Comparative Medicine
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Vol 58, No 1
February 2008
Pages 51–56

Polyomaviruses of Nonhuman Primates: Implications for Research

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Polyomaviruses are a family of small nonenveloped DNA viruses that infect birds and mammals. At least 7 nonhuman primate polyomaviruses that occur in macaques, African green monkeys, marmosets baboons, and chimpanzees have been described, as well as 4 polyomaviruses that occur in humans. *Simian virus 40* (SV40), which infects macaques, was the first nonhuman primate polyomavirus identified as a contaminant of early polio vaccines. Primate polyomaviruses cause inapparent primary infections but persist in the host and can cause severe disease in situations of immunocompromise. This review describes the primate polyomaviruses, and the diseases associated with the viruses of macaques. In macaques, the greatest current concerns are the potential confounding of study results by polyomavirus infections and the zoonotic potential of SV40.

Abbreviations: PML, progressive multifocal leukoencephalopathy; SV40, *Simian virus 40*

Polyomaviruses were previously members of the family *Papovaviridae*, which included (and derived its name from) rabbit papilloma virus (*pa*), mouse polyoma virus (*po*), and simian vacuolating virus (*va*). Papovaviruses are nonenveloped viruses, with double-stranded circular DNA and an icosahedral capsule. Since the 1980s, studies of *Simian virus 40* (SV40) and mouse polyomavirus have demonstrated that these viruses have smaller capsids (45 nm versus 50 nm), smaller genomes (5 kb versus 8 kb), and a different genomic organization than those of papillomaviruses. SV40 and mouse polyomavirus now form an independent family, *Polyomaviridae*.[18]

More than 13 members of *Polyomaviridae* infect mammals and birds. The first polyomavirus was discovered in 1953 in mice[28] and was so named because it caused tumors at multiple sites in neonatal mice. Indeed oncogenicity is a common feature of polyomaviruses, particularly tumor production in non-native hosts. Various members of the group transform cell lines and immortalize primary cell cultures as well as induce tumors in susceptible animals. SV40 was identified in 1960 in primary macaque kidney cell cultures, as a contaminant of polio vaccines.[68] In 1971, the human polyomaviruses BKV[23] and JCV[54] were identified (both are named after the initials of the patients in which they were first recognized). JCV was discovered in the brain of a patient with progressive multifocal leukoencephalopathy, and BKV was found in the urine of a renal transplant patient. Recently, 2 additional polyomaviruses of the nasopharynx of humans, KIV and WUV, have been identified[2,25] through the use of molecular techniques. KIV was found in nasopharyngeal samples from patients with respiratory disease, and WUV initially was detected in a child with pneumonia. KIV and WUV are closely related genetically and may form a new subfamily of polyomaviruses: their early coding regions (T antigens) are similar to those of other primate polyomaviruses, but their late regions (structural proteins) differ.[7,25] Both KIV and WUV appear to be geographically widespread.

The capsids of the polyomaviruses contain 3 structural proteins: VP1, the major capsid protein, and VP2 and VP3, which enclose a single molecule of viral DNA. The viruses also encode regulatory proteins, the T (tumor) antigens. SV40 and other primate polyomaviruses encode 2 T antigens, large T and small t, whereas mouse polyomavirus and some of the other family members have a third, middle T antigen. The T antigens of SV40, BKV, and JCV have about 75% amino-acid homology.[58] The T antigen of SV40 is essential for initiation of viral DNA replication and promotes transformation and immortalization of host cells, partially through binding to and inhibiting tumor suppressor proteins p53, p107, p130 (pRb2), and pRb (reviewed in reference 10).

Table 1. Polyomaviruses of nonhuman primates

Virus (species)	Year identified	Reference
SV40 (rhesus macaque)	1960	68
Simian virus 12 (baboon)	1977	71
B-lymphotropic polyomavirus (LPyV; African green monkey)	1979	80
Baboon polyomavirus 2	1989	24
Cynomolgus polyomavirus	1999	74
SV40-CAL (marmoset)	2004	79
Chimpanzee polyomavirus	2005	33

Primate Polyomaviruses

To date, at least 7 nonhuman primate polyomaviruses in macaques, African green monkeys, marmosets, baboons, and chimpanzees have been described (Table 1). SA12 (*Simian virus 12*) and B-lymphotropic polyomavirus (*African green monkey polyomavirus*) were first isolated from kidney and a lymphoblastoid cell line, respectively, from African green monkeys.[80] Later, SA12 was identified as a baboon polyomavirus,[71] and an additional polyomavirus was isolated from baboon kidney.[24] SV40-CAL was isolated from kidney extracts from a Goeldi's monkey with glomerulonephritis;[79] the nucleic acid sequence of SV40-CAL is similar to that of a strain of SV40-T302.[38] No association between SV40-CAL and renal or

Received: 29 Oct 2007. Revision requested: 5 Dec 2007. Accepted: 16 Jan 2008.
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other disease has been made as yet. The nucleic acid sequences of SA12 and B-lymphotropic polyomavirus have been obtained.[11,55] Phylogenetic analysis places these primate polyomaviruses and SV40 close to BKV and JCV, with B-lymphotropic polyomavirus less closely related.[57] In addition, PCR analysis revealed a chimpanzee polyomavirus in the feces of a juvenile monkey with diarrhea,[33] but attempts to isolate virus were unsuccessful.

Some authors refer to all of the macaque and baboon polyomaviruses as SV40 or SV40-like, and the vast majority of studies feature the macaque viruses. Although our understanding of polyomavirus transmission and pathogenesis is incomplete, we do know that exposure generally occurs early in life, followed by transient viremia and viruria[3] and then latency in kidney, lymphoid, and other tissues. In a group of free-ranging rhesus macaques in Nepal, more than 75% of juveniles and more than 90% of adults were seropositive for SV40.[34] In breeding colonies of macaques where infection is endemic (that is, most conventional colonies), 90% to 100% of breeding adults are seropositive,[40,43,46] and more than 80% of captive adult baboons have antibodies to SV40.[56] Similarly, more than 80% of healthy adult humans are seropositive for JCV or BKV or both.[77] Little is known regarding the protective effects of the antibodies and the cellular immune response to SV40 and other polyomaviruses.

Transmission generally is thought to be horizontal, occurring after weaning, with no clinical signs after primary infection in healthy nonhuman primates. Among several cohorts of weanling cynomolgus macaques from seropositive dams, most animals were seronegative at the time of initial sampling (at 6 to 12 mo of age), when groups of weanlings were formed.[49] Seroconversion followed rapidly, with nearly 100% seroconversion within 3 mo in 2 of 3 groups. For unknown reasons, only about 30% of the animals in the third cohort (n = 16) had seroconverted by 300 d. In another study,[56] a group of infant baboons was followed with biannual serum sampling. More than half of the animals remained negative until they were older than 1 y, and nearly 67% had seroconverted by 3 y, the approximate age at which they reached puberty.

Viremia follows infection in rhesus macaques,[60] and the virus can be found in the kidney and urine of healthy macaques;[52] in kidney, brain, lung, and peripheral blood mononuclear cells in SIV-infected rhesus macaques;[29,31,39,52,62] and in kidney, ureter, and small intestine in cynomolgus macaques after renal transplant.[74] Infectious virus has been recovered from cage waste (urine, feces, and food residue) from seropositive cynomolgus macaques.[9]

The pathogenesis of the human polyomaviruses JCV and BKV has been studied somewhat more intensively than that of SV40, although the exact mode of transmission for the human viruses remains unknown currently. After infection, viral DNA can be found in kidney, lymphocytes, bone marrow, tonsil (JCV),[50] and possibly brain (JCV) and other tissues, suggestive of hematogenous spread. Virus is shed intermittently in the urine of infected persons; in the United States, the urinary prevalence of BKV is about 15%, compared with about 40% for JCV.[43] As yet, no clear association between KIV or WUV and respiratory (or other) disease has been made.[53]

Disease Due to Macaque Polyomavirus Infection

Disease due to SV40 is associated with immunocompromise. In studies of SIV-infected rhesus macaques, 10 of 229 animals[62] and 18 of more than 1000[52] had SV40-related disease. The central nervous system, kidney, and lung can be affected.[29,62]

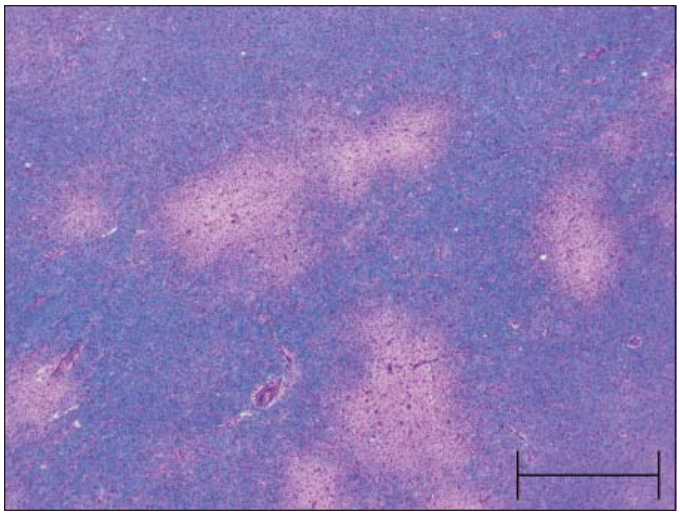

Figure 1. PML. Low-magnification photomicrograph of cerebrum from an SIV-infected rhesus macaque. The pale foci in the blue-stained white matter are areas of demyelination due to SV40 infection. Luxol fast blue–hematoxylin and eosin stain; bar, 1 mm.

The SV40 lesion identified most frequently in SIV-infected rhesus macaques is progressive multifocal leukoencephalopathy (PML), which is essentially identical to the lesion caused by JCV in immunocompromised humans.[59] PML is a demyelinating lesion of the white matter of the central nervous system, due to infection of oligodendrocytes, with characteristic pale foci in tissue stained for myelin (Figure 1). With time, the areas of demyelination are filled with a dense infiltrate of macrophages containing myelin debris (gitter cells). These foci of demyelination are surrounded by gliosis, with cells containing enlarged nuclei with smudgy intranuclear viral inclusions and characteristic large, gemistocytic astrocytes with enlarged and sometime multiple nuclei (Figure 2). Astrocytes can be infected also, at least in macaques,[1,62] as well as, possibly, macrophages and smooth muscle cells.

In addition to PML, SV40 causes a meningoencephalitis, a lesion of the meninges and superficial gray matter, without marked demyelination. The meninges are variably thickened by edema and inflammatory cells, largely around vessels. The inflammation extends into the superficial gray matter, along blood vessels with hypertrophied endothelial cells. Like PML, SV40 meningoencephalitis is characterized by reactive astrocytes and cells with large, smudgy, intranuclear inclusions. Meningoencephalitis typically occurs in macaques infected with SV40 after SIV infection[62] and can occur in concert with PML under certain circumstances.[4]

Other manifestations of primary SV40 disease in SIV-infected rhesus macaques include interstitial pneumonia and nephritis, often occurring in concert with meningoencephalitis.[29,62] In addition, cynomolgus polyomavirus has been associated with renal disease and immunosuppression after renal transplantation[17,74] in cynomolgus macaques. The renal lesions of SV40 and cynomolgus polyomavirus are similar and are characterized as interstitial nephritides with enlarged tubular epithelial cells containing intranuclear inclusions, particularly in collecting ducts (Figure 3). Ureteral involvement with occasional stenosis has occurred in renal transplant recipient cynomolgus macaques.[74] Similar to those

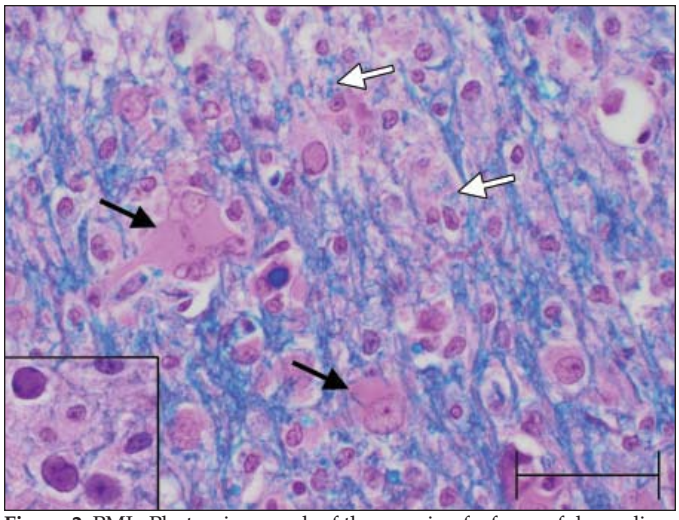

Figure 2. PML. Photomicrograph of the margin of a focus of demyelination in the cerebrum of an SIV-infected rhesus macaque. Large, gemistocytic astrocytes (black arrows), with bizarre, sometimes multiple, nuclei; macrophages containing phagocytized myelin debris (white arrows); and nuclei of glia with smudgy intranuclear inclusions of SV40 (enlarged in inset). Luxol fast blue–hematoxylin and eosin; bar, 50 µm.

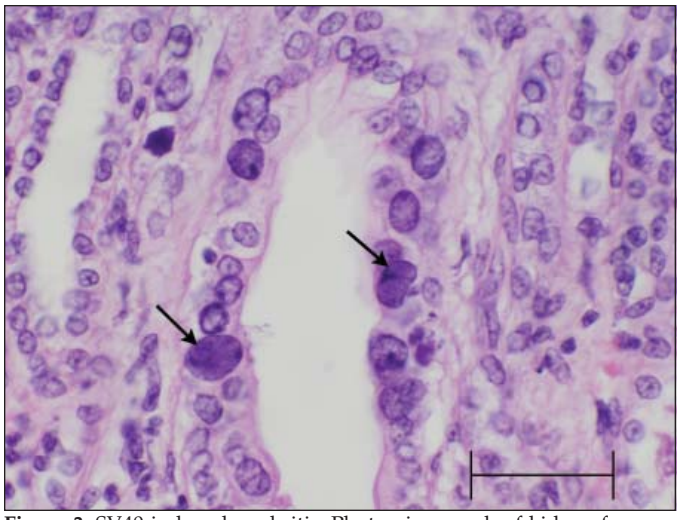

Figure 3. SV40-induced nephritis. Photomicrograph of kidney from an SIV-infected rhesus macaque. Arrows indicate enlarged nuclei of a collecting duct with intranuclear polyomaviral inclusions. Hematoxylin and eosin stain; bar, 50 µm.

due to SV40 and cynomolgus polyomavirus, BKV causes renal lesions (including ureteral stenosis) in human renal transplant recipients.[70] There is a single case report of disseminated BKV infection in a patient with AIDS, who had meningoencephalitis similar to SV40-associated lesions in macaques, as well as pulmonary and renal lesions.[72]

Research Implications

Thus far, reported polyomaviral lesions in nonhuman primates have occurred almost exclusively in macaques immunosuppressed with SIV or post-transplantion medications. However, a few cases of PML in human patients on treatment regimens that are not so clearly immunosuppressive have been described.[35,37,73] The patients received natalizumab, either alone or in combination with other drugs. A retrospective analysis of patients given natalizumab in various clinical trials estimated the risk for development of PML to be 1 in 1000 after a mean of 18 mo of treatment.[78] Natalizumab is a monoclonal antibody directed against α_4 integrin, which inhibits binding of activated mononuclear leukocytes to endothelial cells in the brain and other tissues that use α_4 integrin (VLA4), thus preventing trafficking of these cells into those tissues. No other infections typical for the setting of immunosuppression were noted in the treatment trials. Although several hypotheses have been proposed to account for the apparent association of PML and natalizumab,[5,41,67] no definitive explanation has been reported. Because drug regimens often are tested in macaques before their use in humans, polyomavirus disease might appear in macaques under conditions similar to those associated with disease in humans.

Zoonotic Potential

SV40 was first identified as a contaminant of macaque kidney cell cultures that were used in making polio and other vaccines. From 1955 to 1963 and probably later,[20] more than 30 million people were potentially exposed to SV40 through the Sabin (modified live) and Salk (formalin-inactivated) polio vaccines and an adenoviral vaccine given to US military personnel.[64] SV40 is tumorigenic in rodents[14,15,20] and can transform many cell types. For example, SV40 DNA was found in a malignant astrocytoma in an SIV-infected macaque.[30] In addition, the abilities of SV40 to infect human cells in culture[8,61] and replicate in humans[20,48,51] have long prompted interest in SV40 infection of humans and its association with subsequent disease, particularly cancers. Over the past several decades, multiple studies (described in following paragraphs) have found evidence of SV40 exposure in humans, by using serology, immunohistochemistry, or probes for nucleic acid in human tumors, particularly mesotheliomas, osteosarcomas, brain tumors, and non-Hodgkin lymphomas, which are the tumor types found in SV40-inoculated rodents.

Antibodies to SV40 have been found in the sera of healthy persons who likely received contaminated polio vaccines and those who did not[10,27] and in mesothelioma patients.[10] For example the seroprevalence of SV40 among cancer patients (5% to 10%) is similar to that among their controls, suggesting no association with SV40.[47]

Some caution is warranted in interpreting serology studies, particularly early ones, because the T antigens of SV40, BKV, and JCV strongly cross-react with the same antisera, whereas less cross-reaction is seen with the structural proteins.[47] When tested by a plaque neutralization assay,[65] the sera from most subjects were negative for SV40 (31 of 34 mesothelioma patients, 32 of 33 osteosarcoma patients, and 34 of 35 controls). In a study comparing the reactivity of rhesus macaque and human sera to VP1 virus-like particles from SV40, BKV, and JCV,[75] both macaque and human sera demonstrated cross-reactivity between SV40 and BKV and (to a lesser extent) JCV. Both SV40 and BKV virus-like particles blocked the reactivity of macaque sera to SV40, BKV, and JCV (human sera not tested), and the SV40 and BKV activities correlated. Because no SV40-negative macaque sera demonstrated reactivity with BKV, the BKV reactions of the macaques were considered to be due to SV40 antibodies, rather than to indicate BKV infection of the rhesus macaques. The SV40 and JCV reactivities of the macaque sera were not significantly correlated. In humans,

SV40 titers tended to be low, and the prevalence of SV40 antibody was much lower than the prevalence of antibodies to one or both of the human polyomaviruses (JCV and BKV). In a study of 109 zoo workers that combined the use of virus-like particles with competitive inhibition,[22] 9% of workers with regular exposure to nonhuman primates had specific anti-SV40 antibodies, as did 3% of workers with infrequent or previous exposure. Another study[16] using virus-like particles found anti-SV40 antibodies in 6.6% of human sera; however, the reactivity disappeared after preincubation of the sera with BKV or JCV virus-like particles, indicating that the response to SV40 was due to cross-reactivity. Using different techniques, another group[44] found no SV40-cross-reactive antibodies in South American Indians with JCV and BKV titers but who were unlikely to have been exposed to SV40 nor in Japanese with high titers to JCV or BKV, thus arguing against cross-reactivity as the source of SV40 reactivity in human sera. The serologic evidence for SV40 infection in humans remains controversial.

Multiple studies have found evidence of SV40 nucleic acid[6,12,26,32,69] or T antigen[13] in human tumors, most often in a high percentage of mesotheliomas. These data are not without controversy, because other studies[21,36, 42,45,66] have found no evidence of SV40 in human tumors. Interpretation of the various studies is complicated by the use of different techniques and different sources of tumors.[13] An additional concern is possible contamination due to the ubiquitous presence of SV40 nucleic acid in most vectors frequently used in PCR laboratories.[13,76] Most epidemiologic studies show no increased risk for tumors in populations potentially exposed to SV40 in vaccines.[19,27,39,64] The Immunization Safety Review on SV40 Contamination of Polio Vaccine and Cancer[63] concluded that the evidence is inadequate to accept or reject a causal relationship between SV40-containing polio vaccines and cancer and recommended further studies with improved and standardized techniques.

Summary

Nonhuman primates often are infected with polyomaviruses, with disease potential in situations of immunocompromise, whether from post-transplantation therapy, infection with an immunosuppressive retrovirus (simian retrovirus, SIV), or drug toxicity studies. The greatest current concern regarding macaques is the potential confounding of study results by polyomaviruses. Because most macaques from conventional breeding colonies are infected with polyomaviruses, the possibility of associated disease is of concern, both in situations of known immunosuppression and in unpredictable circumstances. Polyomaviruses can be eliminated from breeding colonies by careful selection and culling of animals. By use of these techniques, macaques free of polyomaviruses will become more readily available to investigators in the future.

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