Infection with JC Virus and Possible Dysplastic Ganglion-Like Transformation of the Cerebral Cortical Neurons in a Case of Progressive Multifocal Leukoencephalopathy

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Abstract. Infection of the cerebral cortical neurons with JC virus (JCV) with possible dysplastic ganglion-like alteration of the infected neurons found in a case of progressive multifocal leukoencephalopathy (PML) is described. The patient was a 21-year-old man with common variable immunodeficiency who died of PML after a 9-month clinical course. At autopsy, the white matter of the cerebrum, brainstem, cerebellum, and spinal cord exhibited extensive demyelination and necrosis. Numerous inclusion-bearing oligodendrocytes and bizarre astrocytes were found. In the occipital and temporal cortex, thick band-like aggregates of dysplastic ganglion-like cells (DGLCs) were found. These DGLCs showed immunohistochemical properties of neurons, and nuclei of some DGLCs were immunoreactive for large T antigen of SV40/JCV and p53, but not for capsid protein JCV VP1. In situ hybridization for mRNA of JCV large T antigen revealed positive signals in the nuclei of some DGLCs. These results indicate that JCV infected neurons and it is suggested that binding of the large T antigen with cellular proteins could have resulted in the dysplastic, ganglion cell-like change of the infected neurons, although the possibility that the aggregates of DGLCs represent a pre-existent malformative lesion of the cortex cannot be excluded completely.

Key Words: Dysplastic change; JC virus; Neuronal infection; Progressive multifocal leukoencephalopathy.

INTRODUCTION

Progressive multifocal leukoencephalopathy (PML) is an uncommon demyelinating disorder caused by an infection with JC virus (JCV) (1, 2) and almost exclusively affects immunologically compromised patients (3). In the central nervous system (CNS), JCV replicates mainly within nuclei of oligodendrocytes and induces multifocal loss of the myelin sheaths in the white matter. Affected oligodendrocytes show a characteristic enlargement of the nuclei, which are filled with numerous viral particles. Bizarre and gigantating astrocytes are frequently seen in and around the demyelinating foci and some of these astrocytes have been demonstrated to be infected with the virus (4–7). It has been a subject of long-term controversy whether neurons are infected with JCV or not, and a few recent studies have suggested the possibility of infection of cerebellar granule cells (8) and also cerebral cortical neurons (9, 10).

JCV is well known to be oncogenic in the CNS of the experimental animals (11–16), and various types of neuroectodermal tumors, such as glioblastoma (11–13) and medulloblastoma (11, 13, 14), have been produced by an intracerebral inoculation of the virus. Furthermore, there have been several human autopsy cases of PML reported in which malignant astrocytoma (17–19) or primary malignant lymphoma of the CNS (20–22) has been found in association with demyelinating lesions, thus suggesting the possibility of virus-induced oncogenesis in human brain. Moreover, JCV DNA sequences and expression of the viral oncoprotein has recently been detected in human oligoastrocytoma (23), pleomorphic xanthoastrocytoma (24), and medulloblastoma (25).

We report here a very unusual and intriguing autopsy case of PML in which, in addition to extensive demyelinating and necrotizing lesions involving the white matter of almost the whole CNS, we describe the appearance of a large number of dysplastic or dysmorphic ganglion-like cells in the cerebral cortex. These cells showed unequivocal immunohistochemical properties of neurons, and the immunohistochemical and in situ hybridization (ISH) studies demonstrated that these neurons were infected with JCV and expressed large T antigen, but not the capsid protein VP1.

Case Report

The patient was a 21-year-old man who had been diagnosed as having common variable immunodeficiency (26, 27) at the age of 1 year and 4 months. The serum Ig G was 70 mg/dl at that time and thereafter he had received a replacement treatment with regular monthly injections of immunoglobulin for about 20 years. He had developed well without any neurological and psychiatric abnormalities and had become a university student. He had never experienced any epileptic seizures and his family history was unremarkable. In January 1997 at the age of 20, he noticed clumsiness of his hands such that he was unable to write and developed difficulty in speaking. Repetitive speech and unsteadiness of gait developed in February and these symptoms progressively worsened. On admission in March, neurological examination revealed motor aphasia, Gerstmann’s sign, right homonymous hemianopsia, muscle weakness of the right upper and lower extremities, and paresis of the right facial
nerve. Spastic gait on the right side was also noted and deep tendon reflexes were exaggerated. Laboratory studies on admission showed low values of serum Ig G and Ig A: Ig G 462 mg/dl, and Ig A less than 6 mg/dl. The Ig M was normal at 154 mg/dl. Cerebrospinal fluid (CSF) examination showed the following results: cell counts 6/μl, protein 29 mg/dl, glucose 70 mg/dl, Ig G 1.0 mg/dl, Ig G index 0.67, myelin basic protein 6.5 mg/dl. No viruses were detected from the cultivation of the CSF. Computed tomography (CT) scan of the brain revealed multiple extensive low-density areas involving the white matter of the left parietal, temporal and occipital lobes, and the putamen and thalamus. On magnetic resonance imaging (MRI), these abnormal areas showed low signal intensity without contrast enhancement in T1-weighted images and high signal intensity in T2-weighted images. Findings suggestive of the presence of malformative lesions such as focal cortical dysplasia, cortical tuber, or ectopic gray matter were not seen.

The administration of a neutralizing antibody for enterovirus was attempted, since chronic persistent infection with enterovirus is known to occur frequently in patients with hypogammaglobulinemia (28). The administration of acyclovir and steroid pulse therapy were also performed successively for a suspected herpesvirus infection and angiitis of the CNS, respectively, but the symptoms did not improve. A stereotactic needle biopsy of the left occipital lobe was done in April and the microscopic examination revealed necrosis of the white matter accompanied by an infiltration of lipid-laden macrophages and hypertrophy of astrocytes.

Although oligodendrocytes containing intranuclear inclusion bodies were not found, the gene analysis study of both CSF and brain specimen using polymerase chain reaction (PCR) revealed some rearrangements of the regulatory region of JCV genome that were compatible with that of the Mad-4 type, and the diagnosis of PML was established. The details of the gene analysis study were reported in the previous paper in which a number of the patient was described as O-1 and the designated name of JCV as Osaka-1 (29). Drug therapy using interferon-α and cytosine arabinoside was instituted but the response was minimal. He exhibited an apallic posture of the extremities in June. CT scan and MRI examination revealed an extensive spread of the lesion over almost the whole supratentorial white matter and also the brainstem. Since July 1997, paralysis of the bilateral third, sixth, and seventh cranial nerves appeared successively and the patient died of respiratory arrest due to the brainstem lesion in October, some 9 months after the onset of neurological symptoms. Autopsy findings outside the CNS were unremarkable.

MATERIALS AND METHODS

The brain and spinal cord were fixed in 20% formalin for 2 weeks and then cut sequentially in horizontal planes. Appropriate specimens for paraffin sections were taken from many regions and examined light microscopically using the following stains: hematoxylin and eosin (H&E), luxol fast blue-periodic acid Schiff, modified Bielschowsky, and Nissl (cresyl violet) stains. Specimens for the ultrastructural study were retrieved from a paraffin block of the thalamus, which contained aggregates of inclusion-bearing oligodendrocytes. They were processed in the usual manner after the refixation in osmium tetroxide.

The immunohistochemical studies were performed on the paraffin sections of the occipital and temporal lobes. Monoclonal (mono) or polyclonal (poly) antibodies to the following substances were employed: neurofilament (mono, DAKO, Glostrup, Denmark, 1:100), neuron-specific enolase (NSE, mono, DAKO 1:100), synaptophysin (mono, DAKO, 1:200), microtubule-associated protein-2 (MAP-2, mono, Boehringer Mannheim, Indianapolis, IN, 1:400), 14–3–3 brain protein (originally raised monoclonal antibody MAb#9 (30), 1:100), glial fibrillary acidic protein (GFAP, mono, DAKO, 1:100), JCV VP1 (originally raised polyclonal antibody against the BC loop of JCV VP1, 1:3,000 [31]), SV40 large T antigen (mono, Ab-2, OncoGene Research Product, Cambridge, MA, 1:50), Ki-67 (mono, MIB-I, Immunotech, Marseille, France, 1:200), and p53 protein (mono, DO7, DAKO, 1:100). A labeled streptavidin-biotin complex method was used for the immunostaining, and the reaction products were visualized with diaminobenzidine as the chromogen. Pretreatment with microwave processing was utilized for some of the antibodies. For a simultaneous demonstration of large T antigen and neuronal markers of NSE, MAP-2, and 14–3–3 brain protein on the same section, the double immunostaining method utilizing 4-nitroblue tetrazolium chloride (NBT) and aminoethylcarbazole was used.

In Situ Hybridization (ISH)

cRNA probes for ISH were prepared by excision of a DNA clone encoding 950 bp of JCV large T antigen (nucleotides 4308–3360), which exhibits only 54.4% homology to the SV40 T gene. The excised DNA was subcloned into the pBluescript II SK(+) vector (Stratagene, La Jolla, CA).

The direction of each fragment was determined by restriction enzyme digestion and direct sequencing. Large T cRNA probes for both antisense and sense were transcribed with T3 and T7 RNA polymerase, respectively, in the presence of digoxigenin (DIG)-labeled uridine triphosphate. ISH was performed according to the method described by Suzuki et al (32). Briefly, tissue sections from formalin-fixed, paraffin-embedded specimens of the occipital and temporal lobes were deparaffinized, washed in 0.1 M phosphate buffer, treated with proteinase K (10 µg/ml) at 37°C for 30 min, and incubated with DIG-labeled probes at a concentration of 1 µg/ml in hybridization buffer. The slides were washed in 5×SSC (0.75 M sodium chloride and 75 mM sodium citrate, pH 7.0), 50% formamide in 2×SSC, and treated with 20 µg/ml RNaseA in TNE (10 mM Tris-HCl pH 7.6, 500 mM NaCl, 1 mM EDTA) for 30 min at 37°C, before they were stringently washed in 2×SSC, 0.2×SSC at 50°C. For detection of DIG-labeled hybrids, sections were blocked with 1.5% blocking solution and then reacted with anti-DIG antibody conjugated to alkaline phosphatase (1:500) for 30 min at room temperature. The signal was visualized by staining with freshly
RESULTS

Neuropathological Findings

Gross Appearance: The brain weighed 1,340 grams. Although it was symmetrically swollen and felt abnormally soft as a whole, no other abnormalities were noted on external examination. On cut sections, the cerebral cortex was as a whole diffusely atrophic. The cortices of the right parahippocampal and calcarine gyri were rather thick and showed an irregularity of the contours. The cerebral white matter showed an extensive diffuse and very severe softening, and the tissue components in the centrum semiovale were almost lost, leaving only friable, necrotic and gliotic tissue (Fig. 1). The basal ganglia and thalamus also exhibited a remarkable softening with a brownish discoloration. Similar alterations extended caudally into the brainstem and, less severely, into the spinal cord. The cerebellar white matter was also severely necrotic in the central region. The peripheral white matter of the folia was relatively well preserved.

Histopathology

Cerebral White Matter: The greater part of the cerebral white matter demonstrated diffuse and severe involvement with demyelinating and necrotizing processes observed. Myelin sheaths, oligodendrocytes, and axons were severely affected and almost completely disappeared, leaving only blood vessels and a coarse meshwork of astroglial fibers filled by numerous lipid-laden macrophages (Fig. 2). Bizarre and gigantic astrocytes were scattered in the lesions. Perivascular lymphocytic cuffing of a mild degree was rarely seen. Many oligodendrocytes in the marginal areas of the lesions had remarkably enlarged nuclei containing homogenous and basophilic inclusion bodies (Fig. 3). An ultrastructural study of these inclusion bodies revealed dense aggregates of numerous round viral particles, each measuring about 40 nm in diameter, admixed with an elongated filamentous form (Fig. 4).

Cerebral Cortex: Neurons of the cerebral cortex were remarkably reduced in number, and the cortical neuropil showed a moderate rarefaction. A few neurons exhibiting central chromatolysis, probably due to axonal injury in the subjacent white matter, were found. Proliferation of hypertrophic astrocytes in association with microvascular proliferation was pronounced. An unusually large number of inclusion-bearing oligodendrocytes and bizarre astrocytes were found in the deep cortical layers.

The most prominent and unique finding was the appearance of a number of large polygonal cells resembling dysplastic or dysmorphic ganglion cells, such as those seen in gangliocytoma or ganglioglioma (Fig. 5). These abnormal cells, which we have tentatively termed dysplastic ganglion-like cells (DGLCs) in this article, were especially prominent in the calcarine cortex of the right occipital lobe and the right parahippocampal cortex of the temporal lobe, where large numbers of them formed thick band-like aggregates and replaced the whole layer of the atrophied cortex. However, they did not form obvious grossly distinct nodules. DGLCs were not found in the subcortical white matter. They possessed large, round, and vesicular nuclei with prominent basophilic nucleoli and abundant cytoplasm of amphophilic and finely granular appearance (Fig. 6). Coarse or fine granules of purple color were seen in the cytoplasm with Nissl stain (Fig. 7). Binucleate DGLCs were not found. Between the DGLCs, numerous, strongly argyrophilic, thick nerve fibers were seen (Fig. 8), a few of which appeared to arise from DGLCs. In other regions, a small number of DGLCs were seen scattered sparsely in the atrophied cortex, but without forming cellular aggregates.

Basal Ganglia, Thalamus, and Brainstem: Extensive and confluent, demyelinating and necrotizing lesions similar to those found in the cerebral white matter were seen. Atrophied residual axons were found in many places, and neurons in the midbrain, pontine base, and olivary nucleus were relatively well preserved. Scattered aggregates of inclusion-bearing oligodendrocytes were found in the thalamus and cerebral peduncle and bizarre astrocytes were also present.

Cerebellum: In the central white matter the severity of demyelinating lesions was the same as that in the cerebral white matter. The dentate nucleus was severely affected but the neurons were relatively well preserved. In the peripheral white matter of the folia, the lesions were less severe in degree and showed multifocal and discrete appearance. The cerebellar cortex retained an approximately normal appearance.

Spinal Cord: The lesions exhibited typically multifocal features of PML and discrete small foci of demyelination and necrosis were found in the white matter and also in the gray matter. The pyramidal tracts bilaterally showed severe secondary degeneration.

Immunohistochemical and In Situ Hybridization Studies

With anti-JCV VP1 antibody, the nuclei of inclusion-bearing oligodendrocytes were strongly immunostained. The antibody was generated by synthetic peptides containing 16 amino acid residues of JCV VP1 BC loop. The peptides showed only 31% homology to SV 40 VP1, and unlikely to detect SV 40. They were not only scattered widely in the white matter, being especially numerous in the marginal areas of the demyelinating foci, but also were observed frequently in the deep layers of the cerebral cortex (Fig. 9). With anti-SV40 large T antigen antibody, which is known to cross-react with JCV
Fig. 1. The cerebral white matter was severely destroyed, leaving only necrotic and gliotic tissue. The basal ganglia and thalamus were also severely affected. The cerebral cortex showed diffuse atrophy. The right parahippocampal and calcarine cortices were thick and showed an irregularity of the contours.

Fig. 2. The tissue components of the white matter were replaced by lipid-laden macrophages and a coarse meshwork of glial fibers. Several atypical, bizarre astrocytes were seen. Corpus callosum, H&E, ×50.

Fig. 3. Many oligodendrocytes in the marginal areas of demyelinating lesions contained basophilic intranuclear inclusion bodies. Frontal white matter, H&E, ×100.

Fig. 4. Nuclei of the infected oligodendrocytes were filled with numerous round viral particles. Thin filamentous form was also found. ×30,000.
T antigen (16), even the larger numbers of immunoreactive nuclei of oligodendrocytes were observed. The nuclei of most of the inclusion-bearing oligodendrocytes and of bizarre astrocytes were also immunoreactive with anti-p53 protein antibody.

The cytoplasm of DGLCs was strongly and diffusely immunoreactive for NSE (Fig. 10), MAP-2, and 14–3–3 brain protein. The immunoreactivity for NSE of DGLCs was much stronger than that in normal cortical neurons. With anti-neurofilament antibody, numerous thick nerve fibers among DGLCs were strongly immunostained, and, in addition, the perikaryal cytoplasm of some DGLCs was diffusely immunoreactive (Fig. 11). With anti-synaptophysin antibody, the cytoplasmic surface of DGLCs was decorated by stippled, very fine positive granules (Fig. 12). With anti-GFAP antibody, DGLCs were not immunoreactive, but many thick glial fibers between DGLCs and the cytoplasm of hypertrophic or gigantic astrocytes were strongly immunostained. Nuclei of DGLCs were not immunoreactive for JCV VP1 antibody, but they were positively immunostained by anti-SV40 large T antigen antibody. The double immunostaining for large T antigen and neuronal markers clearly demonstrated that some of DGLCs having NSE- or 14–3–3 brain protein-positive cytoplasm had nuclei that were immunostained for large T antigen (Fig. 13). Nuclei of some DGLCs were immunoreactive for p53 protein (Fig. 14), but not for Ki-67.

ISH for mRNA of JCV large T antigen demonstrated that, in addition to the inclusion-bearing oligodendrocyte nuclei, the nuclei of some DGLCs also showed positive signals (Fig. 15). ISH for mRNA of large T antigen combined with immunohistochemistry for MAP-2 confirmed that certain of DGLCs, whose cytoplasm was immunoreactive for MAP-2, had nuclei showing positive signals for JCV large T mRNA. In these ISH experiments we chose a region that has relatively weak homology (54.4%) to the SV40 T genome as a probe, with RNAase treatment after hybridization that eliminates nonspecific binding to other mRNAs. Thus, it is highly likely that the signal is detecting JCV T genome.

**DISCUSSION**

The patient described in this report had been afflicted with common variable immunodeficiency (26, 27) since infancy and died of PML at the age of 21 after a 9-month neurological illness. Common variable immunodeficiency is a poorly defined, probably heterogenous group of disorders of humoral immunity having in common severe hypogammaglobulinemia (26, 27). It is usually sporadic and characterized clinically by variable degrees of impaired antibody responses. Circulating B cells are normal in number in most patients, but they are functionally defective and cannot differentiate into plasma cells producing Ig G or Ig A. Patients with this type of immunodeficiency are therefore prone to various infections, and diverse immunological and hematological disorders develop at later stages (26, 27). In the CNS, various types of encephalomyelitis occur frequently and enteroviruses are considered to be the major etiological agents (28).

There have been only a few reported cases of PML in association with common variable immunodeficiency (28, 33, 34) or congenital hypogammaglobulinemia (35).

This case of PML exhibited some atypical pathological features, and in particular, as shall be discussed later, the appearance of many DGLCs. First, the white matter lesions were very severe and showed a great tendency toward necrosis. The distribution of the lesions was diffuse and widespread, involving almost the whole white matter of the cerebrum, cerebellum, and brainstem. A multifocal, discrete nature, which is typical of PML lesions, was noted only in the spinal cord and cerebellar folia. The diffuse and unusually widespread distribution of the lesions might be related to the relatively long clinical course (9 months). Secondly, we found an unusually large number of oligodendrocytes infected with JCV in the deep cerebral cortical layers using the immunohistochemistry for JCV VP1. This finding suggests that perineuronal satellite oligodendrocytes were infected extensively with JCV. A similar finding has been reported by other investigators (7, 36, 37).

However, the unique and the most important neuropathological finding in the present case was the appearance of many large polygonal cells, DGLCs, in the cerebral cortex. These cells not only formed thick, band-like cellular aggregates in the occipital and temporal lobes but also were sparsely distributed in the cortex of the other areas. Immunohistochemical studies clearly demonstrated that these had a phenotype of neuron: they were immunoreactive for NSE, neurofilament, MAP-2, synaptophysin, and 14–3–3 brain protein. They were immunohistochemically negative for GFAP. To our knowledge, the appearance of many neuronal cells with features similar to those of DGLCs has never been documented in PML.

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**Fig. 5.** The normal architecture of the cerebral cortex was lost and replaced by thick band-like aggregates of numerous DGLCs, thick nerve fibers, and proliferating small blood vessels. Occipital cortex, H&E, ×25.

**Fig. 6.** DGLCs had large vesicular nuclei with prominent nucleoli and abundant cytoplasm, closely resembling “ganglion cells” seen in gangliocytomas or gangliogliomas. Occipital cortex, H&E, ×50.
Fig. 7. Coarse or fine, cresyl-violet-positive granules were seen in the cytoplasm of DGLCs. Occipital cortex, Nissl, ×100.

Fig. 8. Thick, randomly oriented, argyrophilic fibers were found between DGLCs. The cytoplasm of some DGLCs was also strongly argyrophilic. Occipital cortex, modified Bielschowsky, ×50.

Fig. 9. An unusually large number of oligodendrocytes in the deep layers of the cortex were immunostained by an anti-JCV VP1 antibody. Occipital cortex, ×50.

Fig. 10. The cytoplasm of DGLCs was strongly immunoreactive for NSE. Occipital cortex, ×50.

Fig. 11. Thick fibers between DGLCs and also the perikarya of some DGLCs were immunoreactive for neurofilament. A multinucleated, bizarre astrocyte, which is not immunoreactive, is seen in the lower center. Temporal cortex, ×100.

Fig. 12. The cytoplasmic surface of DGLCs was decorated by very fine granules that were immunoreactive for synaptophysin. Occipital cortex, ×100.

Fig. 13. Double immunostaining. Nuclei of some DGLCs were immunoreactive for large T antigen and the cytoplasm for 14–3–3 brain protein. Occipital cortex, ×200.
The possibility that the aggregates of these DGLCs represent a neoplasm, such as gangliocytoma or ganglioglioma, or a kind of congenital malformation that was pre-existent incidentally, needs to be scrutinized carefully. Each DGLC showed a close morphological resemblance to dysplastic or dysmorphic ganglion cells seen in gangliocytoma or ganglioglioma, and, on the other hand, the thick, band-like aggregates of DGLCs had caused a distinct cytoarchitectural derangement of the involved cortex which was reminiscent of focal cortical dysplasia (FCD) (38).

Although the cell density of DGLCs appeared to be higher than the normal neuronal population in the involved cortex, the possibility that these aggregates of DGLCs represent a gangliocytoma or ganglioglioma is unlikely for the following reasons. First, DGLCs did not form a grossly distinct nodular lesion. Secondly, despite the appearance of many DGLCs, the patient did not have any neurological or psychiatric symptoms and signs (including the history of seizures) until the onset of PML. 9 months prior to death and the CT scan and MRI did not reveal any abnormalities suggestive of the presence of a neoplasm.

The possibility that these DGLCs represent a congenital malformative lesion such as FCD (38) cannot be excluded in view of the apparently high-density, haphazard orientation and dysplastic or dysmorphic features of DGLCs. The absence of the history of seizures and neuroradiological abnormalities does not necessarily rule out the presence of a malformative lesion. However, as shall be discussed below, the fact that the neuronal infection with JCV was detected only in DGLCs but not in the surrounding normal cortical neurons suggests that the development of the DGLCs could be related to the transforming capability of JCV and argues against the view that they had been present prior to the onset of PML, although abnormal dysplastic neurons in a malformative lesion could have been more susceptible to JCV infection.

Another possibility, albeit remote, is that this lesion could be an exaggerated form of the cortical tubers that are seen in cases of tuberous sclerosis (39). However, the histopathological features in the present case are unlike those of the classical tubers. Cortical tubers usually contain, in addition to neuronal cells, many giant eosinophilic cells that show divergent glioneuronal differentiation (39). Furthermore, the present patient and members of his family did not have any stigmata suggestive of tuberous sclerosis.

The DGLCs could be unequivocally demonstrated to be infected with JCV. Nuclei of DGLCs were immunohistochemically positive for anti-SV40 large T antigen. T antigen is known to be well conserved among polyomaviruses and T antigen of JCV cross-reacts with that of SV40 (16). Using a probe for mRNA of JCV large T antigen, ISH showed positive signals in nuclei of DGLCs. In PML, the cell population in the CNS, which is predominantly infected with JCV, has been clearly shown to be oligodendrocytes (1, 6, 7, 33). Astrocytes are also known to rarely contain virions or viral genome (4–7, 18) or to express large T antigen (33). In addition, Houff et al (40) have described JCV-infected B lymphocytes in the Virchow-Robin spaces. The possibility of neuronal infection by JCV has been extensively investigated and most investigators have been unable to demonstrate the presence of virions or viral genome in neuronal nuclei (6, 7, 36, 41, 42). However, there have been several reports that have suggested the possibility of neuronal infection with JCV. Among the earlier studies, Richardson (3) noted a peculiar nuclear enlargement of cerebellar granule cells in 3 out of 10 cases of PML. A similar alteration of granule cells was subsequently described by Kuchelmeister et al (43), although they could not detect JCV DNA in these cells with ISH. Mázdó and Stoner (8) detected cerebellar granule cells with darkly stained nuclei and an accumulation of amorphous material in the nucleoplasm of these abnormal granule cells in a case of PML in acquired immune deficiency syndrome. This result suggested an abortive or latent infection of granule cells with JCV. With regard to infection of cerebral neurons, Chou et al (9) reported the presence of viral particles ultrastructurally in neuronal nuclei, and Nakamura et al (10) found papovavirus DNA in cortical neurons of apparently normal configuration with ISH. Moreover, the human neuroblastoma cell line IMR-32 has been shown to be susceptible for JCV proliferation in vitro (44). We demonstrated clearly the occurrence of the infection of cerebral cortical neurons with JCV in the present case, although this must be considered to be an exceptionally rare event.

DGLCs in the present case expressed large T antigen but not JCV capsid protein VP1, indicating that the replication of virus had not occurred in the nuclei of the DGLCs (45). This finding contrasts with that of oligodendrocytes that express both T antigen and VP1 protein, and suggests different mechanisms of infection in oligodendrocytes and neurons, one being cytolytic and the other transforming. Experiments using JCV T antigen transgenic mice have demonstrated that in the absence of viral

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**Fig. 14.** Nuclei of some DGLCs were immunoreactive for p53 protein. Occipital cortex, ×200.

**Fig. 15.** ISH for mRNA of JCV large T antigen. Nuclei of some DGLCs showed positive signals. Temporal cortex, ISH with eosin, ×200.
replication, T antigen is capable of transforming the infected cells and inducing neoplasms (46, 47). Furthermore, nuclei of these DGLCs showed an immunoreactivity for p53 protein. p53 protein is a tumor suppressor gene product that downregulates the cell cycle, and it is known that in PML, JCV T antigen causes bizarre morphological alterations of the infected astrocytes through its binding with and the subsequent stabilization/inactivation of the p53 protein (48–50). Taken together, it is reasonable therefore to consider that, in the present case, JCV could have infected the cerebral cortical neurons and, in the failure of the expression of structural protein genes in the neuronal nuclei, large T antigen could have caused a derangement of the control of cell growth by binding to intranuclear p53 protein, thus inducing a phenotypic transformation of the infected neurons into DGLCs. An apparently high cell density of DGLCs in comparison with the normal neuronal population is difficult to explain. It may be due to an actual proliferation of these dysplastic neuronal cells. However, another possibility, which was mentioned earlier, that the latent infection with JCV occurred in dysplastic neurons in a malformative lesion, cannot be denied, and the elucidation of this problem awaits an accumulation of additional reports of similar cases.

The infection of neurons with JCV and the possible subsequent transformation of them into DGLCs found in the present case has some important pathological implications in view of the well-known oncogenic potential of JCV in the CNS of experimental animals (13). Glioblastoma (11–13), cerebral neuroblastoma (13), and primitive neuroectodermal tumor resembling medulloblastoma (13–16) have been produced by inoculating JCV in the brains of animals, and in these JCV-induced brain tumors, large T antigen is expressed in the tumor cells without the production of virions (12, 14). In humans, at least 3 autopsy cases have been reported in which multiple malignant astrocytomas developed in brains having lesions of PML (17–19), but no neoplasms of neuronal origin have been found in association with PML.

Although most studies have failed to detect either viral DNA sequences or the expression of large T antigen in human CNS neoplasms (51–53), a few recent reports have provided findings suggestive of an etiologic role of SV40 or JCV in the pathogenesis of various kinds of human glial neoplasms (54, 55). Rencic et al (23) demonstrated the presence of JCV DNA and RNA by PCR and detected immunohistochemically large T antigen in tumor cells in a case of oligoastrocytoma. The presence of JCV DNA in tumor cells was also detected in a case of pleomorphic xanthoastrocytoma (24) and many cases of medulloblastoma (25). These results indicate that JCV and SV40 viruses may play important etiopathogenetic roles in certain human glial and primitive neuroectodermal neoplasms. However, low frequency of SV40, JC, and BK polyomavirus sequences in human medulloblastomas, meningiomas, and ependymomas have also been published recently (56). Although attempts to discover JCV genome in human CNS neoplasms of neuronal origin have been unsuccessful, the finding of DGLCs containing JCV mRNA in the nuclei, as in the present case, raises the possibility that JCV does play a role in the genesis of some neuronal neoplasms, and especially gangliocytoma and ganglioglioma. This possibility should be investigated more extensively using more sensitive techniques.

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