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Owl Monkey Astrocytoma Cells in Culture Spontaneously Produce Infectious JC Virus Which Demonstrates Altered Biological Properties

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A tumor cell suspension of an explanted JC virus (JCV)-induced owl monkey glioblastoma was inoculated intracranially into four recipient juvenile owl monkeys. Twenty-eight months following inoculation one owl monkey developed a glioblastoma, which was explanted into tissue culture. DNA from both the tumor tissue and tumor cells in culture hybridized to a JCV DNA probe by Southern analysis, indicating that free, as well as integrated, viral DNA may be present. At the time of the second culture passage, viral JCV DNA was extracted from these cells and cloned into a plasmid vector. Nucleotide sequencing of the regulatory region of the cloned DNA demonstrated homology with the prototype Mad-1 strain of JCV and revealed a 19-base-pair deletion in the second 98-base-pair tandem repeat that eliminated a second TATA box. This deletion is characteristic of the Mad-4 strain of JCV, which is highly neurooncogenic. By the third culture passage, 100% of the cells were T-antigen positive. Approximately one-third of the cells in culture hybridized to a biotinylated JCV DNA probe when in situ hybridization was used, a technique that only detects high-copy-number of replicating viral sequences. By the culture passage 5 and continuing through culture passage 14, viable JC virions could be recovered. The T protein synthesized by this virus, now termed JCV-586, differed from both the Mad-1 and Mad-4 strains in that it formed a stable complex with the cellular p53 protein in the tumor cells. Also, the JCV-586 T protein reacted to several monoclonal antibodies made to the simian virus 40 T protein that were not recognized by either the Mad-1 or Mad-4 strains.

The human viral pathogens BK virus (BKV) and JC virus (JCV) belong to the papovavirus family and, similar to simian virus 40 (SV40) and mouse polyomavirus, can produce tumors when inoculated into animal hosts (22). For example, BKV can induce tumors in hamster that have cells which sometimes contain episomal as well as integrated viral DNA associated with the chromosome of the cells (3, 21, 25).

The neurotropic human papovavirus JCV can induce brain tumors in hamsters that can be transplanted into inbred hosts without release of infectious virus or evidence of free viral DNA. There is one report in which JCV was rescued from a hamster glioma by fusion with primary cultures of human fetal glial (HFG) cells (23). Unlike BKV, JCV can also induce tumors (glioblastomas) in the nonhuman primate owl and squirrel monkeys, but long latent periods (18 to 30 months) are usually required for tumor formation (9). Examination of tumor cells or explants grown in culture has provided evidence for the presence of the entire JC viral genome (16) and its T-protein expression (10) with no evidence of free viral DNA or virion formation. Because of the long latent period for tumor formation in nonhuman primates, primate inoculation with JCV is a poor experimental model system for the study of brain tumors. We wished to test whether transplantation of tumor cells from donor to recipient owl monkeys might result in tumor formation at a more rapid rate and therefore provide a more useful model for studying gliomas in primates. The lack of production of free viral DNA or virus in owl monkeys in their brain tumors or in owl monkey cells in culture suggested that this host is nonpermissive. Transplantation rejection problems arising from an outbred owl monkey colony could be overcome by using intracranial inoculations.

Here we report the formation of a glioblastoma in a recipient owl monkey that received a donor tumor cell suspension. The latent period for tumor formation was 28 months. Unexpectedly, we found that the explanted tumor cells grown in culture possessed free viral DNA and released infectious JCV. The data describe the molecular characterization of this virus (JCV in owl monkey 586 [JCV-586]) in relation to the prototype JCV strain Mad-1 and neurooncogenic virus JCV strain Mad-4 and SV40.

MATERIALS AND METHODS

Owl monkey inoculations. Owl monkeys were inoculated intracerebrally (9) with a total volume of 0.30 ml of JCV that was prepared as a cell lysate from a pool of infected primary human fetal cells (supplied by B. Padgett and D. Walker, University of Wisconsin, Madison). This pool was described as containing the JCV Mad-1 strain. Owl monkeys that received tumor cell suspensions were also inoculated intracerebrally with 1×10^6 to 5×10^6 cells that were dissociated mechanically from tumor tissue that was received immediately at the time of autopsy.

Cell culture. Explants of tumor tissue were minced mechanically and planted in poly-D-lysine (100 μg/ml)-treated culture flasks with Eagle minimum essential medium supple-

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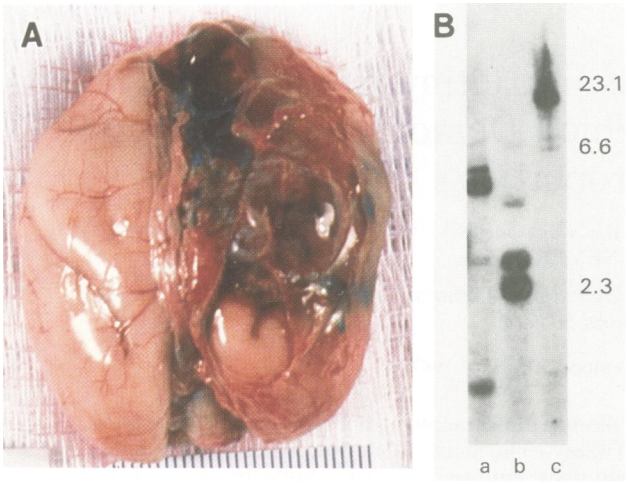


FIG. 1. (A) Owl monkey 586 brain with tumor mass on the right cerebral hemisphere. Photograph was taken directly after removal of the brain at the time of necropsy. (B) Southern analysis of the total DNA extracted from approximately 10% of the tumor mass after dissection to remove nontumor tissue components. A total of 15 μg of DNA was placed in each lane of a 0.7% agarose gel after endonuclease digestion with *Bam*HI (lane a), *Bgl*II (lane b), and *Xho*I (lane c). Hybridization was made to a ^{32}P-labeled JCV DNA probe for 24 h. Development of the autoradiograph was done following a 16-h exposure. Size markers were *Hin*dIII λ DNA digest fragments, noted as kilobase pairs to the right of the gel.

mented with 10% fetal bovine serum and 0.05% glucose. Passage of tumor cells was done by 0.01% trypsin-EDTA disruption of cultured cells when they were nearly confluent, which usually required 7 to 10 days in culture. Establishment of HFG cells and infection with various strains of JCV were done by previously described procedures (11). Routinely, 50 to 100 hemagglutination units of virus (hemagglutinating units with human type O erythrocytes) were adsorbed to confluent cell cultures for 90 min and refed with medium containing 1% fetal bovine serum.

Antibodies. Monoclonal antibodies to the SV40 T protein PAb-108, -100, -101, -106, and -115 were provided by Tucker and Elizabeth Gurney University of Utah, Salt Lake City (6). Monoclonal antibodies PAb-416 and -419 were purchased from Oncogene Sciences, Inc., New York, N.Y. (7). Hamster tumor serum was raised against SV40-transformed SVT2 hamster tumor cells, as described previously (12). Antiserum to astroglial cell-specific glial fibrillary acidic protein (GFAP) was purchased from Lab Systems, Helsinki, Finland. Antisera to oligodendroglia cell-specific galactocerebroside (gal C) were raised in rabbits as polyclonal hyperimmune sera by the protocol described by Fry et al. (5).

T-protein detection. Immunofluorescent assays were done on acetone-methanol-fixed glass cover slips of owl monkey tumor cells or infected HFG cells. Preparation of the cover slips, fixation, staining reaction, and reading of the slides have been described previously (12). With some samples, the intensity of the fluorescent reaction is described as either strong or weak, with no attempt to quantitate the results further. For immunoprecipitation assays [^{35}S]methionine (1,200 Ci/mmol; New England Nuclear Corp., Boston, Mass.), metabolically labeled cell cultures were used (12). In these experiments we used protein A (Pansorbin; Calbiochem-Behring, La Jolla, Calif.) to augment the precipitation reaction. It was not necessary to use *N*-ethylmaleimide to clearly resolve the JCV-586 T protein, as described previously for the prototype JCV T protein (13).

DNA assays. Total cellular DNA was extracted with 1.0% sodium dodecyl sulfate and 50 μg of proteinase K per ml. DNA was prepared following RNase treatment, phenol-chloroform extractions, and ethanol precipitation, essentially as described by Maniatis et al. (14). Detection of the presence of the JCV genome in DNA from tumor cells was done by Southern analysis (20). Hybridization of DNA transferred from agarose gels to nitrocellulose filters was done in 40% formamide for 24 h with an α-^{32}P-labeled deoxynucleoside triphosphate (10^8 cpm/μg) JCV DNA probe at 37°C. Filters were washed with 2× SSC buffer (0.6 M NaCl plus 0.06 M sodium acetate [pH 7.4]) and 0.5% sodium dodecyl sulfate at room temperature for 30 min and then for 3 h at 68°C. Selected restriction enzyme digests were done to distinguish fragments generated by recognition sites in cellular or viral DNAs.

In situ DNA hybridization was done essentially as described by Brigati et al. (2) and modified in our laboratory for cell cultures, as described previously (1, 11). A biotinylated JCV DNA probe was prepared by the nick-translation incorporation of biotin-labeled dUTP (Enzo, Inc., New York, N.Y.) into the total JCV genome with an efficiency of approximately 22% substitution. The hybridization reaction included 50% formamide, 10% dextran sulfate, 40 μg of herring sperm DNA per ml, 20 μg of probe DNA per ml in 2× SSC. Denaturation of probe and cellular DNA occurred at 85°C for 10 min. The hybridization period was 16 h (37°C). Detection of the hybrid was made by using streptavidin-biotin horseradish peroxidase complex in the presence of diaminobenzidine and H_2O_2.

Molecular cloning of JCV-586 DNA was initially done at the *Eco*RI site of pBR322 by using conventional recombinant DNA techniques. DNA sequencing of part of the genome involved subcloning of the replication origin and the 98-base-pair tandem repeats into the M13 mp18 for the dideoxy sequencing method.

RESULTS

Tumor induction in owl monkeys. To produce additional glioblastomas as a model for the study of the pathology of primate brain tumors, JCV from a pool of infected human fetal glial cell lysates was inoculated intracerebrally into juvenile owl monkeys in 1981. Twenty-two months later, owl monkey 241 developed signs of tumor formation, including weight loss, hemiparesis, anorexia, and lethargy. The animal was necropsied, and a tumor mass located along the site of virus inoculation was found. The tumor was identified histologically as an astrocytoma similar to other JCV-induced tumors (16). Tumor tissue at the time of autopsy was prepared as a cell suspension for inoculation into recipient owl monkeys and was also planted in cell culture. The owl monkey 241 tumor cells survived in culture for only two passages but showed papovavirus T antigen by fluorescent antibody staining. Rapid decline and loss of viability is not uncommon when JCV-induced glioblastomas are grown in tissue culture. Therefore, loss of owl monkey 241 tumor cells in culture, even after they were shown to be T-protein positive, was not surprising. Further investigation of tumor 241 was not possible due to an insufficient quantity of tissue and the inability to maintain the glioma in culture. Approximately 5×10^6 tumor cells were directly inoculated intracranially into four monkeys. One recipient owl monkey

died 2 weeks following inoculation due to causes incidental to the inoculation or the cell burden. Three other animals survived the postinoculation period without difficulty.

Twenty-eight months following the intracranial inoculation, one of the three owl monkeys (owl monkey 586) developed a tumor mass that pressed on the right cerebral hemisphere cortex at the inoculation site (Fig. 1A). The tumor mass was easily removed from the surrounding brain tissue and prepared for histological examination, tissue culture, and liquid nitrogen storage. The two remaining owl monkeys were still under observation 46 months following intracranial inoculation.

Total cellular DNA was subsequently extracted from a fragment of the tumor, representing approximately 10% of the total tumor mass. The DNA was analyzed by the procedure described by Southern (20) following restriction endonuclease digestion, transfer to nitrocellulose filters, and hybridization to a ^{32}P-labeled JCV DNA probe. In Fig. 1B the Southern pattern indicating the presence of the JCV genome associated with the chromosome is shown. Strongly hybridizing JCV DNA bands that were generated by a single viral *Bam*HI cleavage site and two *Bgl*II cleavage sites may indicate integration as a tandem copy, as described previously for other JCV-induced tumors (16, 24), or the possible presence of free viral DNA. Less prominent bands hybridized to the JCV DNA probe indicating other, random integration sites. It was not possible to definitively map the integration pattern of JCV DNA in the tumor tissue or to evaluate this tissue for the presence of episomal viral DNA due to insufficient quantity of DNA extracted from the dissected tumor.

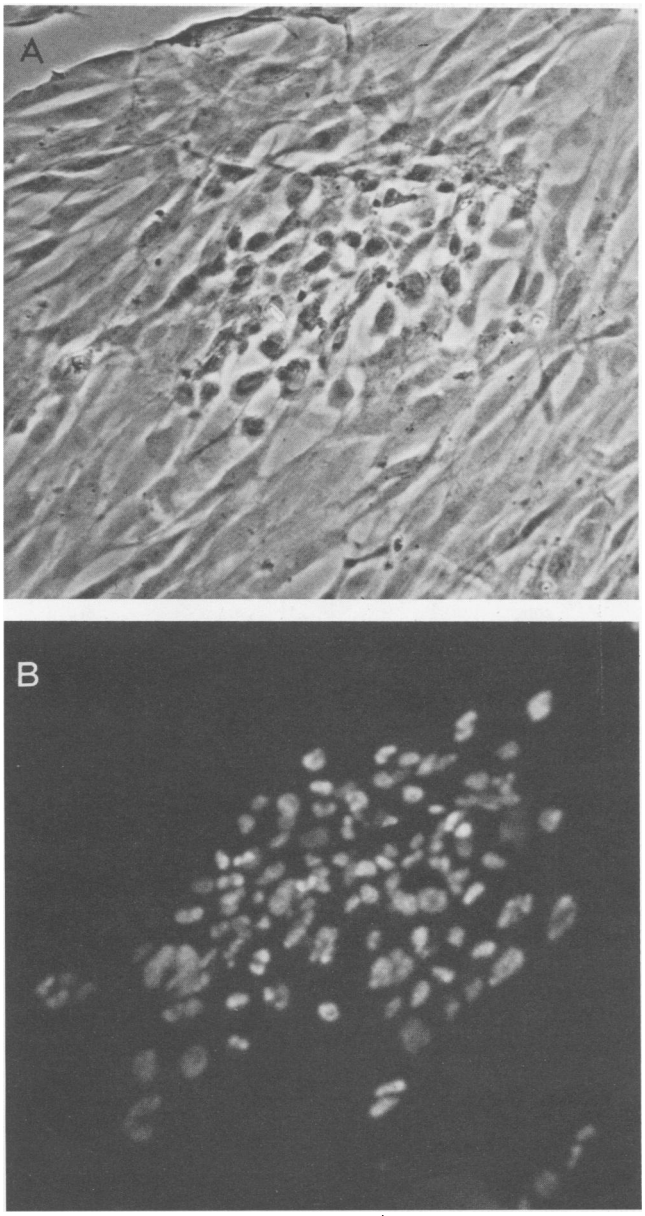


FIG. 2. Owl monkey 586 tumor cells at time of second passage in culture. (A) Phase-contrast micrograph of bright-field view of cells. (B) T-protein-stained cells of identical view as shown by phase-contrast microscopy. Magnifications, ×160.

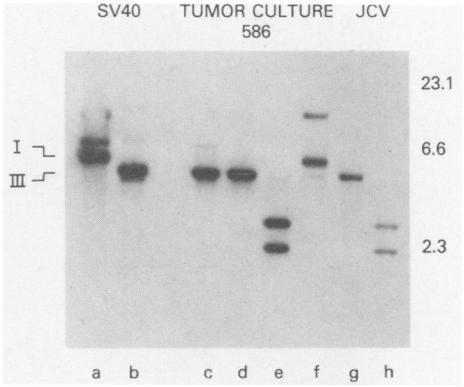


FIG. 3. Southern analysis of cellular DNA extracted from owl monkey 586 tumor cells at passage 3 in culture. A total of 5 μg of DNA was placed in each lane of 0.6% agarose gel (SeaKem, GTG) after endonuclease digestion with *Eco*RI (lane c), *Bam*HI (lane d), *Bgl*II (lane e), and *Xho*I (lane f). Low-molecular-weight JCV DNA extracted by the method described by Hirt (8) was digested with *Bam*HI (lane g) and *Bgl*II (lane h) for comparison with cellular DNA. SV40 DNA was either undigested (lane a, form I) or digested with *Bam*HI (lane b, linear form III) as viral DNA markers. *Hin*dIII λ DNA digest fragments were used as size markers, noted as kilobase pairs to the right of the gel. Hybridization conditions were similar to those described for Fig. 1B.

Virus release from tumor cells in culture. Unlike the 241 tumor cells in culture, the 586 tumor cells grew rapidly when explants were planted in Eagle minimal essential medium with 10% fetal bovine serum. The cells were assayed by immunofluorescence for the presence of either GFAP or galactose C to determine their identity as glial cells (19). The culture cells demonstrated the intermediate filament GFAP but not galactose C, indicating their derivation as astrocytes similar to the donor tumor cell. JCV T protein was identified in passage 2 cells by immunofluorescence with cross-reacting SV40 hamster tumor serum. In Fig. 2A a photomicrograph of one area of the culture by phase-contrast microscopy is shown, and that same area stained for T protein is shown in Fig. 2B. Not all cells at passage 2 were T-protein positive. By passage 3 sufficient numbers of cells had propagated so that DNA extraction for Southern analysis was possible. The hybridization pattern of total DNA to a ^{32}P-labeled JCV DNA probe is shown in Fig. 3. Digestion of owl monkey 586 cellular DNA with restriction endonucleases *Bam*HI and *Eco*RI, which cleaves JCV DNA once, resulted in fragments that migrated as linear viral DNA

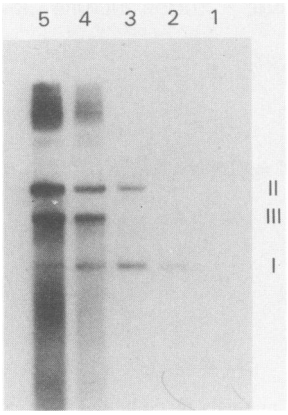


FIG. 4. Extraction of DNA by the method described by Hirt (8) from owl monkey 586 tumor cells from passages 1 to 5 (lanes) analyzed by Southern transfer and hybridization to a ^{32}P-labeled JCV DNA probe. Development of the autoradiograph followed exposure to x-ray film for 36 h. Markers on the right indicate the migration of viral closed, circular, and supercoiled form I DNA; linear form III DNA; and nicked circular form II DNA run in a 1% agarose gel. Differences in the concentration of forms I, II, and III among the five samples are probably artifacts due to variations in cell concentration, amount and quality of the DNA that was extracted, and the concentration of DNA used for the transfer blot.

(Fig. 3, lanes c and d) and comigrated with linear SV40 (Fig. 3, lane b) and JCV (Fig. 3, lane g) DNAs. Digestion of owl monkey 586 DNA with *Bgl*II enzyme, which cleaves JCV DNA twice, resulted in fragments that migrated at 2.8 and 2.3 kilobases (Fig. 3, lane e), similar to JCV DNA (Fig. 3, lane h); and digests with *Xho*I, which does not cleave JCV DNA, revealed a viral band (Fig. 3, lane f), which comigrated with form I SV40 DNA (Fig. 3, lane a). Interpretation of this Southern analysis suggests that free viral DNA is present in the tumor cells. Determination of the presence of free viral DNA was examined by collecting cells from independent passages 1 through 5. Cells that detached from their culture flasks were harvested. Low-molecular-weight DNA was extracted by the method described by Hirt (8) and analyzed by Southern blotting. Nonintegrated viral DNA could be detected at passages two through five (Fig. 4). The DNA samples at passage 5 actually showed viral DNA bands on the ethidium bromide-stained gels. Because only detached cells were used for Hirt (8) extracts, to preserve cells at low passage levels for further culture expansion, it is possible that cells at passage 1 contained free viral DNA but that too few cells were harvested for detection. By passage 5, some cells in culture demonstrated cytopathic effects, including nuclear enlargement, rounding, and detachment from neighboring cells. The total number of cells in 75-cm^2 flasks were harvested from independent passages 2 through 5 and treated with sodium deoxycholate to release virions from cell lysate debris. The resultant samples were assayed for virus by hemagglutination of human type O erythrocytes. Passage 5 samples were positive with a hemagglutination titer of 128. Increasing titers were seen as more cells were harvested from further passages in culture through passage 14. We did not examine these cultures past passage 14. Virus that was collected from independent passages 5 through 10 was inoculated onto HFG or kidney cells and subsequently tested for the multiplication of viruses. Only the infected glial cultures produced new virus.

Identification of viral DNA and virions released as JCV. Many cells survived continued passage in culture so that a persistent type of infection apparently was established. To determine the relative number of cells that replicated viral DNA, and therefore that probably released virions, and to identify the DNA as JCV and not BKV or SV40, we hybridized the cells in situ with a biotinylated JCV DNA probe. We used this technique extensively for both brain tissue samples of patients with progressive multifocal leukoencephalopathy (1), as well as JCV-infected tissue culture cells (11), to identify the presence of replicating JCV genome. In Fig. 5 is shown an in situ hybridization to passage 3 cells, with positive signals in many cells showing the characteristic cytopathic effect observed in these cultures. Because these probes are highly specific and do not cross-react with SV40 or BKV DNAs (data not shown), we were certain that the cells were replicating JCV DNA and releasing JCV virions. Neither biotinylated BKV nor SV40 DNA probes hybridized to passage 3 owl monkey 586 cell cultures.

The free viral DNA isolated from passage 3 cells was subsequently cloned into an *Eco*RI site of pBR322. The regulatory region of the genome was then subcloned into the

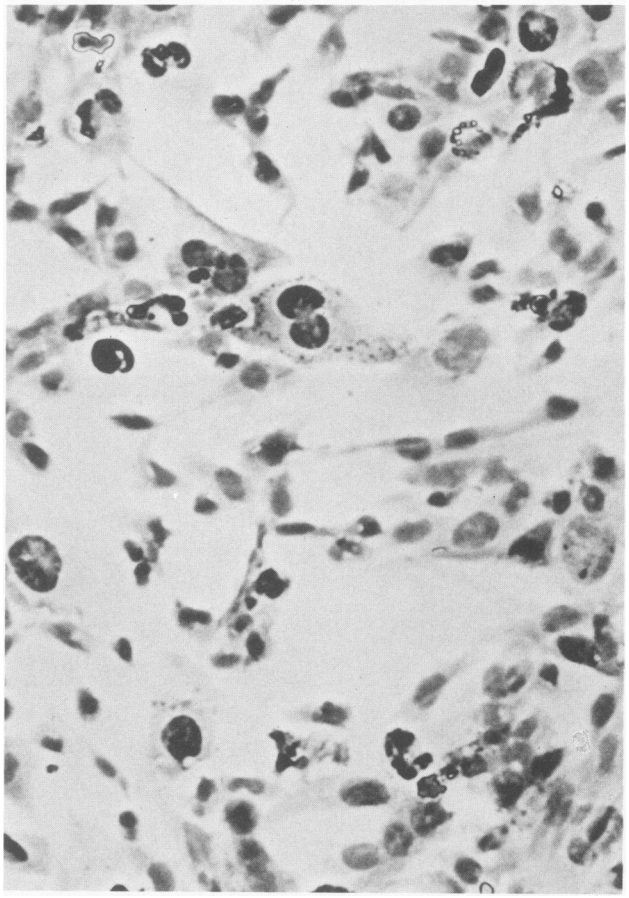


FIG. 5. In situ DNA hybridization of owl monkey tumor cells from passage 3 to a biotin-labeled JCV DNA probe. Cell nuclei containing hybrids were detected following hybridization for 16 h and treatment with streptavidin-biotin-horseradish peroxidase in the presence of diaminobenzidine and H_2O_2. Dark brown precipitates form in the nuclei of positive cells. Magnification, ×160.

mp18 vector for sequence analysis. This *Hin*dIII-*Pvu*II fragment, np 5112-268, contains the replication origin and the 98-base-pair tandem, direct repeats. This region of the JCV genome has been shown to be hypervariable by endonuclease assays and nucleotide sequencing and has become diagnostic for strain differentiation (15). The sequence of the JCV-586 DNA was similar to that of the prototype Mad-1 strain (4), except for a 19-base-pair deletion in the beginning of the second TATA box region of the tandem repeat. This sequence was identical to that of the Mad-4 strain (15), which is more neurooncogenic than other JCV strains (17). Restriction endonuclease analysis of the JCV-586 DNA with *Bam*HI, *Eco*RI, *Bgl*II, *Pvu*II, *Nco*I, and *Sst*I enzymes did not reveal any other gross alterations in the genomic DNA.

JCV-586 T protein. To characterize the JCV-586 T protein better, we immunoprecipitated protein extracts from owl monkey 586 cells at passages 4 and 10 using the same antisera reagent that was used for the fluorescent antibody assays. In Fig. 6 (lanes b and c) is shown a protein pattern which indicates that the T protein migrates as a 94-kilodalton protein, which is similar to that in Mad-1 in another owl monkey tumor cell line 26 (12) or in lytically infected HFG cells (13). The protein precipitation pattern, however, unlike owl monkey 26 cells or infected HFG cells, demonstrated a 53-kilodalton band, presumably the cellular oncogene p53. To test whether this band represented the authentic p53, we immunoprecipitated similar lysates with PAb-122, a monoclonal antibody to p53 (Fig. 6, lanes d, e, and f). From the results of this experiment it is clear that the primate host cell p53 protein is recognized by PAb-122 and forms a complex with the JCV-586 T protein. This result was surprising because previous attempts to recognize a JCV T protein-p53 complex in owl monkey or human glial cells were unsuccessful (12, 13).

Because it is thought that the carboxy-terminal end of the T protein is responsible for complex formation with p53, we next immunoprecipitated owl monkey 586 protein extracts

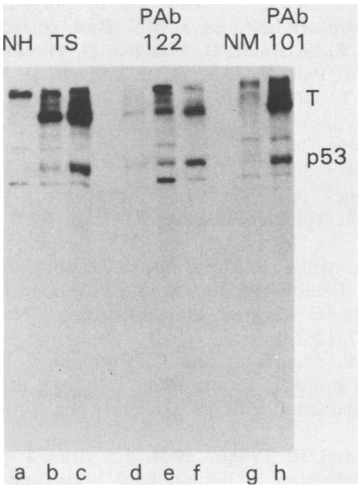
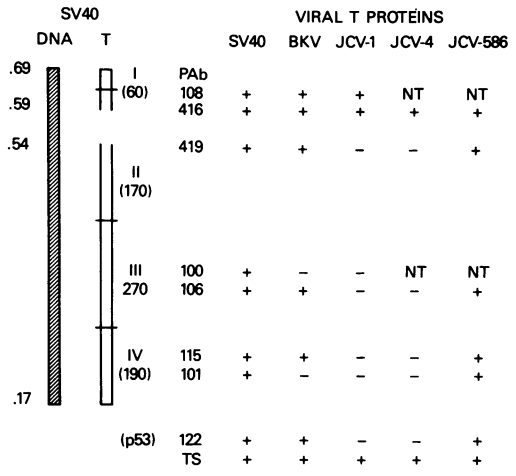


FIG. 6. [^{35}S]-methionine-labeled protein extracts were made from owl monkey 586 tumor cells in culture and immunoprecipitated with normal hamster serum (NH), hamster SV40 tumor serum (TS), monoclonal antibody to cellular p53 protein (PAb-122), normal mouse serum (NM), and monoclonal antibody to SV40 T protein (PAb-101). The precipitated extracts were separated by electrophoresis on a 12% polyacrylamide-SDS gel and exposed to x-ray film for 48 h. Lanes a, b, and e were extracts made from passage 4 cells; lanes c, f, g, and h were made from passage 11 cells; and lane d was made from passage 2 cells.

TABLE 1. Immunofluorescent antibody assays with mouse monoclonal antibodies made to the SV40 T protein and reacted with JCV-infected HFG cells or JCV-induced owl monkey glioma cells[a]



[a] The activity of an SV40 hamster tumor bearing sera that cross-react to BKV and JCV is also included in these tests. The ability of the mouse monoclonal antibody PAb-122 to recognize a T-protein-p53 complex is also indicated. Schematic diagrams of the SV40 DNA and the SV40 T protein were given as a relative indication of the location of the epitopes recognized by these antibodies (6, 7). Numbers in parentheses reflect the approximate number of amino acid residues present in the JCV T protein. Abbreviations: JCV-1, JCV strain Mad-1; JCV-, JCV strain Mad-4; NT, not tested.

with PAb-101, a monoclonal antibody to the carboxy-terminal end of the SV40 T protein that does not react with either JCV or BKV T proteins (6). It is shown in Fig. 6 (lane h), however, that PAb-101 recognizes owl monkey 586 T protein and its complex with p53. This observation may indicate that the JCV-586 T protein is structurally different from the prototype Mad-1 JCV strain. To test this further, we used other monoclonal antibodies to the SV40 T protein against HFG cells that were infected with JCV strain Mad-1, JCV strain Mad-4, and JCV-586. Previous results showed that PAb-108 cross-reacts with JCV strain Mad-1 and BKV T proteins (12) and that PAb-106 and -115 recognized BKV T protein (6). These observations, as well as the results of the experiments reported here, are presented in Table 1 as a summary of immunofluorescent assays. Results of the experiments described here show that PAb-416 recognizes all strains of JCV and BKV T proteins. The epitope of this antibody is reportedly located in the amino-terminal region of the large T proteins, in which sequence homology among primate papovaviruses is greatest (4, 6). PAb-419 antibody reacts to BKV and JCV-586 T protein but not JCV strain Mad-1 or JCV strain Mad-4 T proteins. PAb-106 and -115 antibodies showed extremely weak activity to JCV strain Mad-1 and JCV strain Mad-4 T proteins, which are considered negative in Table 1, but strong activity to JCV-586 T protein. It is apparent that the JCV-586 T protein, because of its binding to the cellular p53 protein and reactivity to SV40 T protein monoclonal antibodies, differs from the other JCV T proteins that were tested.

DISCUSSION

The data presented in this report indicate that the human polyomavirus JCV is released from an owl monkey tumor cell line that was established from explants of a transplant-

able JCV-induced owl monkey astrocytoma. It is not possible to determine, however, whether virus was released in vivo from the original donor tumor cells (owl monkey 241) or was released only from the JCV-586 tumor cells once placed in tissue culture. Because both the donor owl monkey 241 and recipient owl monkey 586 were females and more detailed karyotype information is not available, determination of the exact origin of JCV-586 is difficult. A long period of time elapsed between cell suspension inoculation and tumor formation (28 months). Therefore, owl monkey tumor 586 may have arisen from virus released by the inoculated cells and not from donor cell proliferation. Evidence that the virus, now termed JCV-586, represents a new strain comes from characteristics of its T protein. One prominent feature of JCV-586 is its T protein antigenic reactivity to monoclonal antisera made to SV40 T protein that do not recognize the T proteins of either the prototype Mad-1 or neurooncogenic Mad-4 strains of JCV (Table 1). Because monoclonal antibodies can serve as probes to determine structural details of proteins, it seems reasonable to assume that the JCV-586 T protein differs from those of other JCV isolates. Currently, we are determining the nucleotide sequence of the early region of the JCV-586 genome to examine those differences at the gene sequence level.

Another interesting feature of the JCV-586 T protein is presented by data described in Fig. 6. Results of these experiments indicate that the T protein forms a stable complex with the cellular p53 protein. Because the prototype Mad-1 strain of JCV does not form such a complex in primate cells, based on results of previous assays carried out in this laboratory (12, 13), the fact that JCV-586 T protein does form a complex with p53 adds further evidence for the structural differences that exist among these JCV T protein isolates. The possibility that JCV-586 is actually a SV40 variant seems very unlikely because of the hemagglutination ability of JCV 586, its host preference for growth to human glial cells, and the restriction pattern of its genome DNA. All of these properties are consistent with the fact that JCV-586 represents another strain of JCV and is biologically different from SV40. It is possible that the JCV-586 tumor cells produce multiple variants of JCV DNAs which, in turn, may synthesize different forms of T protein. We cloned DNA from several passages of JCV-586 tumor cells and from virions purified by gradient centrifugation. In some cases the regulatory region was subcloned and sequenced. Only genome-length, infectious viral JCV DNA was identified in these experiments, indicating that the majority of viral DNA produced by JCV-586 tumor cells is homogeneous.

It is tempting to suggest that the T protein of JCV-586 is necessary for the release of virus from the tumor cell line by allowing replication of JCV DNA in a host cell previously thought to be nonpermissive. To determine whether JCV-586 can initiate a productive infection in owl monkey glial cells, currently we are culturing owl monkey fetal brain to isolate the glial elements and introduce JCV-586 into these cultures. Also, because the nucleotide sequence of the JCV-586 regulatory region is identical to the neurooncogenic Mad-4 strain, based on our sequence determination of the *Hin*dIII-*Pvu*II DNA fragment, which contains the regulatory region, the different properties of JCV-586 could be related to the T protein. The unusual host restriction of JCV growth to glial cells, then, could be related to functions that are associated with the T protein as well as the recognition of viral regulatory sequences in glial cells.

The observations that the JCV-586 T protein reacts with PAb-101, a monoclonal antibody to the carboxy-terminal end of the SV40 T protein, and forms a stable complex with the cellular p53 protein suggest that some alterations may have taken place that effect the carboxy-terminal end of the JCV-586 T protein. Mutations of SV40 DNA located in this region of the T-protein gene have given rise to host range alterations (18), strengthening the idea that the different biological properties of JCV-586 could be related to T-protein functions governed by sequences in this region.

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