, Aoki N, Shimada H, Tajima M, Kato K (1992) Detection of JC virus in the brains of aged patients without progressive multifocal leukoencephalopathy by the polymerase chain reaction and southern hybridization. Neurosci Lett 141: 151–155
10. Padgett BL, Walker DL (1976) New human papovaviruses. Prog Med Virol 22: 1–35
11. Padgett BL, Walker DL, ZuRhein GM, Eckroade RJ (1971) Cultivation of papova-like virus from human brain with progressive multifocal leukoencephalopathy. Lancet I:1257–1260
12. Price RW, Nielsen S, Horton B, Rubino M, Padgett B, Walker D (1983) Progressive multifocal leukoencephalopathy: a burnt-out case. Ann Neurol 13: 485–490
13. Quinlivan EB, Norris M, Bouldin TW, Suzuki K, Meeker R, Smith MS, Hall C, Kenny S (1992) Subclinical central nervous system infection with JC virus in patients with AIDS. J Infect Dis 166: 80–85
14. Richardson EP Jr (1961) Progressive multifocal leukoencephalopathy. N Engl J Med 265: 815–823
15. Selhorst JB, Ducy KF, Thomas JM, et al. (1978) PML: remission and immunologic reversals. Neurology 28: 337
16. White FA, Ishaq M, Stoner GL, Frisque RJ (1992) JC virus DNA is present in many human brain samples from patients without progressive multifocal leukoencephalopathy. J Virol 66: 5726–5736
17. Willoughby E, Price RW, Padjett BL, et al. (1980) Progressive multifocal leukoencephalopathy (PML): in vitro cell mediated immune responses to mitogens and JC virus. Neurology 30: 256–262
18. ZuRhein GM, Chou SM (1965) Particles resembling papovaviruses in human cerebral demyelinating disease. Science 148: 1477–1479
19. ZuRhein GM (1969) Association of papova-virions with a human demyelinating disease (progressive multifocal leukoencephalopathy). Prog Med Virol 11: 185–247