Association of Simian Virus 40 with a Central Nervous System Lesion Distinct from Progressive Multifocal Leukoencephalopathy in Macaques with AIDS

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The primate polyomavirus SV40 is known to cause interstitial nephritis in primary infections and progressive multifocal leukoencephalopathy (PML) upon reactivation of a latent infection in SIV-infected macaques. We now describe a second central nervous system manifestation of SV40: a meningoencephalitis affecting cerebral gray matter, without demyelination, distinct from PML. Meningoencephalitis appears also to be a primary manifestation of SV40 infection and can be seen in conjunction with SV40-induced interstitial nephritis and pneumonitis. The difference in the lesions of meningoencephalitis and PML does not appear to be due to cellular tropism, as both oligodendrocytes and astrocytes are infected in PML and meningoencephalitis, as determined by *in situ* hybridization or immunohistochemistry for SV40 coupled with immunohistochemistry for cellular determinants. This is further supported by examination of SV40 nucleic acid sequences from the ori-enhancer and large-T-antigen regions, which reveals no tissue- or lesion-specific variation in SV40 sequences. (Am J Pathol 1999, 154:437–446)

The primate polyomaviruses include JCV¹ and BKV,² which infect humans, and the closely related SV40³ of macaques. In immunocompetent hosts, primary infection with JCV or BKV occurs early in life, with mild or absent clinical signs. The virus then persists as a latent infection in kidney and also probably in the central nervous system (CNS) and circulating lymphocytes.^{4–7} Reactivation with viral shedding can occur with pregnancy, chronic diseases, or immunocompromise and may or may not be associated with clinical signs. JCV causes progressive multifocal leukoencephalopathy (PML),¹ a demyelinating

disease of the CNS due to infection of oligodendrocytes. PML is seen in up to 5% of AIDS patients^{8,9} and is an AIDS-defining lesion. BKV is associated with interstitial nephritis and/or hemorrhagic cystitis, particularly in transplant recipients.² Similarly, SV40 is associated with both PML and nephritis in SIV-infected macaques with simian AIDS¹⁰ and rarely with an interstitial pneumonia.^{11,12}

Previous studies indicated that SV40 causes interstitial nephritis in primary infections and PML upon reactivation of a latent infection in SIV-infected macaques with AIDS.¹⁰ We now describe a second CNS manifestation of SV40: a meningoencephalitis affecting cerebral gray matter, without demyelination, distinct from PML. This lesion can be a primary manifestation of SV40 in conjunction with SV40-induced interstitial nephritis and pneumonitis. In this report we describe this new CNS lesion and characterize it with respect to disease already associated with SV40 in immunosuppressed macaques.

Materials and Methods

Case Selection, Tissue Collection, and Processing

The SV40 cases described herein were selected from macaques dying with SIV-induced AIDS during the period June 1990 through October 1997 ($n = 229$) at the New England Regional Primate Research Center (NERPRC). SV40-associated meningoencephalitis (ME) was first identified in an SIV-infected rhesus monkey (7NE) that died with SV40-associated interstitial nephritis. Once we recognized a lesion in the gray matter associated with SV40-infected cells, but differing from PML, we carefully re-examined earlier cases. We found that two animals previously diagnosed with renal and/or pulmonary SV40 (1NE and 6NE)¹³ additionally had mild ME,

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Table 1. Antibodies Used in Immunohistochemistry

Specificity (source)	Clone/serum fraction	Tissue fixation	Source
SV40 Tag	Pab101 (IgG2a)	P, F	Santa Cruz Biotechnology
p53 (human)	DO-7 (IgG2b)	P, F	Dako
GFAP (cow)	Purified Ig	P, F	Dako
NSE (human)	Purified Ig	P	Dako
Macrophage (human)	HAM-56 (IgM)	P, F	Dako
CD3 (human)	Purified Ig	P, F	Dako
CD20 (human)	L26 (IgG2a)	P, F	Dako
CD79a (human)	HM57 (IgG1)	P, F	Dako
κ light chains (human)	Purified Ig	P, F	Dako
λ light chains (human)	Purified Ig	P, F	Dako
Oligodendrocyte (rat)	MAB328 (IgM)	F	Chemicon

P, formalin-fixed, paraffin-embedded tissue; F, tissue snap-frozen in OCT.

confirmed by *in situ* hybridization for SV40, and one more case (9NE) was later identified. Eleven animals housed at the NERPRC (designated 1NE to 11NE) diagnosed with SV40 infection after postmortem examination were selected for further study. Four additional cases from the Tulane Regional Primate Research Center (designated 12T to 15T) were included in the study. Of the 15 animals with SV40-associated lesions, 14 were rhesus monkeys (*Macaca mulatta*) infected with SIV and euthanized with terminal AIDS. One additional animal, a Formosan rock macaque (*Macaca cyclopis*; 1NE) died with immunodeficiency of undetermined origin (possibly SIV or type D retrovirus).¹² Formalin-fixed, paraffin-embedded tissues were sectioned and stained with hematoxylin and eosin (H&E) for routine histological examination. In addition, a Luxol fast blue stain for myelin was performed on selected sections of brain. Selected tissues were snap-frozen in optimal cutting temperature (OCT) compound (Miles Scientific, Elkhart, IN) for immunohistochemical labeling.

In Situ Hybridization

In situ hybridization for SV40 or SIV was performed as previously described.¹⁴ The SV40 probe contained the entire genome in plasmid pUC19.¹³ The SIV probe was a combination of two plasmids, which together make up essentially the entire SIVmac239 genome.^{15,16} Briefly, the probes were labeled with digoxigenin-dUTP by random priming. The hybridization was performed under denaturing conditions to identify both DNA and RNA. After hybridization, tissue sections were immunostained to localize the digoxigenin-labeled nucleic acids using nickel-enhanced diaminobenzidine (DAB) as the chromogen.

Immunohistochemistry

Immunohistochemistry for SV40 was performed on formalin-fixed, paraffin-embedded or frozen sections using a monoclonal antibody directed against SV40 T antigen (Table 1), as previously described^{10,17} with DAB as the chromogen, and counterstained with Mayer's hematoxylin. As accumulation of the tumor suppressor gene p53 in nuclei in JCV infection has been demonstrated by several groups,^{18–20} and SV40 T-antigen is known to bind p53,

we also immunostained selected tissues for p53 as an additional marker for SV40 infection. Sections of brain with significant inflammation noted on histopathological examination were immunostained with antibodies to identify B or T lymphocytes and monocyte/macrophages (Table 1).

Co-Localization of Viral and Cellular Determinants

Selected tissues were labeled by immunohistochemistry with antibodies to identify astrocytes (GFAP), oligodendrocytes, macrophages (HAM-56), or neurons (NSE; Table 1), followed or preceded by *in situ* hybridization or immunohistochemistry for SV40. Briefly, after immunostaining with the first antibody, slides were immunostained with the second antibody, or *in situ* hybridization for SV40 was performed. The chromogen was also changed for the second procedure, eg, standard DAB (brown) followed by nickel-enhanced DAB (black) or Vector Red (Vector Laboratories, Burlingame, CA) to allow differentiation of the two labels.

Molecular Cloning and Sequencing

SV40 was isolated from frozen tissues (6 animals, 1NE to 6NE), or DNA was isolated from frozen or formalin-fixed, paraffin-embedded tissues (6 animals, 7NE to 9NE; 12T, 13T, and 15T, for a total of 12 of the 15 cases) for PCR, cloning, and sequencing. All routine cloning procedures were performed essentially as described earlier.^{21,22} Epicurian Coli XL1-Blue MRF' Supercompetent Cells (Stratagene Cloning Systems, La Jolla, CA) were used for the transformation and plasmid growth. Each of the resulting plasmids was sequenced at least twice through the viral insert. PCR-amplified viral fragments from infected tissues were treated with polynucleotide kinase and cloned into pUC18/*Sma*I-cut vector (Pharmacia, Piscataway, NJ) or PCRScript (Stratagene) according to the manufacturer's recommendations.

All nucleotide primers were custom-made by GIBCO BRL (Grand Island, NY). Primers 5'-GGTTTTTCAGT-TAACCTTCTGG-3' (513 to 491), 5'-GTATCTTCCC-CTTCACAAAATTG-3' (468 to 446), 5'-ATACACAAA-

Table 2. Location and Lesion(s) of SV40

Animal	SV40-related histology (SV40-derived sequences)	Localization of SV40 by immunohistochemistry and/or <i>in situ</i> hybridization		
		Brain	Kidney	Lung
1NE	ME, pneumonia, nephritis (K-I)	+	NA	NA
2NE	PML (B-I)	+	—	—
3NE	Nephritis (K-I)	—	+	—
4NE	PML (K, B-I)	+	—	—
5NE	PML (B-I)	+	—	—
6NE	ME, nephritis* (K-I)	+	+	—
7NE	ME, nephritis (K-P)	+	+	NA
8NE	PML (B-P)	+	—	—
9NE	ME, pneumonia (B-P)	+	—	+
10NE	PML	+	—	—
11NE	PML	+	—	—
12T	PML (B-P)	+	NA	NA
13T	ME (B-P)	+	NA	NA
14T	PML	+	NA	NA
15T	ME, pneumonia, nephritis (K, L, B-P)	+	+	+

NE, New England RPRC; T, Tulane RPRC; NA, tissue not available; source of SV40 sequences: B, brain; K, kidney; L, lung; method of obtaining SV40 for cloning: I, virus isolation; P, PCR.

*Rare inclusion only in kidney. This animal also had SIV encephalitis.

CAATTAGAATCAGTAG-3' (4577 to 4601), and 5'-GCTTTAAATCTCTGTAGGTAG-3' (4644 to 4664) were used for PCR amplification of the SV40 origin/enhancer region. Primers 5'-GAGGAGGTTAGGGTTTATGAGG-3' (2492 to 2513), 5'-ACACAGAGGAGCTTCCTGGG-3' (2514 to 2533), 5'-ACAGGCTCTGCTGACATAGAAG-3' (3683 to 3662), and 5'-TGGATGGCTGGAGTTGCT-TGG-3' (3659 to 3639) were used for PCR amplification of the carboxyl-terminal part of SV40 T-antigen. Universal and reverse M13/pUC19 primers were used for sequencing through the amplified region.

PCR amplification and DNA sequencing were performed essentially as described earlier.^{21,23} Each sequence was confirmed by two or more independent PCR reactions. A thermal cycler produced by MJ Research (Watertown, MA) was used for PCR amplification. DNA sequencing was performed using an ABI Prizm automated sequencer. Sequencing reactions were performed with the help of AmpliTaq FS sequencing kit from Perkin-Elmer Cetus (Norwalk, CT), which was used according to the manufacturer's recommendations on either MJ Research or Hybaid Omnigene (SunBioscience, Branford, CT) thermal cyclers.

Nucleotide sequences were analyzed and aligned using MacVector version 5.0.1. software (International Biotechnologies, New Haven, CT). The sequence of SV40 was taken from Genebank²⁴ (accession number JO2400).

Serology

Serum samples were assayed for the presence of antibodies to SV40 by a dot-immunobinding technique (Virus Reference Laboratory, San Antonio, TX), as previously described.²⁵

Results

Of 229 macaques that died of SIV-induced AIDS at the NERPRC between June 1990 and October 1997, 10 animals (4.4%) were found to have lesions associated with SV40. For the purposes of this study, five additional cases were included, four from the Tulane RPRC and one macaque from the NERPRC with immunodeficiency of undetermined origin¹² (Table 2). SV40-associated disease was recognized only in immunocompromised animals.

Histopathology

Two CNS lesions associated with SV40 were identified in immunosuppressed macaques: progressive multifocal leukoencephalopathy (PML)^{8,10,26,27} and meningoencephalitis (ME). PML is a demyelinating lesion primarily of the white matter, due to infection of oligodendrocytes (Figure 1, A, C, and E). In contrast, SV40-associated ME is a lesion of the meninges and superficial gray matter, without significant demyelination (Figure 1, B, D, and F). The meninges are variably thickened by edema and infiltrates of lymphocytes, macrophages, and eosinophils, largely around vessels. The inflammation extends into the gray matter along blood vessels, which have hypertrophic endothelium. As in PML, distinctive features of ME include cells with enlarged nuclei with smudgy amphophilic intranuclear inclusions and large, bizarre, gemistocytic astrocytes, with enlarged and sometimes multiple nuclei. The mildest ME lesions may consist entirely of a few gemistocytic astrocytes and occasional intranuclear viral inclusions. ME can be focal in mild cases or diffusely involve the superficial cerebral cortex and periventricular gray matter. In one case with severe ME (9NE), lesions focally extend into white matter, and those foci have associated demyelination.

Figure 1. Histological comparison of PML (A, C, and E) and ME (B, D, and F). In lesions of PML, multiple foci of demyelination are seen in cerebral white matter (A). At higher magnification, cells with intranuclear inclusions of SV40 (C, arrow) are evident within the foci of demyelination, and occasional multinucleated astrocytes are in the midst of macrophages that have phagocytized myelin debris (E). Luxol fast blue/H&E; magnification, ×20 (A), ×220 (C), and ×440 (E). In contrast, in ME there is no demyelination but irregularity in the superficial cerebral gray matter (B). These areas are characterized by the presence of many gemistocytic astrocytes underlying meninges thickened by edema and inflammation (D). Occasional multinucleated astrocytes are present in foci of mononuclear cell inflammation in gray matter (F). B: Luxol fast blue/H&E; magnification, ×20. D and F: H&E; magnification, ×220 (D) and ×440 (F).

A dense infiltrate of macrophages (gitter cells) is present in the center of regions of demyelination in PML, often containing phagocytized myelin in their cytoplasm (Figure 1E). A more diffuse infiltrate of macrophages is seen in ME, increasing with the severity of the lesion. The morphology of the macrophages varies, with those in the demyelinating PML lesions more ovoid with fewer cytoplasmic processes than those in the ME lesions (Figure 2, A and B).

Table 2 indicates the lesion(s) associated with SV40 in the 15 affected animals and the tissues in which SV40 was identified. Eight animals had PML, six had ME, and in one animal (3NE) SV40 was localized to the kidney only, both by histopathology and *in situ* hybridization. In the remaining cases, pulmonary and/or renal SV40 was associated exclusively with ME. Of the 10 cases for which we have epidemiological data, 2NE to 11NE, the incidence of SV40-associated lesions was 4.4% of AIDS cases (10/229). Among these 10 animals with SV40-associated lesions, PML was most common (6/229, 2.6%) followed by ME (3 cases, 1.3%), nephritis (1.3%), and pneumonia (0.4%) (Table 2). These 10 animals comprise all of the cases of SV40-associated disease that we have recognized in SIV-infected macaques at the New England Regional Primate Research Center (NERPRC) through October 1997. Of the six cases of ME, five, including all of the cases of ME at the NERPRC, had SV40-associated lesions in two or more locations.

Serology

Serology for SV40 was performed on 14 of the 15 animals at multiple times (Table 3), including time of inoculation with SIV, date of death, and one or more times in between. All animals with PML were seropositive for SV40 at the time of SIV infection, with two stipulations. Animal 2NE was spontaneously SIV-infected, so date of inoculation was unknown. This animal was seropositive for SV40 at least 4.5 years before death. Animal 10NE was given an attenuated strain of SIV and then challenged 3.5 years later with virulent SIV, 2 years before death. This animal was seronegative for SV40 at the time of the original SIV infection but seroconverted at some time in the next 16 months, so was seropositive at the time of challenge with pathogenic SIV and for 4 years before death. All animals with ME, nephritis, and/or SV40 pneumonia seroconverted to SV40 after SIV infection. Once an animal seroconverted to SV40, every additional sample tested was also positive. The average survival of those animals was 2.01 years after SIV infection (Table 3). All of the animals with PML were SV40 infected before SIV infection, or for more than 4 years before death, and they survived an average of 2.53 years after SIV infection. The ME/renal cases averaged 3.53 years old when they died (range, 2.06 to 5.66 years; only one animal was over 5 years), and the PML cases averaged >7.5 years (range, 4.8 to 12 years; only one animal was under 5 years).

In Situ Hybridization, Immunohistochemistry, and Co-Localization of Viral and Cellular Determinants

To confirm that the lesions were due to SV40, *in situ* hybridization or immunohistochemistry was performed. SV40 nucleic acid and/or T antigen was found in multiple nuclei of oligodendrocytes and astrocytes in the brain in both ME and PML (Figure 2, C and D). SV40-infected neurons were not seen in any case. Immunostaining for p53 protein labeled the nuclei of similar numbers of cells with a similar distribution in brain and kidney as *in situ* hybridization and immunohistochemistry for SV40. This finding confirms that SV40, like JCV, binds p53 protein *in vivo*.

In addition to oligodendrocyte and astrocyte infection, SV40 antigen and/or nucleic acid was found in mononuclear cells within the cellular infiltrates. SV40-infected cells were also found in the meninges and rarely in choroid plexus, and in one animal with severe ME (9NE), in cells in the wall of meningeal and parenchymal vessels (Figure 2B). Macrophages occasionally contained SV40 nucleic acid in their cytoplasm, and in one case of ME, nuclear labeling with SV40 co-localized with cytoplasmic labeling for macrophages (Figure 2B). In several animals, prominent perivascular cuffs of mononuclear cells were seen in association with both PML and ME. CD3⁺ T lymphocytes were observed in the perivascular cuffs, as well as in aggregates in areas of demyelination in PML (Figure 2, E and F); in ME they were in the meninges and around vessels, generally in lower numbers. Using an antibody directed against CD20, B lymphocytes were observed in low numbers in only one of five PML cases examined and were not found in the one case of ME with significant perivascular inflammation (Figure 2F). Antibodies recognizing CD79a and κ or λ light chains identified a larger number of B cells than CD20, but the number relative to T cells remained quite small.

In situ hybridization for SIV was performed on sections with SV40 lesions in 13 of 15 animals. None of the sections from any animal had SIV and SV40 in the same focus of inflammation. One animal, 6NE, had SIV encephalitis^{28,29} with SIV nucleic acid in multiple cells within the encephalitis but none in the regions of ME. An occasional SIV-containing cell was found in a section of lung from one other case with SV40 pneumonia, 9NE, but again, SIV was not present within the SV40 lesion.

SV40 DNA Sequencing

Ilyinskii et al¹³ reported previously on the sequences of the ori-enhancer and T-antigen carboxy terminus of SV40 isolated from six of the animals (1NE to 6NE). We now have sequences from an additional 6 animals, for a total of 12 of the 15 cases (Table 2).

We examined an additional 17 clones of the ori-enhancer region from 6 animals (Figure 3) and confirmed our previous finding of a single 72-bp element in all of the animal isolates, in contrast to the laboratory strain of SV40, 776, which contains a tandem repeat of the 72-bp

Table 3. SV40 Serology and Age at Death in SIV-Infected Macaques

Animal	SV40-related histology	Age at death/length of SIV infection (years)	Serology; pre-SIV/terminal
6NE	ME, nephritis	3.52/2.24	-/+
7NE	ME, nephritis	4.85/2.82	-/+
9NE	ME, pneumonia	2.06/1.16	-/+
13T	ME	5.66/3.35	-/+
15T	ME, pneumonia, nephritis	2.69/1.53	-/+
3NE	Nephritis	2.4/1.0	-/+
	Average for ME, nephritis	3.53/2.01	
2NE	PML	>12/unknown	?/+*
4NE	PML	4.8/1.77	+/+
5NE	PML	>12/2.06	+/+
8NE	PML	5.85/3.72	+/+
10NE	PML	6.39/1.88	+/+†
11NE	PML	6.38/4.99	+/+
12T	PML	7.27/1.41	+/+
14T	PML	5.43/1.88	+/+
	Average for PML	>7.5/2.53	

NE, animal housed at NERPRC; T, Tulane RPRC.

*Spontaneous infection, so date of infection with SIV is unknown. This animal was seropositive for SV40 4.5 years before death.

†This animal was seronegative 5.5 years before death, when infected with an attenuated strain of SIV, but had seroconverted within 16 months (4 years before death) and was seropositive when challenged with a virulent SIV 2 years before death.

element. The nucleotide sequences of the new clones were essentially identical to those previously reported, with one exception. All of the clones from the brain, lung, and kidney of animal 15T lacked one of the usual complement of three 21-bp repeats seen in strain 776 and all other clones examined from the animals.

Considerably more sequence variation was seen in the 3' end of the T antigen (Figure 4) than in the ori-enhancer. SV40 from one animal, 12T, had the same sequence as laboratory strain 776 over the 200-bp region examined. Unlike previous findings,¹³ in which kidney-derived SV40 had a consensus sequence in this region and the various brain isolates had more variation, sequences of the additional clones examined contained more varied sequences, with no identifiable relationship to the tissue from which the SV40 was derived (brain, kidney, or lung). In animals with SV40 derived from two or three tissues (4NE and 15T), one sequence was predominant in all tissues, although both animals had a minor variant in brain-derived SV40.

Discussion

PML occurs in up to 5% of AIDS patients, and in our study SV40-associated disease of any type had a similar incidence (10 of 229, 4.4%) in SIV-infected rhesus monkeys at the NERPRC. In addition to PML, we now recognize a second CNS manifestation of SV40, meningoencephalitis, and SV40 is also associated with interstitial nephritis and pneumonia. Meningoencephalitis affects the menin-

ges and gray matter of the cerebrum and brain stem, without the classic demyelination of PML.

The critical difference in SV40 lesion type appears to be the temporal relationship of SV40 infection to SIV infection and the age of the affected animals. All of the animals with ME, renal, and/or pulmonary SV40 lesions were infected with SIV before SV40 infection. They survived an average of 2 years after SIV infection and averaged 3.5 years old at death. Animals with PML survived longer after SIV infection (2.5 years) and were older when they died (>7.5 years on average). Using a different serological technique, virus neutralization, for antibodies to SV40 on animals 12T to 15T, another study³⁰ had similar results, with two exceptions: 13T was found to be SV40 positive at the time of SIV inoculation, whereas 15T was never seropositive for SV40. Our data suggest that 1) PML is a reactivation of SV40, whereas ME, along with SV40-associated nephritis and pneumonia, is a manifestation of primary infection, and 2) older animals with perhaps a more indolent course of SIV infection are more likely to have SV40-associated PML.

Data from humans are consistent with our findings in macaques. PML patients have IgG antibody to JCV, evidence that PML results from reactivation of latent JCV infection.³¹ And despite seroepidemiological studies suggesting that the majority of individuals are infected with JCV during childhood, PML is rare in children with immunocompromise, including HIV infection.^{27,32}

Lesions in gray matter in AIDS patients with PML have been reported,³³ and we observe them as well, particu-

Figure 2. *In situ* hybridization and immunohistochemistry for SV40 and cellular antigens. *In situ* hybridization for SV40 coupled with immunohistochemistry for the macrophage marker HAM56 (A and B) demonstrates many gitter cells immunostained for HAM56 (brown), along with several SV40-infected cells (black) in PML (A, enlarged in inset). In B, note the SV40 nucleic-acid-containing cells in vessel wall and meninges and HAM56-positive macrophages in the meninges of an animal with ME. Arrow indicates a cell with nuclear labeling for SV40 and cytoplasmic immunostaining for HAM56 (B). *In situ* hybridization for SV40 with nickel-enhanced DAB, immunohistochemistry for HAM56 with DAB; magnification, ×220. SV40 infection of both astrocytes (C) and oligodendrocytes (D) was detected in both PML and ME. In C, *in situ* hybridization for SV40 (black) coupled with immunohistochemistry for GFAP demonstrates several gemistocytic astrocytes, including an infected astrocyte (arrow). Brown; magnification, ×220. Immunohistochemistry for SV40 (brown) and for oligodendrocytes (red) identifies an infected oligodendrocyte (arrow). Magnification, ×220. A perivascular cuff in PML contains many T cells (E) and no B cells (F). E: CD3. F: CD20 immunohistochemistry, ABC technique with DAB, hematoxylin counterstain; magnification, ×220.

Figure 3. The ori-enhancer (regulatory region) of SV40 from SIV-infected macaques contrasted to strain 776. The darkly shaded boxes represent the 21-bp tandem repeats seen in strain 776 and all but one of the animal isolates. The lightly shaded boxes represent the tandem 72-bp repeats seen in strain 776 only.

larly by extension at the junction of gray and white matter and adjacent to ependyma. However, the gray matter lesions seen in cases of PML are not seen isolated from foci of demyelination, and the meningeal involvement seen in ME is never present. We are aware of one description³⁴ of an AIDS patient with disseminated BKV infection. This patient had BKV-associated pulmonary and renal lesions and meningoencephalitis without white matter involvement identical to the lesions seen in ME caused by SV40 in macaques, with the exception that BKV-infected oligodendrocytes were not identified.³⁴ This is in contrast to our cases of SV40-associated ME, as well as PML, in which many of the infected cells are oligodendrocytes.

If the report of Vallbracht et al³⁴ represents a true difference in pathogenicity for the CNS between JCV and BKV, several explanations are possible. Although both BKV and JCV can be found in kidney and are shed in the urine, only BKV has been associated with disease of the kidney and urinary tract.⁸ Despite apparent efficient replication in multiple cell types *in vivo*, growth of JCV is highly restricted *in vitro*, compared with SV40 and BKV,^{35,36} although recent studies have identified JCV-susceptible primary and established cell lines.^{37,38} This restricted cell type specificity is thought to be due to tissue-specific transcription factors that interact with the regulatory region of the JCV genome.^{37,39–41} JCV DNAs from different sites in the same patient show sequence variations in their regulatory regions, suggesting organ-

specific adaptation of the virus.^{42,43} JCV has a regulatory region very different from those of BKV and SV40,^{44,45} and greater variation in this region has been identified in patient isolates.^{36,46} Possibly SV40 is playing the role of both BKV and JCV in different scenarios.

We examined two regions of the SV40 genome, the ori-enhancer region and the T-antigen carboxy terminus, regions previously identified by us and others^{13,30,42,47,48} to be potential sites for tissue-specific polyomavirus replication. Sequence differences in the ori-enhancer (regulatory) region appear not to be responsible for tissue-specific SV40 replication as sequences were virtually identical from brain and kidney isolates. As we and others have noted previously,^{13,30,48–50} all animal isolates contain a single 72-bp enhancer, not the two copies found in the laboratory strain 776. Only one variation was seen in the ori-enhancer region: a loss of one of the usual complement of three 21-bp repeats, seen in all clones from brain, kidney and lung from a single animal in the current study, a variation also noted by others³⁰ in the same animal.

The T-antigen carboxy terminus of SV40 is a variable region and, as such, a potential site to distinguish and identify viral strains.^{13,30} Although sequence variations in the SV40 T-antigen carboxy terminus were more widespread than in the ori-enhancer, no tissue- or lesion-specific changes were noted in this study. Thirteen of sixteen clones from the three Tulane cases had two unique deletions not seen in any of the NE clones, and 29 of 30 sequences from NE clones contained a deletion not seen in any of the Tulane clones (Figure 4). Lednicky and colleagues³⁰ found the same sequence in 12T (identical to strain 776 over this region) and 15T but some variation in the T-antigen sequence from 13T. We obtained two clones of the T-antigen region from the brain of 13T and also sequenced the amplified product directly from the PCR mixture; all approaches gave the same sequence. Lednicky et al³⁰ reported minor variants in the ori-enhancer sequences from this animal, so the T-antigen sequence discrepancy may reflect a real SV40 heterogeneity in animal 13T, as we have observed in other cases.

Polymaviruses probably spread to the CNS via the circulation. The multifocal distribution of early PML lesions and the vascular and perivascular infected cells in ME and PML^{34,51} support a hematogenous entry. Multiple studies have identified peripheral blood mononuclear cells as sites of persistence of BKV and JCV^{5,52–55} and, in the case of JCV, B lymphocytes specifically.^{38,51,56–58} Rhesus monkeys become viremic during primary SV40 infection.⁵⁹ Although the infected circulating cells in primary SV40 infection have not been identified, SV40 can be isolated from peripheral blood mononuclear cells from SIV-infected macaques with SV40-associated disease.³⁰ JCV-infected lymphocytes^{36,51} and macrophages⁶⁰ have been identified in the brains of patients with PML by some investigators but not others.^{26,61} B lymphocytes are rare in both SV40 and SIV-associated CNS inflammation in macaques^{10,62} and in some cases of PML and HIV encephalitis in AIDS patients.^{26,61} We have not observed SV40 co-localized to lymphocytes or in perivascular cuffs of inflammatory cells in PML. This may be due, at least in

Figure 4. Sequence of the T-antigen carboxy terminus of SV40 from brain, kidney, and/or lung from SIV-infected macaques.

part, to the lack in SV40 of sequences in the JCV regulatory region that interact with B cells.^{41,44,45}

Multiple studies have identified cytoplasmic JCV in macrophages after phagocytosis of cellular debris^{60,63–65} in PML, and we have observed this rarely as well (data not shown). We have identified apparent macrophage infection in severe ME, and infected macrophages were found in the reported case of disseminated BKV.³⁴

AIDS patients with PML frequently have HIV-infected cells among the inflammatory cells in the demyelinating lesion.^{26,61,63,66} *In situ* hybridization failed to identify SIV in the SV40-associated lesions in any animal, although one case with SIV encephalitis (SIVE) had abundant SIV nucleic acid in macrophages and multinucleated cells in the SIVE lesions, and other cases had SIV localized to lymphoid tissue. This confirms previous findings in our laboratory that SIV does not co-localize to sites of inflammation due to opportunistic infections in simian AIDS^{67–71} and may represent a difference in pathogenesis of the two lentiviruses, particularly in the terminal stages of disease.

In conclusion, SV40 is associated with both primary (ME) and recrudescence (PML) CNS disease in macaques with AIDS. Sequence analysis of the regulatory region and T antigen from different lesions does not support a tissue- or lesion-specific sequence. Instead, age of the animal, time of SIV infection, and possibly immunological status relative to time of SV40 infection may be the most important factors in determining the SV40-associated lesions of macaques with simian AIDS.

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