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Simian Virus 40-induced Disease in Rhesus Monkeys with Simian Acquired Immunodeficiency Syndrome

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Simian virus 40 (SV40) disease was diagnosed in four rhesus monkeys that died with SIV-induced acquired immunodeficiency syndrome (AIDS). One juvenile monkey seroconverted for SV40 6 months after inoculation with SIV and developed severe bilateral tubulointerstitial nephritis. In contrast, progressive multifocal leukoencephalopathy (PML) occurred in two adult monkeys that were seropositive for SV40 before SIV inoculation, as well as a third adult that was naturally infected with SIV and seropositive for SV40 5 years before death. Large intranuclear inclusions containing abundant polyomavirus particles were limited to either renal tubular epithelial cells or oligodendrocytes. In situ ***DNA hybridization for SV40 large T antigen further demonstrated that SV40 nucleic acid was localized to either kidney or brain tissue. By immunohistochemical analysis, areas of central nervous system inflammation and demyelination were shown to contain CD68$^+$ macrophages (gitter cells), aggregates of CD8$^+$ T lymphocytes, and numerous gemistocytic astrocytes that labeled for glial fibrillary acidic protein. These observations indicate that rhesus monkeys with SIV-induced AIDS are predisposed to polyomaviral disease, in which SV40 nucleic acid is observed in renal tissue in primary infections and brain tissue after viral reactivation. Furthermore, this organ-specific replication suggests that tissue-tropic strains of SV40 may develop in immunodeficient monkeys. (Am J Pathol 1992, 140:1431–1440)***

Infection with human immunodeficiency virus type 1 (HIV-1) or simian immunodeficiency virus (SIV), both members of the lentivirus subfamily of Retroviridae, is associated with development of acquired immunodeficiency syndrome (AIDS) in humans or rhesus monkeys,[1] respectively. In both species, progressive immune dysfunction results in increasingly frequent infection of the host by opportunistic viral, fungal, bacterial, and protozoal pathogens. Diseases that result from recrudescence of latent viruses carried by the host represent a major cause of morbidity and mortality in immunosuppressed individuals.[2,3] Most prevalent in this group are DNA viruses of the herpesvirus family, such as the herpes simplex viruses, varicella-zoster virus, and cytomegalovirus.[2]

Simian virus 40 (SV40) is an oncogenic DNA virus belonging to the Papovaviridae family. PAPOVA is an acronym derived from the names of the three original viruses in this family: *pa*pillomavirus, *po*lyomavirus, and *va*cuolating agent (SV40).[4] The papovavirus family is currently divided into the Papillomavirinae and Polyomavirinae subfamilies, the latter of which includes SV40. Two other polyomaviruses, JCV[5] and BKV,[6] infect humans and are named after the person from whom they were first isolated.

Polyomavirus virions are 40–45 nm in diameter, lack an envelope, and contain double-stranded, closed circular DNA of approximately 5000 bp.[7] The viral genome consists of early- and late-coding regions and a noncoding regulatory region. The early region encodes DNA binding proteins, known as large or small T (tumor) antigens, which bind to the regulatory region of the genome to facilitate replication of late region viral DNA. The late region encodes three capsid proteins (VP1, VP2, VP3), which display genus-specific antigenic determinants.[7] As the name implies, these polyomaviruses cause tumors when inoculated into species (e.g., hamsters) unrelated to their natural host.[8]

Exposure to JCV or BKV in humans occurs early in life, possibly as a respiratory infection during childhood, so that most adults are seropositive.[8] Experimental infection of rhesus monkeys with SV40 is associated with transient

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viremia, viruria, and seroconversion, but no clinical signs.[9] In immunocompetent natural hosts, these DNA viruses remain latent, primarily in renal tissue, where the viral genome is present as a circular episome in a few unidentified cells.[10,11] Periodic, asymptomatic reactivation of latent renal polyomavirus occurs with pregnancy, diabetes, or old age, and is accompanied by urinary excretion of JCV or BKV.[8]

In an immunocompromised host, polyomaviral disease may result from either primary infection or reactivation of a latent infection. Under these circumstances, the lesions reported with JCV and BKV are limited to the central nervous system (CNS) or kidney, respectively.[8] Replication of JCV in oligodendrocytes causes progressive multifocal leukoencephalopathy (PML),[5,12] a fatal demyelinating disease. PML has been associated with JCV infection of children with combined immunodeficiency[13] and from viral reactivation in adults with immunocompromise due to malignancies, immunosuppressive therapy, or, more recently, AIDS.[14–21] In contrast, fatal tubulointerstitial nephritis has been reported in a BKV-infected child with hyperimmunoglobulin M immunodeficiency,[22] and BKV recrudescence is often observed after renal allografts, where it is associated with replication in urothelial cells.[6,23] To date, BKV disease has not been reported with HIV infection, despite serologic evidence of BKV reactivation in AIDS.[24]

In this report, we describe and further characterize polyomaviral disease in four rhesus monkeys that died with SIV-induced simian AIDS.

Materials and Methods

Animals

The animals described in this report were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. All animals were individually housed in isolation facilities, but were periodically handled in small groups for blood or tissue collection.

Polyomaviral disease was diagnosed after postmortem examination of four SIV-infected rhesus monkeys (*Macaca mulatta* [Mm]) that died with simian AIDS (Table 1). One of these animals, Mm 91-78, was identified as naturally infected with SIV during routine screening of the primate colony. Mm 246-88 died 366 days after intravenous inoculation with 0.5 ml of a 1:100 dilution of media from human peripheral blood lymphocytes (PBLs) infected with SIVmac251, the original uncloned isolate of SIV.[25,26] The remaining animals, Mm 124-79 and Mm 54-83, died 752 and 610 days, respectively, after intravenous inoculation with 0.25 ml of media (reverse transcriptase activity = 10^5 cpm/ml) from rhesus monkey PBLs that were infected with SIVmac239, an infectious molecular clone demonstrated to cause simian AIDS.[1]

Two of these animals, adults with SV40 disease in the CNS, were seropositive for SV40 before inoculation with SIV. A third adult with PML was naturally infected with SIV

Table 1. *Simian Immunodeficiency Virus-infected* Macaca mulatta *(Mm) with Simian Virus 40 Polyomaviral Disease*

Animal	Sex	Age	SIV strain	SV40 serology		Location of SV40 lesions	Other diagnoses
				Date	Status		
Mm 91-78	F	>12	natural infection	11-19-85	+*	Brain	Pulmonary acariasis, myocarditis, demodicosis, *M. avium intracellulare* enteritis.
				10-26-89	+		
				05-07-90	+		
				06-07-90†	+		
Mm 124-79	F	>12	SIVmac239‡	10-08-87	+	Brain	Interstitial pneumonia, multicentric lymphoma.
				06-02-89‖	ND¶		
				06-11-90	+		
				06-24-91†	+		
Mm 54-83	F	8	SIVmac239‡	06-02-89‖	+	Brain	Interstitial pneumonia, suppurative dermatitis, pulmonary arteriosclerosis, adenoviral enteritis
				04-09-90	+		
				11-13-91	+		
				03-10-91†	+		
Mm 246-88	M	2	SIVmac251#	10-18-89	–	Kidney	Bacterial pneumonia, cerebral gliosis, pulmonary arteriosclerosis.
				12-02-89‖	ND¶		
				12-20-89	–		
				01-10-90	–		
				05-15-90	+		
				12-03-90†	+		

* Positive at 1:5 dilution. Virus Reference Laboratory, San Antonio, TX.[33]
† Date of death.
‡ SIVmac239 is an infectious molecular clone demonstrated to cause simian AIDS.[1]
‖ Date of inoculation.
¶ Not determined.
# SIVmac251 is the original, uncloned isolate of SIV.[25,26]

and seropositive for SV40 at least 5 years before death. The fourth animal, a juvenile with SV40 lesions confined to the kidney, seroconverted for SV40 6 months after inoculation with SIV.

Histologic Examination

Formalin-fixed, paraffin-embedded tissues were sectioned by routine methods and examined using hematoxylin and eosin stains. In addition, a Luxol fast-blue stain for myelin was performed on sections of cerebrum.

Immunohistochemical Staining

Tissue samples were snapfrozen in (OCT) compound (Miles Scientific, Naperville, IL) and cryostat sections were mounted on organosilane-coated slides, desiccated and stored at −70°C until further processing. Sections of brain or lymph node were then fixed in 2% paraformaldehyde for 10 minutes at 4°C and immunostained using an avidin-biotin-horseradish peroxidase complex (ABC) technique.[27–29] Briefly, tissue sections were incubated sequentially with unlabeled primary monoclonal antibodies against specific antigens, followed by biotinylated secondary antibody and then biotin-conjugated horseradish peroxidase (Vector Laboratories, Inc., Burlingame, CA). Diaminobenzidine (DAB) served as the chromogen for the peroxidase and resulted in deposition of a brown precipitate on antigenic sites labeled by the primary antibody. Kidney sections were immunostained using a peroxidase-antiperoxidase (PAP) technique as previously described.[27–29]

Frozen brain tissue was examined for the presence of $CD20^+$ B lymphocytes (B1; Coulter Immunology, Hialeah, FL), $CD4^+$ T lymphocytes or macrophages (Nu-Th/I; M. M. Yokoyama and Y. Matsuo, Nichirei Research Institute, Tokyo, Japan), $CD8^+$ T lymphocytes (DK25; DAKO Corporation, Carpenteria, CA), $CD68^+$ macrophages (EBM11; DAKO), tumor necrosis factor alpha (TNFα) (B154.4; G. Trinchieri, Wistar Institute, Philadelphia, PA), SIV core protein (p27; Gag) (R1C7; A. A. Minassian and M. Popovic, NIH), and SIV envelope protein (gp120; Env) (Senv 71.1; C Thiriart, SmithKline Beecham Biologicals, Rixensart, Belgium). Kidney and lymph node were immunostained for SIV p27 and gp120. Formalin-fixed, paraffin-embedded sections of brain were immunostained for glial fibrillary acidic protein (GFAP) (DAKO Carpenteria, CA) with a PAP method.

In addition, frozen sections of brain from two macaques (Mm 126-86 and Mm 127-83) with SIV-associated granulomatous meningoencephalitis[30] were immunostained for CD68, TNFα, SIV p27, and SIV gp120.

As negative controls for each antibody, serial sections were processed identically using equivalent concentrations of irrelevant primary antibodies of the same isotype. All sections were counterstained with Mayer's hematoxylin.

In Situ *Hybridization for SV40 Nucleic Acid*

Sections of formalin-fixed, paraffin-embedded brain, lung, liver, kidney, lymph node, and bone marrow from Mm 91-78, Mm 54–83, and Mm 246-88 were mounted on organosilane-coated slides and used in an *in situ* hybridization procedure.[31] This hybridization technique used horseradish peroxidase (HRP)-labeled DNA probes directed against the entire SV40 large T antigen gene (Digene Diagnostics, Inc., Silver Spring, MD). Silver enhancement of heavy metal-modified DAB was then used to produce a black precipitate at the site of nucleic acid hybridization.[31] As a negative control, serial sections were hybridized with a HRP-labeled plasmid vector (Digene Diagnostics, Inc., Silver Spring, MD). All sections were counterstained with nuclear fast red.

In Situ *Hybridization for SIV RNA*

In situ hybridization for SIV RNA was performed on formalin-fixed, paraffin-embedded brain from the three animals with PML, and one animal with SIV granulomatous meningoencephalitis (Mm 126-86) using a ^{35}S-labeled DNA probe and methods previously described.[32]

Electron Microscopy

Formalin-fixed specimens of kidney and brain were processed and embedded in Epon 812 resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined on a JEOL 100S transmission electron microscope.

Serology

Sera samples were assayed at a 1:5 dilution for the presence of antibodies to SV40 by a dot-immunobinding technique (Virus Reference Laboratory, San Antonio, TX), as previously described.[33]

Results

Renal Tissue

Both kidneys of Mm 246-88 were small and firm and had an irregular capsular surface; on cut section they were pale tan. Microscopically, there was diffuse fibrosis, multifocal, nonsuppurative tubulointerstitial nephritis, focal glomerulosclerosis, and glomerular atrophy (Figure 1). The renal medulla was characterized by tubular disorganization, dilatation, and regeneration. Numerous tubular epithelial cells contained enlarged, hyperchromatic nuclei with marginated chromatin, and large basophilic intranuclear inclusions (Figure 2A). These inclusions were frequently present in sloughed epithelial cells forming cellular casts within tubular lumina (Figure 1). By electron microscopy, these degenerating tubular epithelial cells contained abundant polyomavirus particles (40 nm diameter) arranged in paracrystalline arrays within the nucleus (Figure 2C). SV40 infection of these cells was confirmed by *in situ* hybridization for SV40 large T antigen nucleic acid (Figure 2B).

Renal tissue from macaques with brain lesions was histologically unremarkable and contained no SV40 nucleic acid, as measured by *in situ* hybridization. Kidney tissue from all animals tested negative when immunostained for SIV p27 and gp120 antigens.

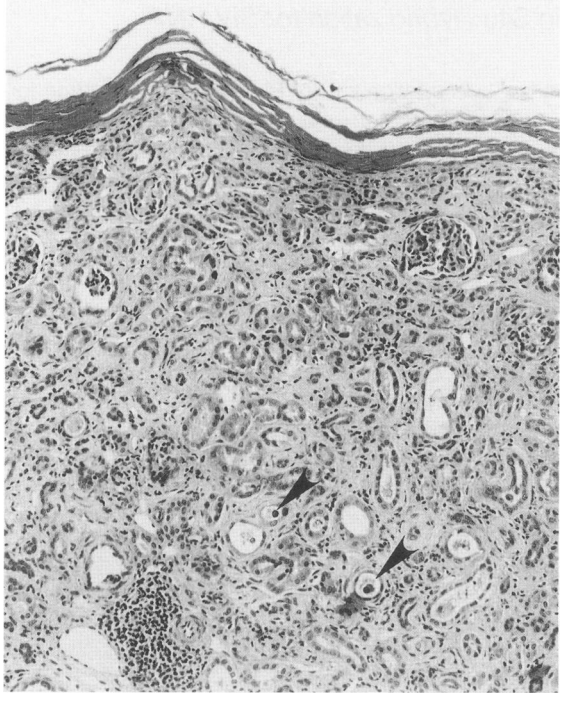

Figure 1. *Section of renal cortex with SV40-induced tubulointerstitial nephritis. There is an irregular capsular surface, diffuse fibrosis, and a multifocal lymphocytic infiltrate. Several tubular luminae contain sloughed epithelial cells (arrowheads) (H&E, ×132).*

CNS Tissue

Macroscopic lesions were similar in Mm 91-78, Mm 124-79, and Mm 54-83. Multiple small (1–3 mm) gray-pink foci were scattered in cerebral white matter; these foci were present in subcortical white matter and occasionally in gray matter of virtually all sections of cerebrum examined.

Histologically, both early and advanced lesions of demyelination and gliosis were present within white matter, most commonly near the boundary with gray matter, and in subependymal gray matter. Early lesions were characterized by microgliosis, with large astrocytes and numerous interfascicular oligodendrocytes that had swollen, atypical nuclei containing marginated chromatin and intranuclear inclusions (Figure 3). By electron microscopic examination, these oligodendrocytes were seen to contain abundant intranuclear polyomaviral particles (40 nm diameter), including filamentous forms (24 nm width) (Figure 4). Areas of more advanced demyelination (Figure 5) were characterized by a dense inflammatory infiltrate of macrophages (gitter cells) laden with phagocytized myelin. Around the periphery of advanced lesions was a collection of large gemistocytic astrocytes with prominent cytoplasm and enlarged, eccentric nuclei (Figure 5B).

Polyomaviral brain lesions were also examined using immunohistochemical procedures. $CD8^+$ T lymphocytes were occasionally present in unaffected brain parenchyma and within Virchow-Robbins spaces, but within areas of demyelination and gliosis they appeared in aggregates (Figure 5C). $CD20^+$ B lymphocytes were not detected, and $CD4^+$ T cells were rare in both early and advanced lesions. Microglial cells throughout the normal cerebral tissue were lightly $CD68^+$. However, in advanced lesions a dense infiltrate of $CD68^+$ macrophages was present. These cells did not contain immunolabeled TNFα.

In areas of unaffected white and gray matter, fibrous and protoplasmic astrocytes were labeled for GFAP (Figure 6A). However, in areas of SV40 replication, dramatic immunostaining occurred for GFAP in large gemistocytic astrocytes with swollen, truncated cytoplasmic processes (Figure 6B). These unusual astrocytes were also moderately positive for TNFα. By *in situ* hybridization, SV40 large T-antigen nucleic acid was localized to the nuclei of many scattered oligodendrocytes and occasionally to the cytoplasm of astrocytes (Figure 7).

Brain tissue from the animal with renal lesions was

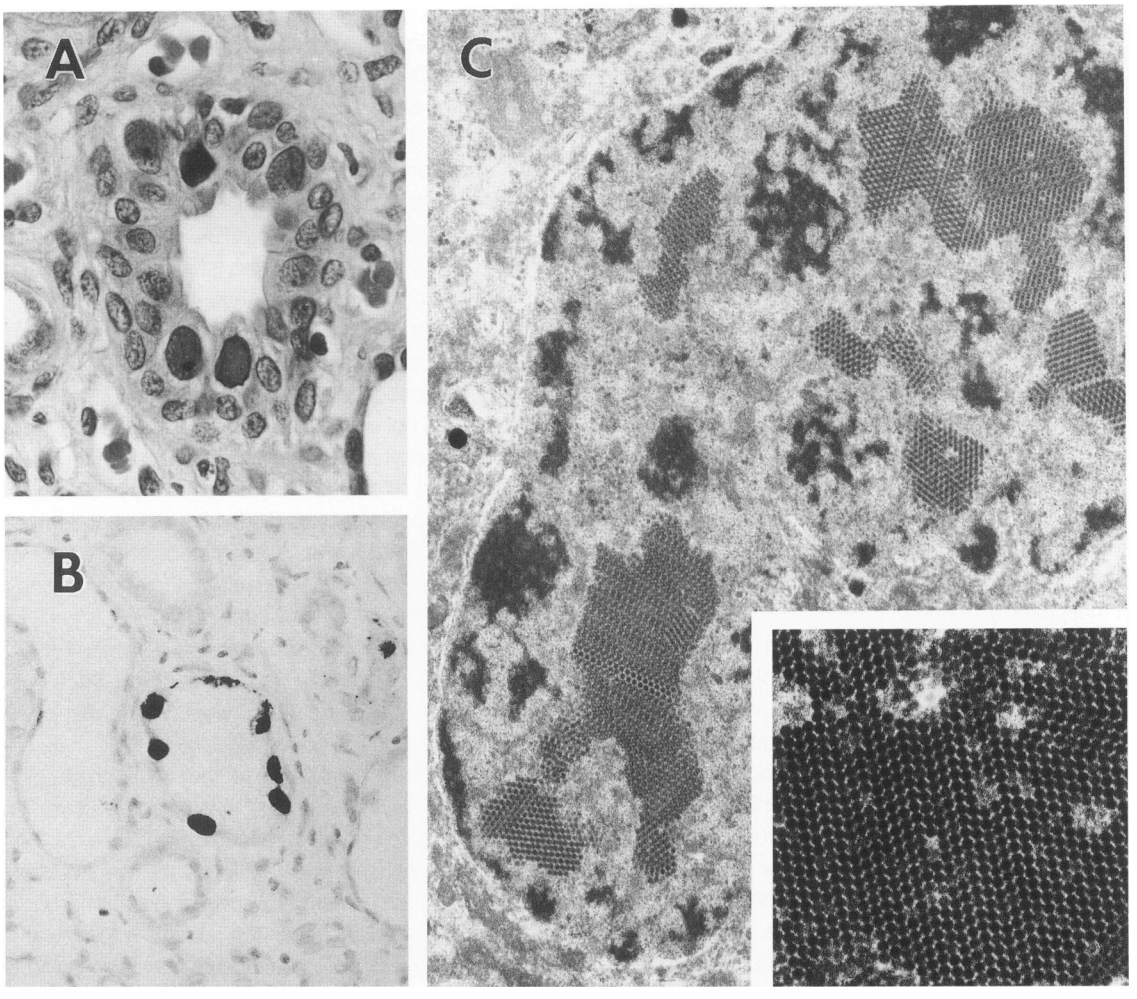

Figure 2. *SV40-induced renal disease.* **A:** *Several medullary tubular epithelial cells contain enlarged, hyperchromatic nuclei with marginated chromatin and large, basophilic intranuclear inclusions (H&E, ×520).* **B:** In situ *hybridization demonstrates SV40 large T antigen nucleic acid in the nuclei of many of these epithelial cells (nuclear fast red, ×260).* **C:** *Transmission electron micrograph of a sloughed renal tubuloepithelial cell. The degenerating nucleus contains abundant spherical polyomavirus particles (40 nm diam.) arranged in paracrystalline arrays (inset) (×22,500; inset, ×50,000).*

marked by diffuse gliosis, but *in situ* hybridization for SV40 nucleic acid did not reveal positive cells.

None of the sections of brain from animals with PML contained cells that immunostained for SIV p27 or gp120; similarly, SIV RNA was not detected by *in situ* hybridization. In contrast, gag and env proteins and SIV RNA were localized to numerous $CD68^+$, $TNF\alpha^-$ macrophages and multinucleate giant cells (MNGCs) present in CNS tissue of macaques with SIV-associated meningoencephalitis.

Other Tissues

SV40 viral inclusions were not seen in lung tissue of any animal, although all of them had concomitant pulmonary disease (Table 1). Similarly, SV40 large T antigen nucleic acid was not detected by *in situ* hybridization in lung, liver, spleen, lymph nodes, or bone marrow from the three animals that were evaluated.

Despite the absence of lentiviral antigens in brain tissue, SIV p27 and gp120 antigens were detected by immunostaining in follicular dendritic cells of lymph nodes from all four animals with SV40 disease.

Discussion

Simian polyomaviral disease (PML) was first identified in a retrospective survey[34] of neurologic lesions in adult (≥ 6 yr) macaques (7 *M. mulatta,* 1 *M. arctoides* [*speciosa*]) from which SV40 was subsequently isolated.[35] These animals had granulomatous pneumonia, avian tuberculosis, and lymphoma — diseases encountered in

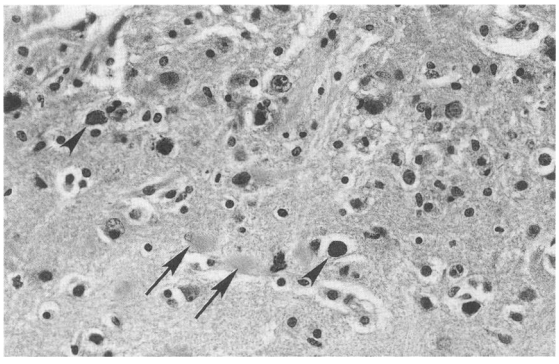

Figure 3. *Early SV40-induced progressive multifocal leukoencephalopathy (PML) within cerebral white matter. There is prominent microgliosis, with several large gemistocytic astrocytes (arrows). Many oligodendrocytes have enlarged, hyperchromatic nuclei with prominent amphophilic to basophilic intranuclear inclusions (arrowheads) (H&E, ×325).*

animals with simian AIDS induced by SIV. PML has been reported in one other adult (9.6 yr) case of *M. arctoides* with malignant lymphoma.[36] SV40 has been further associated with interstitial pneumonia and renal tubular necrosis in three juvenile cases of *M. cyclopis* with an immunodeficiency syndrome[37] and one juvenile case of *M. mulatta*, suggested to have had immune deficiency.[38]

In theory, PML may occur in immunodeficient individuals as a result of 1) primary systemic infection involving brain tissue, 2) reactivation of latent virus in brain, or 3) reactivation of latent virus in the kidney or other organ with subsequent replication in the brain. The fact that three adult rhesus monkeys with PML were seropositive for SV40 before inoculation with SIV or at least 5 years before death from naturally acquired AIDS, suggests that CNS lesions represent recrudescence of latent polyomaviral infections in adults with immune disorders. Reactivation of latent polyomaviruses may be related to loss of cell-mediated immunity and escape from immune surveillance.[39] $CD4^+$ T lymphocytes are known to be depleted in humans and monkeys with AIDS,[1] and we have shown that $CD8^+$ T cells predominate over $CD4^+$ T cells in the inflammatory infiltrate associated with SV40-induced PML. However, polyomaviral recrudescence must not depend solely on a lack of $CD4^+$ T cells, since most individuals with AIDS are likely seropositive for JCV, yet PML is recognized in fewer than 4%.[14]

Although SV40,[40] JCV,[11,13] and BKV[10,11] remain latent in kidney cells, the sequence of events leading to viral recrudescence and productive infection of oligodendrocytes is not well defined. It has been suggested that oligodendrocytes become latently infected after initial exposure,[39,41] since JCV DNA is contained in histologically normal oligodendrocytes distant from sites of demyelination.[41–43] This idea is unsubstantiated, for JCV (and BKV) DNA has not been detected in brain from normal individuals.[11,13] Moreover, investigators did not identify JCV DNA in brains of 24 AIDS patients with non-PML viral encephalitis.[16] Similarly, we did not detect SV40 nucleic acid in brain tissue from a juvenile rhesus monkey with SV40 replication limited to the kidney.

A more likely explanation for the development of PML in seropositive individuals is that polyomaviruses enter the brain hematogenously after reactivation in another organ. Recently, mononuclear cells (B lymphocytes) of spleen and bone marrow were reported to harbor JCV DNA and capsid antigen in two patients with PML, one of whom had AIDS.[43] This finding of latently infected lymphocytes may have important implications for polyomaviral replication if these cells are recruited to an area of inflamed brain. For example, SV40 DNA enhancer se-

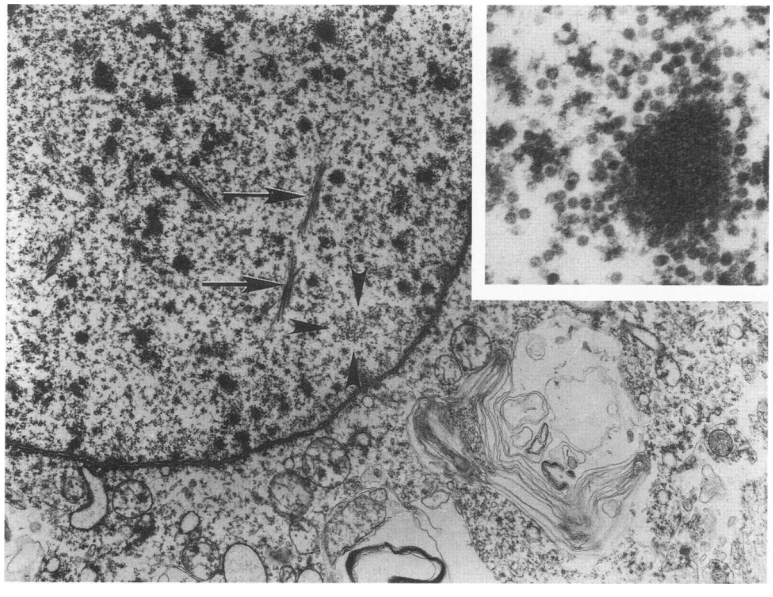

Figure 4. *Transmission electron micrograph of a degenerating oligodendrocyte. The swollen nucleus contains abundant intranuclear polyomaviral particles (40 nm diam.) (arrowheads and inset) and filamentous forms (24 nm diam.) (arrows) (×9,000; inset, ×60,000).*

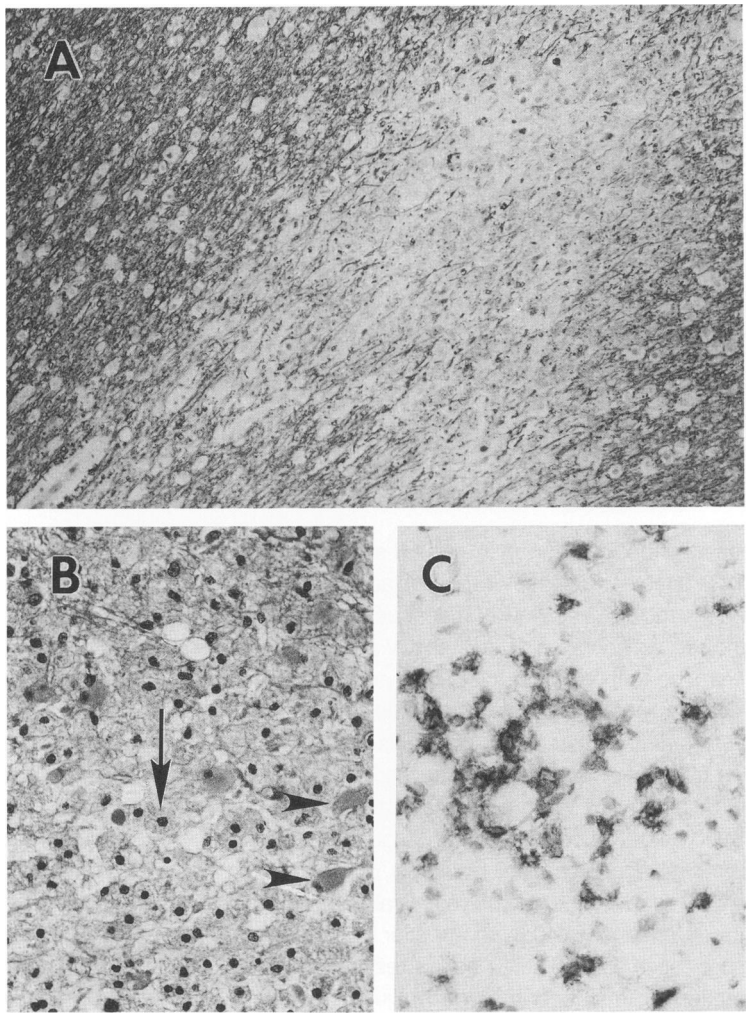

Figure 5. *An advanced lesion of simian PML in cerebral white matter.* **A:** *Lytic replication of SV40 in oligodendrocytes has resulted in severe demyelination (Luxol fast blue, ×160).* **B:** *There is a dense infiltrate of macrophages (gitter cells) (arrow) with numerous gemistocytic astrocytes around the periphery of the lesion (arrowheads) (H&E, ×215).* **C:** *Immunohistochemical staining of regions of demyelination reveals numerous CD8$^+$ T lymphocytes (ABC technique with hematoxylin counterstain, ×325).*

quences are similar to, and can substitute for, B-cell immunoglobulin heavy-chain gene enhancer sequences *in vitro*[44] so that transcriptional factors can bind to both the immunoglobulin heavy-chain enhancer and the SV40 enhancer.[45] Therefore, if SV40 remains latent in lymphoid cells, local or systemic immune activation may stimulate SV40 reactivation or replication within CNS tissue. We have shown that CD8$^+$ T lymphocytes are the predom-

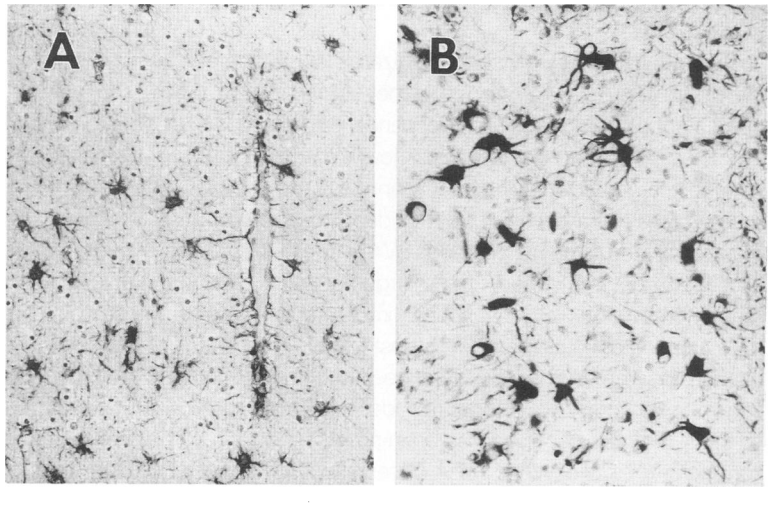

Figure 6. *Immunohistochemical staining for glial fibrillary acidic protein (GFAP).* **A:** *Unaffected white matter contains many fibrous astrocytes, some with cytoplasmic processes extending to a cerebral vein (PAP technique with hematoxylin counterstain, ×162).* **B:** *In an area of SV40 replication within cerebral white matter, there are large, bizarre gemistocytic astrocytes with swollen, truncated cytoplasmic processes and eccentric nuclei (PAP technique with hematoxylin counterstain, ×162).*

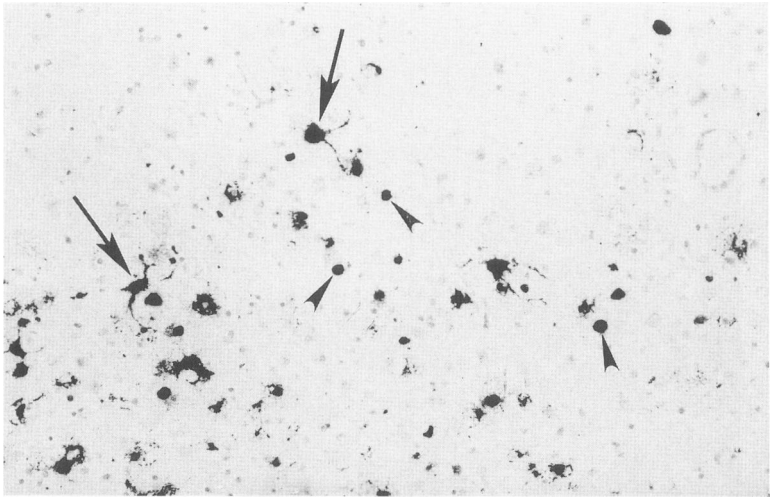

Figure 7. In situ *hybridization demonstrates SV40 large T antigen nucleic acid localized to numerous oligodendrocyte nuclei (arrowheads), as well as the cytoplasm of several gemistocytic astrocytes (arrows), in subependymal white matter (nuclear fast red, ×162).*

inant lymphoid cell in AIDS-related simian PML, but we were unable to detect SV40 nucleic acid in spleen, lymph node, and bone marrow of macaques with PML. Nevertheless, we cannot exclude that JCV and SV40 may gain entrance to the CNS in trafficking lymphocytes carrying low copy numbers of viral transcripts.

We did not observe simultaneous replication of SV40 in both brain and kidney from the macaques in this study. Our finding of PML in adult macaques seropositive for SV40 before inoculation with SIV argues in favor of polyomaviral recrudescence. In contrast, our observation that a juvenile macaque with polyomaviral kidney disease seroconverted for SV40 6 months after inoculation with SIV suggests that renal SV40 lesions result from primary infection of immunocompromised juvenile macaques, much like that reported in an immunodeficient child infected with BKV.[22] We suspect that this juvenile macaque acquired a primary infection as a result of contact with seropositive adults during handling for sample collection. If so, this observation implies that humans, particularly children, with HIV-induced immunodeficiency may be vulnerable to polyomaviral disease after primary exposure.

While serology and *in situ* hybridization substantiate the predilection of SV40 for the kidney during primary infection and brain during recrudescence, the reason for this selective tissue tropism is not known, nor is it clear why the human polyomaviruses JCV and BKV most commonly cause disease in the brain or kidney, respectively. Mutations to the gene that encodes SV40 large T antigen alter viral host cell range,[46] and organ-specific rearrangements in the JCV enhancer-promoter region DNA may affect virus interactions with host cells.[47] The enhancer segment of the JCV genome has further been shown to be homologous to a brain identifier sequence, so that it may be recognized by brain-specific transcription factors.[48] Collectively, these observations raise the possibility that *in vivo* selection of tissue-specific polyomaviral strains may occur in hosts that are immunocompromised.

SV40 replication in SIV-infected macaque CNS tissue elicited a dense macrophage infiltrate without MNGCs. Whereas lentiviral antigens and nucleic acid were prevalent in macrophages and MNGCs in SIV-associated granulomatous meningoencephalitis, we did not detect SIV virions, antigens, or nucleic acid in the macrophage infiltrate accompanying SV40 brain lesions, despite the fact that SIV gag and env proteins were present in lymph nodes from macaques with PML. In contrast, several investigators have demonstrated that macrophages from PML lesions contain HIV virions or antigens.[15,16] Although brain biopsies from AIDS patients with early PML contain few HIV$^+$ macrophages, numerous HIV-infected macrophages and MNGCs are present within PML lesions at the time of death.[15,16] This difference in lentiviral localization in macaque and human PML may be related to the fact that the course and severity of AIDS in these macaques were not modified by therapeutic intervention. Had the disease course been prolonged, additional SIV-infected macrophages may have been recruited, perhaps forming the MNGCs common to SIV encephalitis[30,49] and to one case of human PML in which HIV was demonstrated.[19] Thus, one explanation for the finding of HIV-infected cells in human PML is that AIDS-related encephalopathy[50] may coexist with JCV lesions.[17–19]

There is indirect evidence that simultaneous polyomavirus and lentivirus infection of the brain may enhance replication and dissemination of both viruses *in vivo*. For instance, JCV and BKV, as well as other DNA viruses, have been shown to stimulate the HIV long terminal repeat (LTR) promoter *in vitro*.[51] Interestingly, this transactivation is reciprocal, such that the HIV transregulatory protein, Tat, enhances the activity of the JCV late promoter *in vitro*.[52] Mutual viral transactivation may explain

why human PML lesions are more severe with AIDS than with other immunosuppressive conditions.[19,21]

In conclusion, we have demonstrated that macaques with SIV-induced AIDS are predisposed to primary and recrudescent polyomaviral disease. By DNA *in situ* hybridization, SV40 large T-antigen nucleic acid was localized exclusively to renal tissue in primary infection and brain tissue during reactivation of latent infections. We suggest that SV40 may undergo organ-specific selection of tissue-tropic viral strains in immunodeficient macaques.

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