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Detection of JC virus DNA sequence and expression of the viral oncoprotein, tumor antigen, in brain of immunocompetent patient with oligoastrocytoma

(oligodendroglioma/viral oncoproteins)

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ABSTRACT **We describe molecular and clinical findings in an immunocompetent patient with an oligoastrocytoma and the concomitant presence of the human papovavirus, JC virus (JCV), which is the etiologic agent of the subacute, debilitating demyelinating disease, progressive multifocal leukoencephalopathy. Histologic review revealed a glial neoplasm consisting primarily of a moderately cellular oligodendroglioma with distinct areas of a fibrillary astrocytoma. Immunohistochemical analysis revealed nuclear staining of tumor cells with antibodies against the viral oncoprotein [tumor antigen (T antigen)], the proliferation marker (Ki67), and the cellular proliferation regulator (p53). Using primers specific to the JCV control region, PCR yielded amplified DNA that was identical to the control region of the Mad-4 strain of the virus. PCR analysis demonstrated the presence of the genome for the viral oncoprotein, T antigen, and results from primer extension studies revealed synthesis of the viral early RNA for T antigen in the tumor tissues. The presence of viral T antigen in the tumor tissue was further demonstrated by immunoblot assay. To our knowledge, this is the first report of the presence of JCV DNA, RNA, and T antigen in tissue in which viral T antigen is localized to tumor cell nuclei and suggests the possible association of JCV with some glial neoplasms.**

There are many genetic alterations that can be detected in human brain tumors. Recent observations have established that the authentic papovavirus simian virus 40 (SV40) is associated with some forms of human brain tumors, including choroid plexus and ependymomas (1, 2), loss of heterozygosity for chromosomes 1p, 9q, 10, 13q, 17p, and 19q, amplification of the epidermal growth factor receptor gene, and alterations of the p53 gene, including both mutations and increased accumulation of wild-type p53, have all been detected in various glial tumors (3, 4). In addition, amplification of N-*myc*, *gli*, platelet-derived growth factor receptor, and *mdm2* genes have been detected (5, 6). Although there has been much progress in the characterization of molecular mechanisms of tumorigenesis in glial neoplasms, the epidemiologic factors contributing to the development of these tumors is still unclear. In addition to detection of SV40 in human brain tumors, several sporadic reports (7, 8) have described the presence of the human neurotropic papovavirus, JC virus (JCV), in multiple malignant astrocytomas and primary cerebral malignant lymphoma.

JCV is the etiologic agent of the fatal brain demyelinating disease, progressive multifocal leukoencephalopathy (PML) (9, 10). PML occurs primarily in adults with immunosuppressive conditions and frequently presents with rapidly progressing dementia and weakness. The histopathological changes in PML consist of lytic destruction of oligodendrocytes with resultant foci of demyelination and relative sparing of neuronal cell bodies and axons, intranuclear inclusions in oligodendrocytes, and bizarrely appearing astrocytes.

Similar to other papovaviruses, the genome of JCV consists of three functional domains, the early and the late coding regions separated by the transcriptional control region, which has been shown to be the major determinant of the tropism of this virus for neuroectodermally derived tissues (11–16). The late region encodes capsid proteins produced late in the JCV lytic cycle, whereas the early region encodes the multifunctional oncoprotein, large tumor antigen (T antigen) (17).

Transgenic mouse studies suggest that expression of the viral T antigen may also contribute to the pathology seen in PML. Transgenic mice harboring the regulatory region and T antigen of JCV exhibit immature oligodendrocytes, hypomyelination, and dysmyelination (18–20). Thus, it seems that JCV may cause demyelination directly through the cytolytic infection of oligodendrocytes and indirectly by T antigen-mediated inhibition of myelin production. T antigen-induced dysmyelination may result from a block in maturation of oligodendrocytes and/or from suppression of the genes involved in myelin synthesis. In support of the latter concept, recent studies have indicated that T antigen from SV40, through a regulatory mechanism that includes c-*jun*, may down-modulate production of the myelin-associated P_0 protein (21).

Like the well-characterized papovavirus SV40, JCV also has tumorigenic potential, which is attributed to its early protein, T antigen. The transforming potential of JCV T antigen is likely mediated through its interaction and functional inactivation of tumor suppressor proteins including p53 and the retinoblastoma susceptibility gene product (pRB). These two proteins play integral roles in control of cellular proliferation. As evidenced by coimmunoprecipitation assays in JCV-transformed glial cell lines, the viral early protein interacts with pRB, p53, and p107, which is another member of the pRB family (22–25). This is important in light of previous reports on the association of the retinoblastoma gene product in malignant progression of astrocytoma (26). In addition to its ability to transform cells, expression of JCV proteins in experimental animals induces formation of tumors of neural origin. Intracerebral inoculation of JCV into Syrian hamsters results in cerebellar medulloblastomas, glioblastomas, malignant astrocytomas, and primitive neuroectodermal tumors (27–31). Intracranial inoculation of JCV into owl monkeys led to brain tumor development, primarily malignant astrocytomas, with

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Abbreviations: SV40, simian virus 40; JCV, JC virus; PML, progressive multifocal leukoencephalopathy; T antigen, large tumor antigen.

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one or more integrated copies of the viral genome detected. T antigen was detected in the tumor tissue, as well as in tumor cells that were cultured (32, 33). Four out of five transgenic mouse lines containing the JCV regulatory and early coding regions developed adrenal neuroblastomas, in which high levels of JCV T antigen were present (18). Here we report a case of an immunocompetent patient with an oligoastrocytoma whose histopathological appearance suggested the association of JCV with this neoplasm. Molecular characteristics of the tumor tissue indicated the presence of JCV DNA and expression of the JCV early gene in tumor tissue.

MATERIALS AND METHODS

Tissue Material and Histochemistry. The partial left frontal lobectomy specimen examined by light microscopy either was immediately frozen at −70°C or was fixed in 10% buffered formalin, then embedded in paraffin, and 6-μm sections were cut for structural and immunohistochemical analysis. Paraffin sections were stained with hematoxylin and eosin or luxol fast blue and cresyl violet for histochemical analysis. Immunostaining was performed using the avidin–biotin–peroxidase complex system according to the manufacturer's instructions (Vectastain Elite ABC-Peroxidase Kit, Vector Laboratories). Immunohistochemistry to detect p53 and Ki67 was performed on formalin-fixed paraffin-embedded tissue. Briefly, 6-μm sections were deparaffinized in xylene, incubated in a methanol/3% H_2O_2 solution for 30 min, and rehydrated through graded ethanol to distilled water. For nonenzymatic epitope retrieval, the sections were microwaved in citrate buffer solution (pH 5.5, Biotek Buffer no. 2; Biotek, Santa Barbara, CA) at 800 W for 5 min on high energy setting. Buffer no. 2 was then diluted in distilled water to compensate for evaporation and the samples were microwaved for an additional 5 min. The sections were allowed to cool for 20 min and then rinsed in distilled water followed by PBS. The samples were then blocked with 2% horse serum for 20 min, followed by incubation with antibody to p53 (Oncogene Science), antibody to Ki67 (Immunotech, Westbrook, ME), or normal sera to serve as a control for antibody specificity (data not shown). Incubations were carried out overnight at 4°C in a humidified chamber. Next, biotinylated horse anti-mouse IgG and avidin-biotin peroxidase complex steps were performed according to the manufacturer's instructions (Vector Laboratories). Finally, the sections were developed with DAB substrate (0.02% diaminobenzidine and 0.005% hydrogen peroxide), counterstained with hematoxylin, and mounted.

Immunohistochemistry to detect T antigen was performed on 10-μm-thick frozen sections. Sections were fixed in absolute methanol for 10 min, air dried, rehydrated in PBS, and incubated in PBS with 3% H_2O_2 for 30 min to block endogenous peroxidase activity. Sections were then subjected to nonenzymatic epitope retrieval as described and were then processed with the Vector Elite Mouse IgG Kit according to the manufacturer's instructions (Vector Laboratories). Primary antibody incubation was performed with antibody to SV40 T antigen, which is cross-reactive with JCV T antigen (clone 416, Oncogene Science), or normal sera (data not shown) for 3 h at 37°C. Finally, sections were developed with DAB substrate, counterstained with hematoxylin, and mounted.

PCR Amplification and Viral DNA Analysis. DNA was extracted from sections of paraffin-embedded samples. Tissues were deparaffinized by extraction first with xylene and then with ethanol. After deparaffinization, the tissue was digested with proteinase K (2 mg/ml) from 12 to 18 h in buffer containing 150 mM NaCl, 10 mM Tris·HCl, 1 mM EDTA (pH 8.0), and 0.5% SDS. After phenol/chloroform extraction and ethanol precipitation, DNA samples were used in PCR amplification using two sets of primers derived from JCV Mad-4 (nucleotides 4987–5006, 5′-TTCCTCCCTATTCAGCACTT-3′; nucleotides 219–238, 5′-AAAACAGCTCTGGCTCGCAA-3′) with the potential to produce a 381-bp DNA fragment encompassing the control region of the virus. The second set of primers (nucleotides 2808 to 2828, 5′-CCCCATACCAACATTAGCTTTC-3′; nucleotides 3031 to 3050, 5′-CCAGATTTGTAAGGCAGATAG-3′) amplify a 242-bp fragment corresponding to the viral early genome. PCR was performed in a total volume of 50 μl containing 50 mM KCl, 10 mM Tris·HCl (pH 8.3), 2.5 mM $MgCl_2$, 20% glycerol, 200 mM of each dNTP, 10 pM of each primer, 2.5 units of *Taq* DNA polymerase (Boehringer Mannheim), and 50 μl of mineral oil overlay per reaction. The reactions were denatured for 5 min at 94°C before the addition of *Taq* polymerase. Subsequently, amplification was carried out on a DNA thermal cycler (Perkin–Elmer) for 40 cycles of 1 min each for denaturation, annealing, and extension at 94, 55, and 72°C, respectively. Both negative and positive control reactions were carried out using genomic DNA from normal mice or transgenic mice containing the JCV early region, respectively. The PCR product encompassing the control region was cloned into the pCRII vector using the TA Cloning Kit according to the manufacturer's instructions (Invitrogen). In addition, a Southern blot analysis was performed in which the T antigen PCR product was then blotted and probed using 10^6 cpm/ml ^{32}P-labeled linearized JCV plasmid DNA (34).

Primer Extension Analysis. Primer extension reactions were performed using 50 μg of total RNA isolated by the method of Chomczynski and Sacchi (35). The cell line HJC, derived from a hamster brain tumor expressing JCV T antigen, was used as a positive control. The reactions were hybridized overnight at 37°C with 10^6 cpm of 5′-labeled primer 5′-GGAATGTTCCCCCATGCAGACCTATCAAGG-3′ per reaction in 400 mM NaCl, 40 mM Pipes (pH 6.5), 1 mM EDTA, and 80% formamide (pH 6.1). After hybridization, the samples were then precipitated and treated with 50 units of avian myeloblastosis virus reverse transcriptase at 42°C for 1 h in 50 mM Tris (pH 8.0), 5 mM MgCl, 5 mM DTT, 50 mM KCl, 50 mg/ml BSA, and 160 mM of each dNTPs. After phenol/chloroform extraction, the products were resolved on a 6% denaturing acrylamide gel and visualized by autoradiography.

Immunoprecipitation/Western Blot Analyses. Immunoprecipitation/Western blot analyses were performed using extract made from 500 μg of tissue homogenized in TNN buffer containing 50 mM Tris (pH 7.5), 150 mM NaCl, and 0.05% Nonidet P-40 plus protease inhibitors (aprotinin at 2 mg/ml, leupeptin at 10 mg/ml, pepstatin at 10 mg/ml, phenylmethylsulfonyl fluoride at 100 mg/ml, L-1-tosylamido-2-phenylethyl chloromethyl ketone at 100 mg/ml). Protein (150 μg) in 150 μl of TNN buffer was incubated overnight with 5 μl of SV40 T antigen antibody (clone 416, Oncogene Science), which is cross-reactive for JCV T antigen. The samples were then incubated with 50 μl prewashed Pansorbin (Calbiochem) for 1 h at 4°C. After incubation, the Pansorbin was pelleted by centrifugation, the pellets were washed in TNN, and then resuspended in 25 μl of SDS/PAGE sample buffer containing 200 mM Tris (pH 6.8), 8% SDS, 40% glycerol, and 100 mM DTT, and eluted at room temperature for 15 min. After fractionation of the protein samples on a 1.5 mm 12% SDS/PAGE minigel, proteins were transferred to nylon-supported nitrocellulose (Schleicher & Schuell) in transfer buffer containing 192 mM glycine, 25 mM Tris base, and 20% methanol, and the transblot was incubated for 1.5 h at room temperature in TTBS solution containing 0.1% Tween-20, 100 mM Tris (pH 7.5), 0.9% NaCl, and 0.5% dry milk. After blocking, the blot was incubated with SV40 T antigen antibody (clone 416, Oncogene Science) (1:50 dilution) for 1 h at room temperature, followed by incubation with goat-anti-mouse IgG conjugated to alkaline phosphatase (Pierce) for 1 h at room temperature. Proteins were then visualized using the Alkaline Phosphatase Substrate Kit according to the manufacturer's instructions (Vector Laboratories).

RESULTS

Clinical and Pathological Analyses. A computerized tomography scan of a 61-year-old immunocompetent, HIV-1 negative man experiencing a grand mal seizure revealed a left frontal diffuse noncontrast enhancing lesion that upon biopsy was classified as a low grade oligoastrocytoma. Frequent MRI studies were performed and results showed an increase in the size of the lesion. A T1 weighted (T1WI) MR scan revealed a hypointense lesion within the left frontal lobe (Fig. 1). This mass extended inferiorly in the superior left temporal lobe. Gadolinium-enhanced T1WI demonstrated some new areas of enhancement. The tumor was observed to cause mass effect as evidenced by subfalcine herniation, effacement of sulci, and moderate compression of the left lateral ventricle. Proton density and T2 weighted images were consistent with tumor and diffuse peritumoral edema (data not shown). The patient remained seizure-free and free of neurologic symptoms during the time period of the radiographically documented increase in the size of the lesion. Serological studies indicated immunocompetence as the patient's T and B lymphocytes and CD4 to CD8 ratio were all within expected normal values according to Centers for Disease Control and Prevention (Atlanta) guidelines (48) for T cell determinations.

On the basis of the increase in the size of the lesion, a left frontal lobectomy was performed. Pathologic examination of the left frontal lobe tumor specimen revealed a mixed glial neoplasm consistent with an oligoastrocytoma in the World Health Organization (Geneva) brain tumor classification system (36). The major portion of the specimen consisted of an oligodendroglioma that infiltrated white matter and cortical grey matter. The tumor was composed of neoplastic oligodendroglia with minimal cytoplasm and round-to-ovoid, slightly irregular nuclei and vesicular chromatin (Fig. 2*A*). Focally, the tumor consisted of distinct areas of fibrillary astrocytes. Approximately 10–15% of tumor cells in the oligodendroglial portions of the tumor showed nuclear staining with the proliferation marker, Ki67 (Fig. 2*B*), signifying a high proliferation index for this brain tumor. The particular feature of this tumor that led us to analyze this neoplasm for the presence of JCV was large areas of hypomyelinated white matter in areas of tumor infiltration adjacent to more normally myelinated areas (Fig. 2*C*). Neoplastic oligodendroglia in these areas of hypomyelination and in other portions of the tumor demonstrate strong nuclear staining with monoclonal antibodies to viral T antigen (Fig. 2*D*). Greater than 50% of tumor cells in the oligodendroglial portions of the tumor showed positive T antigen nuclear staining. No detectable T antigen nuclear staining was observed in the astrocytic areas of the tumor. Immunohistochemical staining for the cellular proliferation regulator, p53, showed nuclear staining in less than 2% of tumor cells (data not shown).

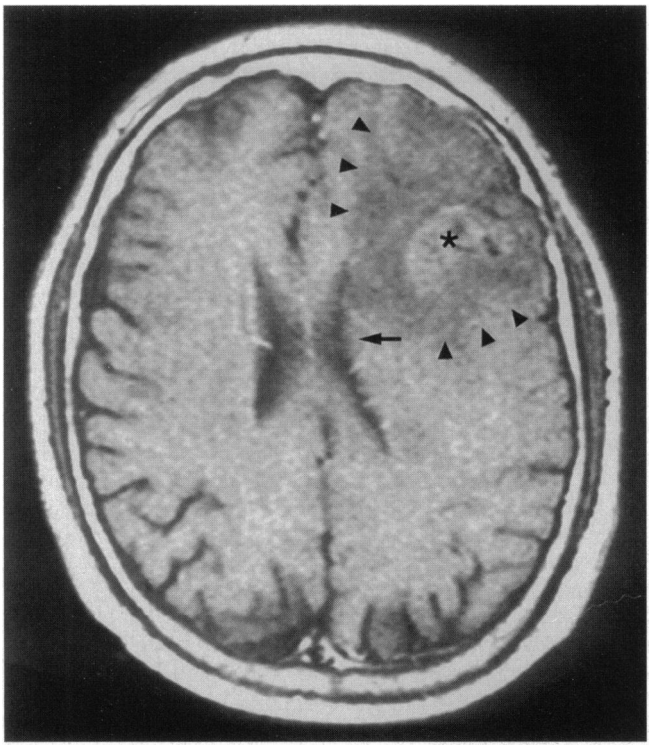

FIG. 1. A spin echo axial T1 weighted image (TR/TE/NEX, 586/20/4) of the patient's brain. Shown is an area of low signal within the left frontal lobe (arrowheads). There is mild compression of the left lateral ventricle (arrow) with a mild-to-moderate subfalcine herniation. Within the center of the lesion there is heterogeneous enhancement with small focal areas of low signal surrounding the enhancing tumor mass (asterisk) is consistent with edema and peritumoral demyelination.

Biochemical and Molecular Biological Analyses. JCV shares significant genome homology, in particular in the protein coding regions, with other polyomaviruses including SV40 and BK virus (37). However, the greatest degree of divergence between JCV and the other polyomaviruses rests in the control region of JCV, which encompasses two 98-bp tandem enhancer repeats in the Mad-1 strain. The most common JCV isolate from PML patients is Mad-4, which exhibits oncogenic properties in experimental animals and has a 19 nucleotide deletion in one copy of the 98-bp enhancer repeats. As described, immunohistochemical analysis of the brain biopsy revealed expression of papovavirus T antigen, most likely from JCV, in tumor cells. To assess the identity of the participant virus, we determined the structural organization of the viral regulatory region. DNA isolated from an area of the tumor was used as a template in a PCR amplification reaction directed with specific primers flanking the JCV control region. The amplified product comigrated with the DNA fragments of 381 bp in size (Fig. 3*A*). The amplified product was isolated and its nucleotide composition was determined by direct sequencing. Analysis of the primary structure from the amplified DNA revealed complete sequence homology to the control region of JCV Mad-4 isolate (Fig. 3*B*).

The presence of the DNA sequence encoding the JCV T antigen in tumor tissue was verified by PCR amplification of a 242-bp DNA fragment using specific primers from the JCV early region (Fig. 4*A*). In this study, genomic DNA from various areas of the biopsy was examined. The identity of the PCR product was confirmed by hybridization using a [^{32}P]-labeled DNA fragment derived from the early region of JCV as a probe and direct sequencing of the PCR product. As shown in Fig. 4*B*, a high level DNA fragment of 242 bp in length was obtained with DNA derived from the tumor area (lanes 1 and 2). The differences in the intensity of the bands observed in lanes 2–5 may be attributed to the unequal distribution of viral DNA within samples prepared from different areas of the tumor specimen. In each of these reactions, negative controls rule out the possibility of laboratory contamination.

To investigate the level of JCV early RNA in tumor tissue, we performed primer extension assays. Toward this end, RNA isolated from two separate areas of tumor tissue was hybridized to the specific [^{32}P] 5′-labeled probe spanning nucleotides 4961 to 4931 as depicted in Fig. 4*A*, and after synthesis of the complementary DNAs by reverse transcriptase the products were analyzed by denaturing urea gel. In this study, RNA from the JCV-induced glioblastoma hamster cell line, HJC, which constitutively produces viral early RNA, served as a positive control. Results from this experiment revealed that, with the exception of two transcripts (5122 and 5082), the other major transcripts of JCV T antigen (5047, 5037, and 5012) (38), which comigrate with those from HJC, are produced in tumor cells.

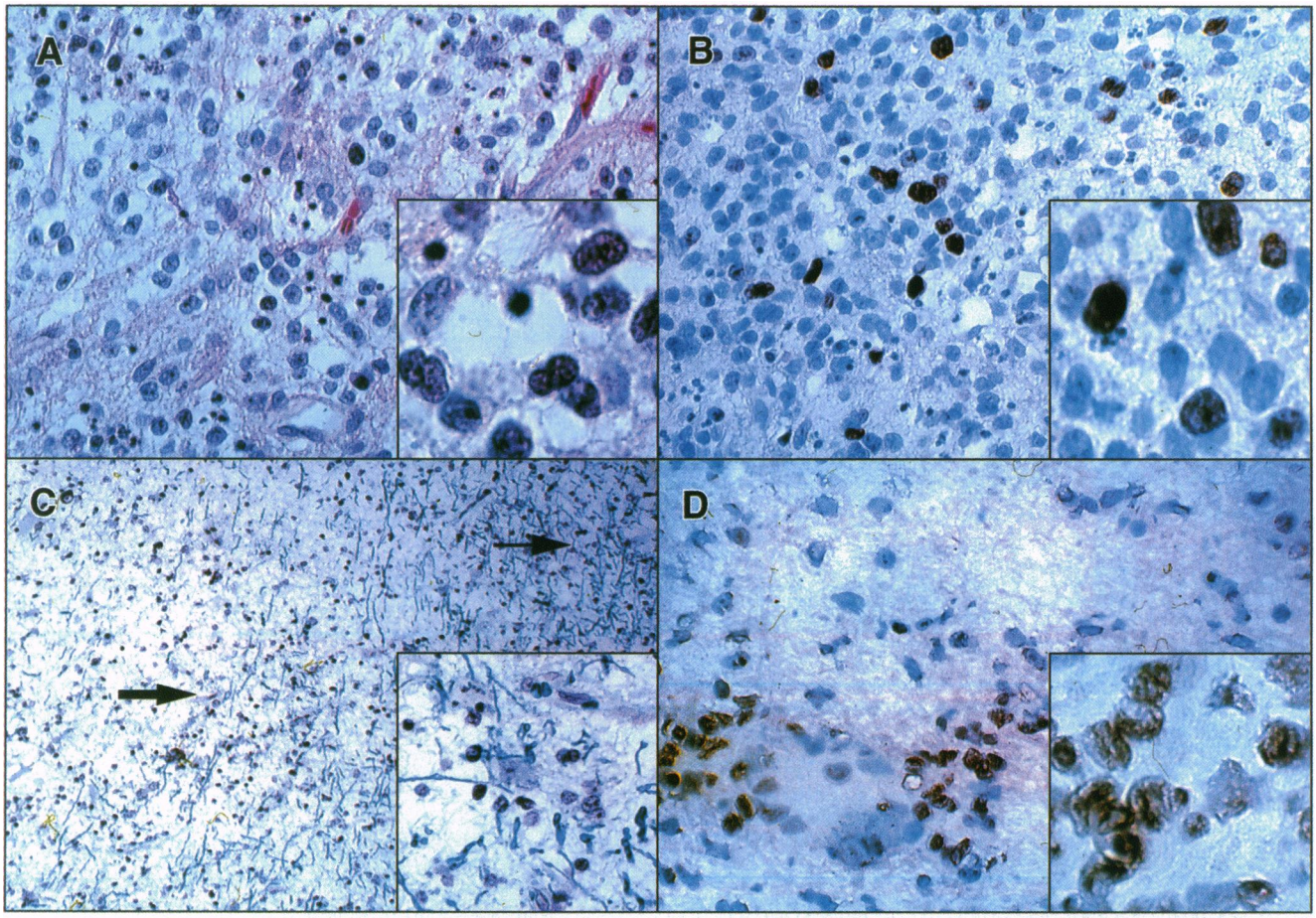

FIG. 2. Hematoxylin and eosin staining and immunohistochemistry for the presence of T antigen in tumor tissue and adjacent white matter. (*A*) The oligodendroglial portion of the tumor showed a homogenous population of tumor cells with round to ovoid nuclei containing vesicular chromatin (hematoxylin and eosin, ×400; *Inset*, ×1000). (*B*) Approximately 10–15% of tumor cells in the oligodendroglial portions of the tumor showed nuclear staining with the proliferation marker, Ki67 (DAB with hematoxylin, ×400; *Inset*, ×1000X). (*C*) Portions of the white matter infiltrated by tumor showed decreased density of myelin (thick arrow) compared with adjacent areas of tumor-infiltrated white matter (thin arrow) (Luxol fast blue, ×200). (*Inset*) Area of decreased myelin density (Luxol fast blue, ×400). (*D*) Approximately 50% of tumor cells in oligodendroglial portions of the tumor showed nuclear staining with immunohistochemical stain to viral T antigen (DAB with hematoxylin, ×400; *Inset*, ×1000).

An additional transcript initiating at 5000, which to our knowledge has not been reported previously, is present in HJC cells and in both tissue samples.

Next, immunoprecipitation/Western blot analysis was performed on extract prepared from several areas of the tumor, one of which demonstrated the presence of JCV T antigen

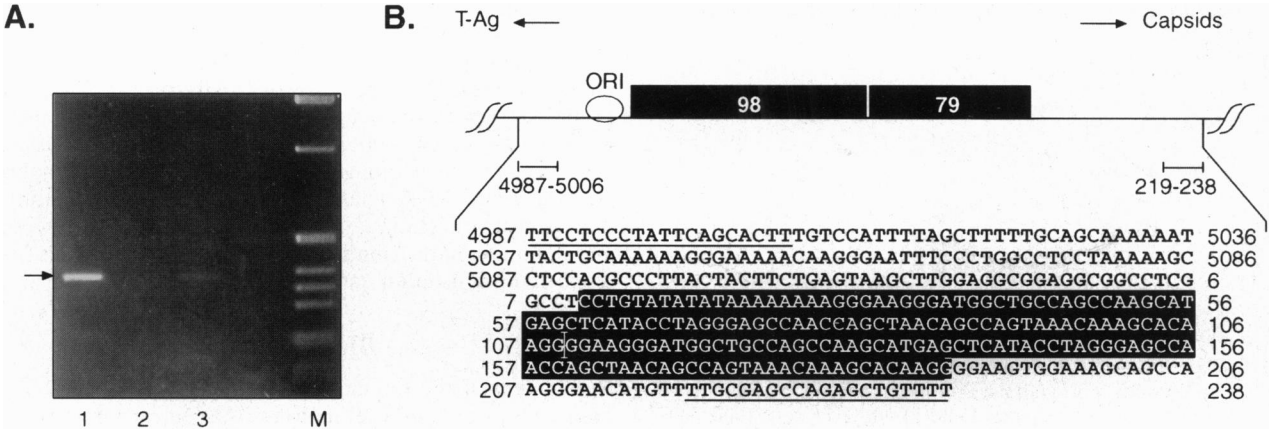

FIG. 3. PCR amplification of JCV regulatory sequence from tumor tissue. (*A*) PCR amplification of the JCV regulatory region from genomic DNA isolated from the tumor tissue. Lanes: 1, (positive control) 381 base pair product amplified from genomic DNA of transgenic mice containing the JCV early region; 2, (negative control) no product present (template DNA used was mouse genomic DNA); 3, 381-bp product amplified from genomic DNA isolated from the tumor tissue; M, 1-kB ladder DNA marker. (*B*) Schematic diagram depicting the regulatory region of the JCV Mad-4 strain. The PCR product from lane 3 of *A* was sequenced and found to have 100% homology to the JCV Mad-4 regulatory region. The primers used (4987–5006 and 219–238), the origin of replication (ORI), the direction of transcription for the capsid proteins and for the viral T antigen, and the modified 98-bp repeat are all denoted.

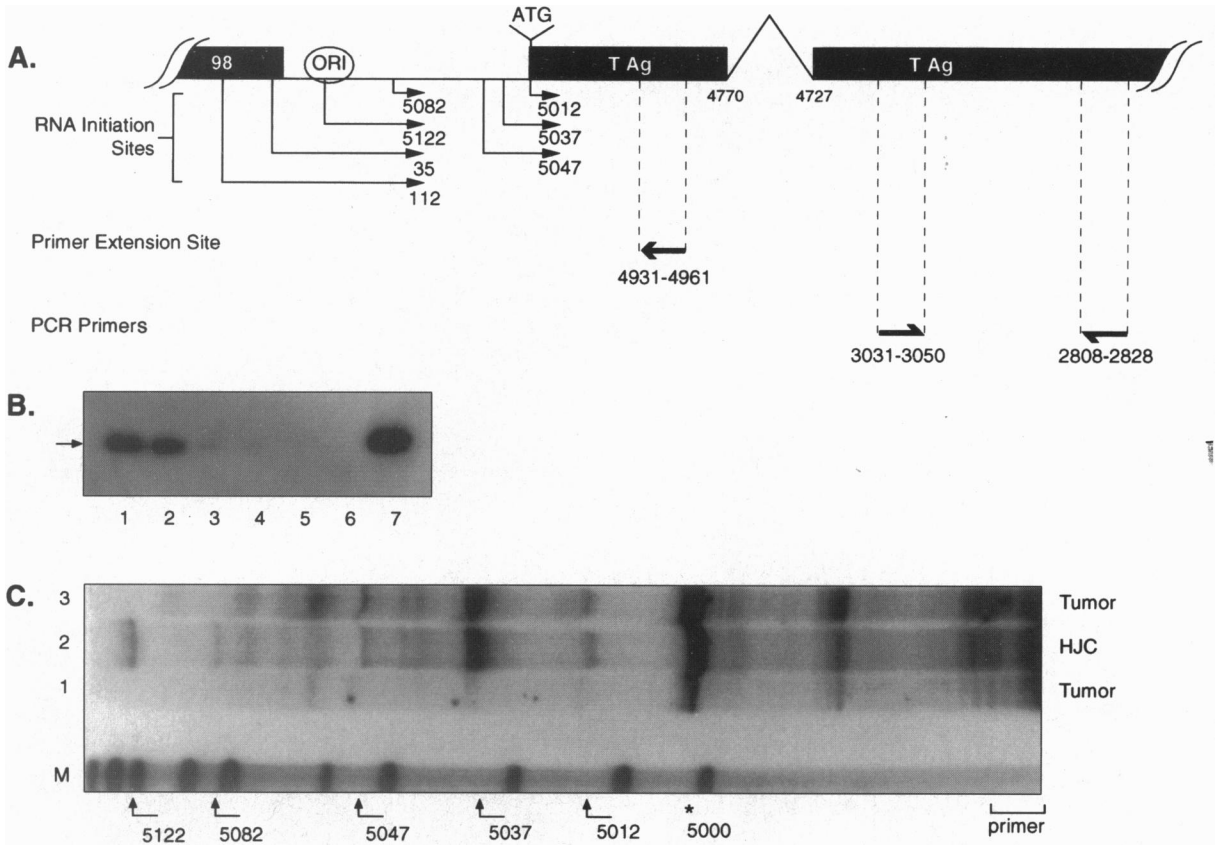

FIG. 4. Detection of T antigen DNA and primer extension of JCV early RNA. (*A*) A schematic diagram depicting the transcription and translation initiation sites for the viral oncoprotein large T antigen. The site of the primer used for primer extension (4931–4961) and the PCR primers used for amplification of a portion of the T antigen encoding DNA (3031–3050 and 2808–2828) are indicated. (*B*) Southern blot for detection of T antigen DNA sequences. Lanes 1–5 contain 242-bp PCR product amplified from a region of genomic DNA encoding T antigen. The template for each lane is from different preparations of genomic DNA from several areas of tumor tissue. Lanes: 6, (negative control) no product present (template DNA used was mouse genomic DNA); 7, (positive control) using genomic DNA of transgenic mice containing the JCV early region. (*C*) Primer extension was performed to detect JCV T antigen RNA. Lanes 1 and 3 contain transcripts produced from 50 μg of total RNA from the patient's tumor. Lane 2 is a positive control containing transcripts produced using 50 μg of total RNA from the JCV T antigen-containing hamster glial cell line, HJC. The various T antigen transcripts are indicated with arrows. The 30 nucleotide primer is seen to the right. Lnae M, DNA marker that includes the DNA fragments obtained upon treatment of pBR322 with *Msp*I followed by [^{32}P]-end-labeling at the 5′ end by T4-polynucleotide kinase.

comigrating with the T antigen present in the JCV T antigen transformed cell line, HJC (Fig. 5). Of note, the tissue sample used in lane 3 represents a section adjacent to that analyzed by immunohistochemistry (Fig. 2*D*), an area confirmed by histology to be of oligodendroglial origin. In addition, low but detectable levels of T antigen in the sample shown in lane 3 may be attributed to a low density of T antigen positive tumor cells in oligodendroglial regions of the tissue or variability in the section of tumor used for preparation of the protein extract. The protein sample analyzed in lane 2 may have contained less T antigen positive cells or may have been prepared from a more heterogenous region of tissue so that the level of T antigen was not in the detectable range by this assay. These observations along with the data from immunohistochemistry and RNA analysis suggest that a combination of more sensitive techniques should be performed to detect low and nonuniform distribution of T antigen positive cells in the suspected brain tumor samples.

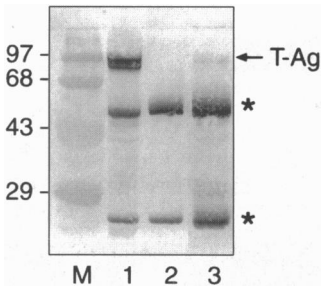

FIG. 5. Detection of JCV T antigen in tumor tissue by immunoprecipitation/Western blot analysis. Protein prepared from two distinct regions of tumor tissue (lanes 2 and 3) and HJC cells (lane 1) (transformed with JCV T antigen) was immunoprecipitated with antibody for SV40 T antigen, which is cross-reactive for JCV T antigen, and the samples were separated by SDS/PAGE. After transfer to nitrocellulose, Western blot was performed using the same anti-SV40 T antigen antibody and detected by alkaline phosphatase. The arrow indicates position of JCV T antigen; M indicates molecular weight protein marker; asterisks indicate IgG heavy and light chains from immunoprecipitation and serve as a control for loading.

DISCUSSION

Replication of the human neurotropic papovavirus, JCV, in brain cells leads to demyelination of the central nervous system as a result of productive infection and replication of the virus in oligodendrocytes, the myelin-producing cells of the central nervous system. Evidently, T antigen, the viral-encoded protein that is produced at the early phase of infection, orchestrates various biological events required for replication of viral DNA and expression of the viral capsid proteins (39). T antigen is an oncoprotein that, in the absence of the entire viral

genome and/or viral DNA replication, has the ability to induce transformation of cells *in vitro* and to cause formation of neuroectodermal origin tumors in experimental animals (23, 40). Previous observations have demonstrated that due to species-specific restrictions of the viral DNA polymerase, JCV DNA cannot efficiently replicate in rodents (41) where the virus is highly oncogenic. Thus, one could speculate that in the absence of viral DNA replication and/or virion formation, T antigen synthesis may promote a cascade of events leading to tumor formation. Because defects in viral replication may also be mediated by sequence alterations in the origin of viral DNA replication, we closely inspected the composition of the viral control region by direct sequencing of the amplified DNA and observed no mutations (as demonstrated in Fig. 3*B*). Of interest, our preliminary analysis of the viral genome corresponding to the coding sequence of Agnoprotein indicated a single base pair mutation that introduces a stop codon in the N-terminal region of this protein (unpublished work). Agnoprotein is an 8-kDa viral late protein that is important for transport of SV40 capsid proteins, VP1–3, from the cytoplasm to the nucleus for virion assembly (42, 43). Thus, it is possible that the mutation in the JCV agnoprotein halts virion formation by maintaining capsid proteins in the cytoplasm.

Although there has been no definitive link made between JCV and human brain tumors, there have been several documented cases of immunosuppressed patients with central nervous system neoplasms and concomitant PML. The first of these, reported by Richardson in 1961 (44), described a 58-year-old man with chronic lymphocytic leukemia and a 15-year history of generalized seizures. At autopsy, the diagnosis of PML was confirmed and an oligodendroglioma was discovered incidentally (44). Davies *et al.* (45) described a primary cerebral neoplasm discovered at autopsy in a patient with PML whereas virus was detected by electron microscopy in one cell that resembled an oligodendrocyte. In addition, another case of PML was diagnosed in a patient having multiple gliomas (46). Here, virus was detected by immunofluorescence, hemagglutination, and electron microscopy (46). GiaRusso and Koeppen (7) reported a case of atypical PML and primary cerebral malignant lymphoma; however, no virus was detectable by immunofluorescence or electron microscopy. Other groups reported (8, 47) concurrence of multiple malignant astrocytomas and gliomas with PML lesions of immunosuppressed patients. In these cases, JCV was detected in PML lesions but not within neoplastic areas.

Identification of a virus in association with the occurrence of a brain tumor does not provide conclusive evidence that the virus is the causative agent. Many malignancies or the treatment regimens themselves may predispose a patient to immunosuppression. Because PML is associated with immunosuppression, the disease may result from tumor-induced or therapy-induced immunosuppression. In this case, there is no known immunosuppressive condition, which is virtually always present during lytic viral infection with JCV. T antigen was detected by immunohistochemistry in oligodendroglial, but not astrocytic, regions of the tumor. Whether or not the production of T antigen in oligodendrocytic cells induces production of secretory proteins that promote the growth of neighboring astrocytic cells remains to be investigated. The present case demonstrates areas of hypomyelination in regions of tumor infiltration consistent with demyelination and the tumor tissue was shown to contain JCV DNA, RNA, and T antigen consistent with the presence of JCV in the tumor. These observations present the first report of an immunocompetent individual in which JC viral DNA, RNA, and protein have been detected in an oligoastrocytoma.

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