

Brief Report

BK-RELATED POLYOMAVIRUS VASCULOPATHY IN A RENAL-TRANSPLANT RECIPIENT

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POLYOMAVIRUSES typically infect a single species and limited types of tissue. Examples include the human BK and JC viruses and simian virus 40 (SV40). These three viruses are approximately 70 percent homologous with each other. They are nonenveloped viruses with a circular, double-stranded-DNA genome of 5 kb and a virion with a diameter of 45 nm.¹ Their genome has an early region encoding the large T and small t proteins, a late region encoding viral capsid proteins, and a noncoding regulatory region.

Primary infection with BK or JC virus usually occurs in childhood and is asymptomatic. More than 80 percent of adults have serologic evidence of infection.^{1,2} In immunocompetent persons, both viruses persist in the epithelial cells of renal tubules without causing disease,³⁻⁵ but they may be reactivated in severely immunocompromised hosts. Reactivated JC virus may infect brain glial cells and cause progressive multifocal leukoencephalopathy. In renal-transplant recipients, BK virus may cause an acute tubulointerstitial nephritis and ureteral stenosis, leading to hemorrhagic cystitis and severe allograft dysfunction.⁶⁻⁸

Fatal multiorgan-system disease due to BK virus is rare. In one case, a child with the hyper-IgM syndrome died of disseminated BK virus infection.⁶ A BK virus-associated interstitial pneumonia developed in an eight-month-old infant after hematopoietic stem-cell transplantation.⁹ BK virus infection restricted to type

II pneumocytes and renal tubular epithelial cells was reported in a 14-year-old patient with the acquired immunodeficiency syndrome.¹⁰ BK virus has been implicated in cases of atypical retinitis in patients with human immunodeficiency virus (HIV) infection,^{11,12} and in one case, a disseminated infection involved multiple cell types.¹³ We report a case of disseminated infection with a BK-related polyomavirus that demonstrated tropism for vascular endothelial cells. The infection resulted in a systemic vasculopathy that led to extensive capillary leakage, myocardial infarction, and death.

CASE REPORT

A 52-year-old man with type 1 diabetes complicated by end-stage renal disease received a cadaveric renal transplant. Acute renal failure developed two weeks after transplantation. Despite clinical evaluation and biopsy, the cause of the renal failure remained unclear. Cyclosporine, which the patient had been receiving, was discontinued, and muromonab-CD3 (OKT3) was administered daily for five days. The serum creatinine level stabilized. Immunosuppressive therapy consisted of prednisone, mycophenolate mofetil, and tacrolimus.

Seven months later, the patient began to have weakness, myalgia, and fatigue in his arms and legs, which progressed until walking became difficult, with the gradual development of dyspnea on exertion. His serum creatine kinase level was 339 mIU per deciliter.

On examination, the patient was confused. He had normal vital signs. His diaphragms were high, with minimal respiratory excursion, but adventitious lung sounds were absent; the heart sounds were distant. There was symmetric edema of his arms and legs, which was more pronounced in his arms, with a moderate degree of proximal and distal weakness. The muscles were tender on palpation. Deep-tendon reflexes were absent, and cutaneous plantar responses could not be elicited.

The white-cell count was 18,200 per cubic millimeter, with 70 percent polymorphonuclear cells. The hematocrit was 35 percent, the erythrocyte sedimentation rate 41 mm per hour, the creatinine level 2.4 mg per deciliter (212.2 μ mol per liter), the albumin level 2.5 mg per deciliter, and the creatine kinase level 629 mIU per deciliter. Urinalysis showed no proteinuria, hematuria, or other abnormalities. Multiple blood cultures were negative. Serologic tests for cryptococcus, cytomegalovirus, rapid plasma reagin, HIV, Epstein-Barr virus, Lyme disease, toxoplasma, mycoplasma, echovirus, adenovirus, and coxsackievirus were negative.

Over the next few days, the patient's albumin level dropped to 1.7 mg per deciliter, and anasarca developed. The creatine kinase level peaked at 1317 mIU per deciliter. The weakness progressed, and severe pain and tenderness developed in the biceps. Mycophenolate mofetil and tacrolimus were discontinued. A lumbar puncture showed normal cerebrospinal fluid; Venereal Disease Research Laboratory and cryptococcal antigen tests were negative, as were bacterial, fungal, and viral cultures.

The patient had an asystolic arrest and required resuscitation with vasopressor support. He had episodes of rapid atrial fibrillation and atrial flutter, and the anasarca worsened; hypotension developed. By the 14th hospital day, the albumin level had dropped to 0.9 mg per deciliter. Monitoring with the use of a Swan-Ganz catheter showed low values for the pulmonary-capillary wedge pressure and cardiac index, which were difficult to maintain, despite the administration of crystalloids and colloids.

Biopsy of a deltoid muscle revealed apoptosis of endothelial cells and nuclear inclusions in endothelial cells, raising the possibility of viral infection (Fig. 1). Ganciclovir, doxycycline, and ciprofloxacin were administered. Despite the provision of maximal physiological support, the patient died. He had gained approximately 25 kg in the 18 days since admission. The family granted permission for an autopsy, with the results available for teaching and research.

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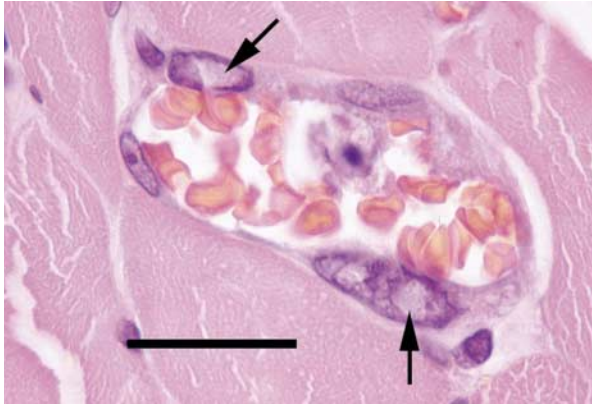


Figure 1. Light Micrograph of a Deltoid-Muscle Specimen Showing Small Blood Vessels with Enlarged, Hyperchromatic Endothelial Cells (Hematoxylin and Eosin).

Many intranuclear inclusions are present (arrows). The nuclear appearance of these cells suggests a transformed state. The bar represents 25 μm .

METHODS

Tissue specimens were embedded in paraffin and stained either with hematoxylin and eosin or immunohistochemically using a standard peroxidase-conjugated streptavidin or avidin technique with a polyomavirus-specific polyclonal antiserum (a gift from Dr. D.L. Walker^{14,15}). A tissue specimen from the deltoid-muscle biopsy was processed for electron microscopy¹⁶ and examined by two independent observers.

For the polymerase-chain-reaction (PCR) assay of BK virus, DNA was extracted from frozen kidney, muscle, and heart tissues. BK virus DNA sequences were identified as described previously.¹⁷ Standard PCR amplification was performed with early-region primers that were specific for the JC and BK viruses: PEP-1, 5'AGT-CTTTAGGGTCTTCTACC3', nucleotides 4392 to 4411 (forward); and PEP-2, 5'GGTGCCAACCTATGGAACAG3', nucleotides 4567 to 4548 (reverse). Dot-blot analysis of PCR products hybridized with oligonucleotide probes that were specific for BK virus (5'GAG-AATCTGCTGTTGCTTCT3') or JC virus (5'TGGGATCCTGT-GTTTTTCATC3') distinguished BK from JC sequences. Plasmids containing viral genomes were used as positive controls, and DNA extraction was controlled by amplifying the β -globin gene.

For sequencing, DNA was extracted from the frozen muscle-biopsy specimen. PCR amplification was performed with the PEP-1 and PEP-2 primers described above, which generate distinct products for the conserved T gene of the BK and JC viruses.¹⁷ Direct sequencing of the amplified product was performed with an automated sequencer.

To amplify the regulatory region of the BK virus, primers BKVR1 (5'GCTCCATGGATTCTCCCTGTTAAG3') and BKVR2 (5'CCTCAGATACTGGCAACTAGGTC3') were used in standard PCR assays. The amplified products were used in a nested PCR reaction with inner primers BK1 (5'GGCCTCAGAAAAGCTTCCACACCCTTACTACTTGA3', nucleotides 5095 to 5130) and BK2 (5'CTTGTCGTGACAGCTGGCGCAGAAC3', nucleotides 307 to 283).

Serum samples from the patient (obtained before transplantation) and the donor were tested for IgG antibodies specific for the BK and JC viruses with the use of the biotin-avidin enzyme immunoassay, as previously described.¹⁸ Titers of IgM antibodies to the two viruses were determined with the use of a similar procedure.

RESULTS

The muscle-biopsy specimen contained enlarged, atypical endothelial cells, with many intranuclear inclusions, lining capillaries and small vessels (Fig. 1). The coarse nuclear chromatin of these cells suggested a transformed phenotype, although the inclusions were indicative of a viral infection. These changes resembled those caused by JC virus infection of glial cells in cases of progressive multifocal leukoencephalopathy, in which oligodendroglia develop nuclear inclusions and the morphologic features of astrocytic nuclei are transformed. Scattered endothelial cells in the muscle-biopsy specimen were characterized by apoptosis, and some vessels had completely necrotic walls and neutrophils. Chronic inflammation was minimal. The morphologic changes in the endothelial cells in the muscle-biopsy specimen were not present in a specimen from a biopsy of the renal allograft performed shortly after transplantation.

Electron micrographs of the muscle-biopsy specimen showed viral particles that were morphologically consistent with papovavirus in vascular endothelial cells (Fig. 2). The muscle fibers were normal. The basal lamina of small vessels was thickened, with focal calcium deposits and cellular debris. Some endothelial cells were normal, whereas others were characterized by hypertrophy, necrosis, apoptosis, and the formation of multinuclear giant cells. Small, round, electron-dense viral particles with a diameter of 40 nm, which were consistent with a human polyomavirus, were present in the nuclei of many endothelial cells (Fig. 2B), and in some instances, the particles formed regular paracrystalline arrays (Fig. 2C and 2D). Smaller numbers of viral particles were also present in cytoplasmic organelles and outside organelles in the interstitial tissues and vessel lumens. Endothelial-cell organelles that contained viral particles included caveolae.

To distinguish between polyomaviruses and papillomaviruses, the other subfamily of papovaviruses, the muscle-biopsy specimen was stained with a polyclonal antiserum that has specificity for polyomaviruses. Commensurate with the light and electron microscopical findings, the nuclei of vascular endothelial cells showed selective and extensive staining by this antiserum (Fig. 3A). The muscle fibers were not stained. Immunoperoxidase staining was negative for human papillomavirus, cytomegalovirus, and herpes simplex viruses 1 and 2.

At autopsy, the thoracic and abdominal cavities had massive effusions. The heart had multiple patchy, white areas indicative of recent infarction. Microscopical examination of the myocardium showed foci of coagulative necrosis associated with necrotic small blood vessels and occasional thrombi. Some vessels had completely necrotic walls; others had enlarged, atypical and apoptotic endothelial cells. Immunoperoxidase staining showed numerous infected endothelial cells in the myocardium (Fig. 3B) and in both the transplanted

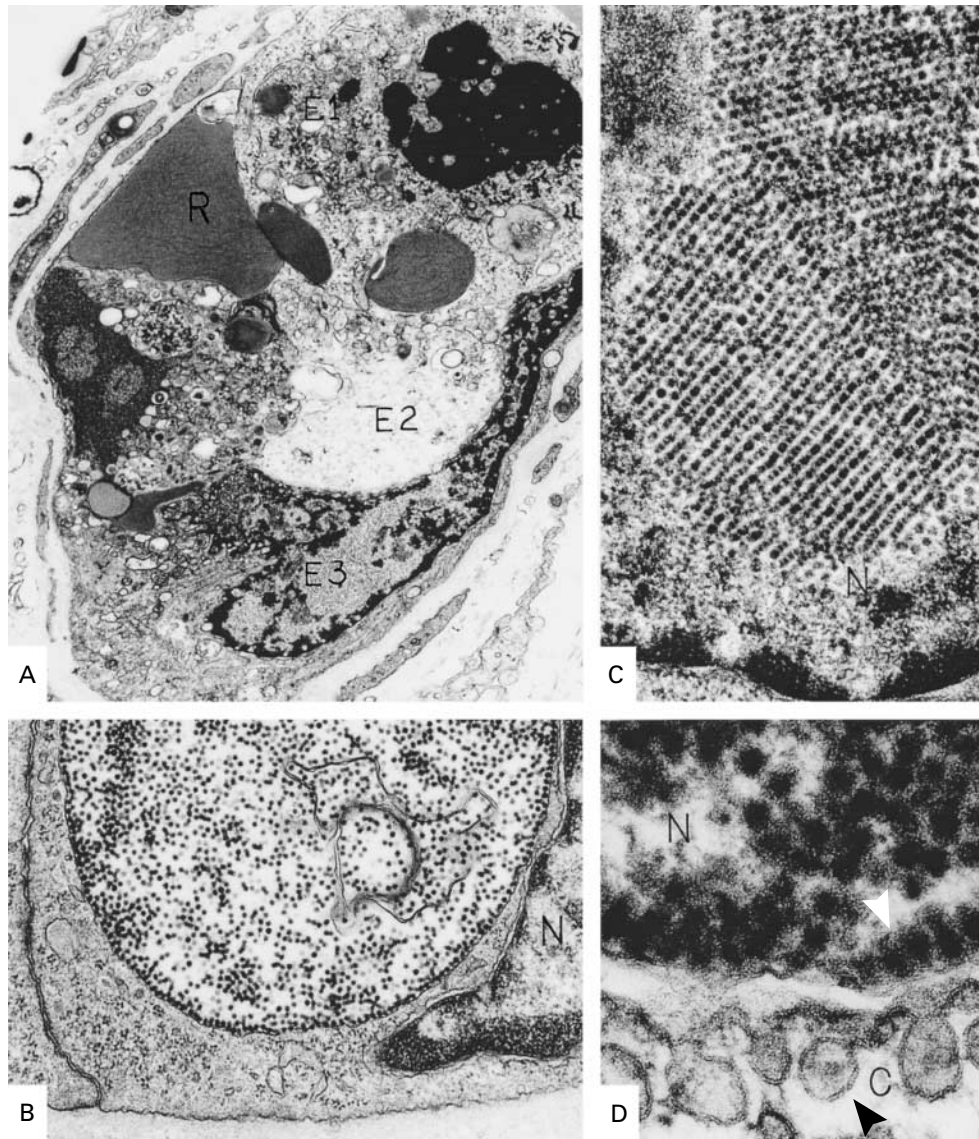


Figure 2. Electron Micrographs Showing Endothelial Cells in Deltoid-Muscle Vessels.

Panel A shows a small venule filled with red cells (R), apoptotic endothelial cells (E1), necrotic endothelial cells (E2), and endothelial cells containing nuclear viral particles (E3) ($\times 6000$). Panel B shows a large, membrane-bound cytoplasmic vacuole in an endothelial cell ($\times 27,000$). The vacuole is filled with viral particles, many of which are lined up along the inner surface. Panel C shows nuclear viral particles in a crystalline array ($\times 45,000$). In Panel D, the viral particles are aligned along the inner surface of the nuclear membrane (white arrowhead), with individual caveolae attached to the outer surface of the perinuclear membrane (black arrowhead) ($\times 145,000$). N denotes nucleus, and C cytoplasm.

and native kidneys. Epithelial cells in the renal tubules showed no immunoreactivity. Vasculopathy was present in the heart, skeletal muscle, and esophagus, whereas in other organs, including the liver, lungs, spleen, pancreas, stomach, and brain, there was no morphologic evidence of viral endothelial infection, although some endothelial cells showed immunoreactivity.

Dot-blot analysis of DNA extracted from heart,

muscle, and renal-transplant tissues and analyzed for both BK and JC virus sequences by PCR showed hybridization of all three tissues with the BK virus-specific oligonucleotide probe but not with the JC virus-specific probe (Fig. 4).

Direct sequencing of a fragment of the conserved T-protein gene of the BK virus genome, generated by PCR amplification with the PEP-1 and PEP-2 primers, yielded a 136-bp fragment with 96 percent homol-

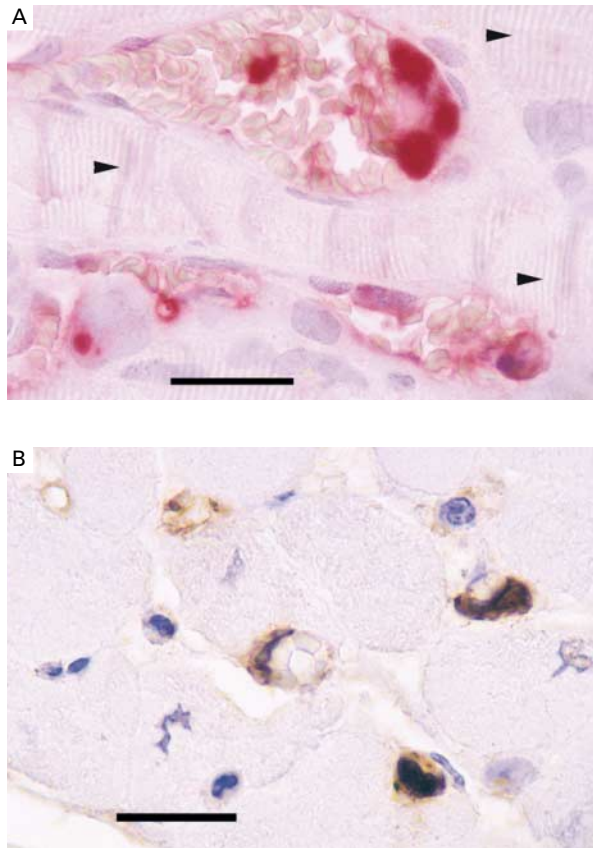


Figure 3. Immunoperoxidase Staining of Deltoid-Muscle and Myocardial Specimens with Polyomavirus-Specific Antiserum. In the deltoid-muscle specimen (Panel A), the staining (deep red) is restricted to the blood vessels and does not involve the striated muscle (arrowheads). The pattern of staining shows that the endothelial cells lining small blood vessels are primarily affected, with some immunoreactivity within the vascular lumen. In the myocardial specimen obtained at autopsy (Panel B), the staining (dark brown) confirms the presence of the virus in the endothelial cells of small vessels. In both panels, the bars represent 25 μm .

ogy with the BK virus Dunlop (BK_{DUN}) strain (nucleotides 4412 to 4547). Of five nucleotide changes, two produced amino acid mutations (with 4 percent variability). Multiple attempts to amplify the BK virus regulatory region were unsuccessful, despite the use of PCR primers specific to the region or to its flanking sequences and the use of BK_{DUN}-plasmid dilutions to control for sensitivity.

Retrospective examination of a specimen from the renal-allograft biopsy performed shortly after transplantation showed immunoreactivity to polyomavirus in capillary endothelial cells and in tubular lumen but not in tubular epithelial cells (Fig. 5). In addition, high titers of IgG antibodies to BK virus were present in

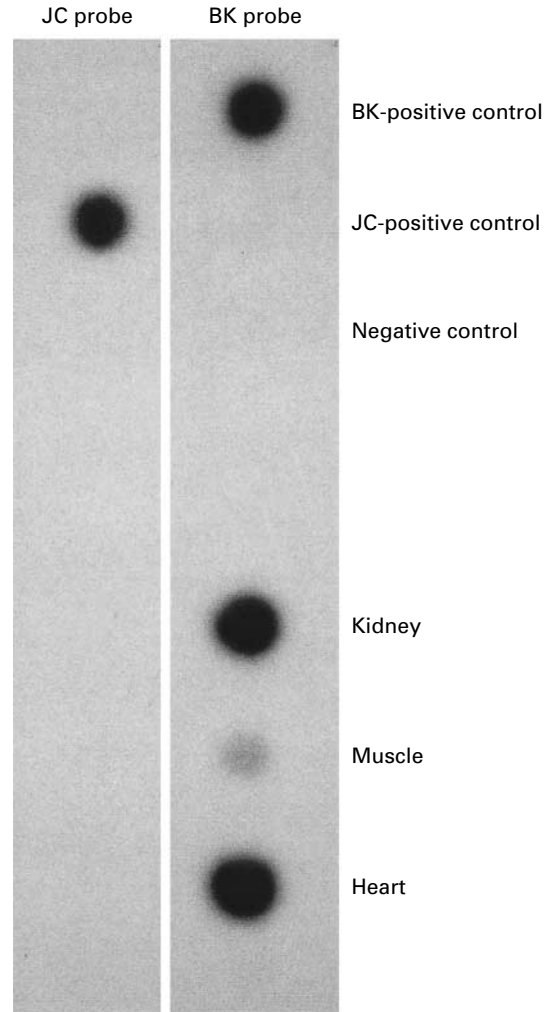


Figure 4. PCR Amplification of BK Virus DNA from the Patient. BK virus DNA was amplified from kidney, muscle, and heart tissues. To establish specificity for the BK virus, as opposed to the closely related JC virus, PCR products were spotted onto duplicate nitrocellulose filters and hybridized with a probe specific for each virus.

the patient's serum both before and after transplantation. The titer of IgM antibodies was low before transplantation but became high during his illness. Titers of IgG and IgM antibodies in the serum specimen from the donor were low (Table 1). All the serum specimens had low titers of IgG and IgM antibodies to JC virus, with the use of the same method of measurement (data not shown). These findings suggest that the patient had a reactivation of a latent viral infection rather than a new infection from transplantation.

DISCUSSION

We describe a patient who had received a renal allograft and seven months later had progressive mus-

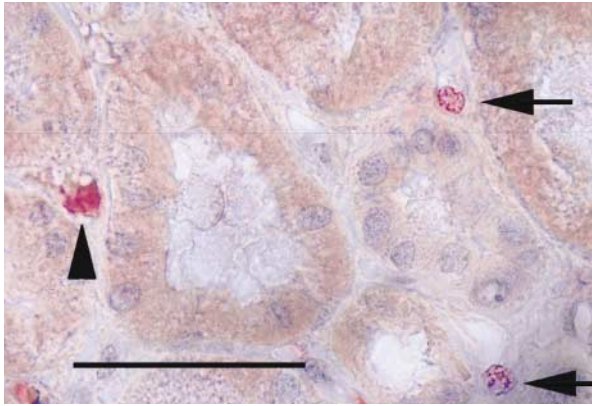


Figure 5. Immunoperoxidase Staining of a Specimen from the Renal-Allograft Biopsy Performed Shortly after Transplantation. Staining (dark red) is present in capillary endothelial cells (arrows) and in vascular lumen (arrowhead) but not in the epithelial cells of the tubules. The hyperchromatic nuclei observed in the muscle-biopsy specimen are absent in this specimen, and the overall immunoperoxidase staining for polyomavirus is less intense. The bar represents 25 μ m.

cle weakness, rapid development of anasarca, and myocardial infarction, which led to his death. A muscle biopsy and autopsy showed a widespread endothelial disorder characterized by a transformed nuclear phenotype, nuclear inclusions containing viral particles, and apoptosis. Further analysis revealed widespread endothelial infection by a BK-related virus. We speculate that the endothelial-cell injury led to capillary leakage and massive edema, as well as microthrombotic events that caused tissue ischemia. The small vessels of the myocardium and skeletal muscle were the chief targets of the viral infection. Other organs, such as the lungs and brain, were minimally affected. Retrospective analysis of a specimen from the renal-allograft biopsy performed shortly after transplantation showed viral proteins in endothelial cells, along with endothelial injury. This case demonstrates a necrotizing endothelial infection by a virus related to the ubiquitous BK virus. Since the BK and JC polyomaviruses do not infect or damage these cells, the infection represents an extension of their usual tissue tropism.

For polyomaviruses to infect cells, the virions must enter the cell, be transported to the nucleus, and replicate. Electron microscopy in this case revealed BK viral particles localized to caveolar organelles; some of the particles were attached to the abluminal surface, and others to the nuclear membrane. These findings suggest that the virions entered the cell and were transported to the nucleus through caveolae. SV40 has a similar mechanism of cellular infection,¹⁹ whereas the JC virus enters glial cells through clathrin-dependent endocytosis.^{19,20}

TABLE 1. RESULTS OF SEROLOGIC TESTS FOR IgM AND IgG ANTIBODIES TO BK VIRUS.*

SERUM SPECIMEN	ANTIBODY TITER	
	IgM	IgG
From the patient		
Before transplantation	1:640	>1:163,840
After transplantation	1:40,960	1:163,840
From the donor	1:160	1:2560

*The test results represent the average of values obtained from two independent measurements of each specimen. As a rough guide to permit a comparison of antibody titers, we classified the results as previously reported¹⁸: negative or low, \leq 1:2560; moderately low, $>$ 1:2560 and $<$ 1:10,240; moderately high, \geq 1:10,240 and $<$ 1:40,960; or high, \geq 1:40,960.

Nuclear replication of polyomavirus requires regulatory and early regions of its genome. The noncoding regulatory regions resemble mammalian promoters and enhancers, suggesting that they act as transcription-factor-binding sites to promote efficient transcription of the viral early region.¹ Nonmutated viruses are most commonly found in urine specimens. Rearranged regulatory sequences and coding-region mutations are associated with altered specificity with respect to target cells, increased pathogenicity, or both.²¹⁻²⁵ In the present case, the fragment of the T gene that was sequenced was 96 percent homologous with the known BK_{DUN} strain, with a change of two amino acids in composition. Although we do not think that these mutations alone are responsible for the altered tissue tropism,²⁵ we have not been able to amplify the regulatory region, suggesting that the virus has additional mutations in this area. Thus, the molecular basis for the tropism of this viral variant is unknown.

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REFERENCES

1. Shah KV. Polyomaviruses. In: Fields BN, Knipe DM, Howley PM, eds. *Fields virology*. 3rd ed. Vol. 2. Philadelphia: Lippincott-Raven, 1996:2027-43.
2. Rziha HJ, Bornkamm GW, zur Hausen H. BK virus. I. Seroepidemiologic studies and serologic response to viral infection. *Med Microbiol Immunol (Berl)* 1978;165:73-81.
3. Koralnik IJ, Schmitz JE, Lifton MA, Forman MA, Letvin NL. Detection of JC virus DNA in peripheral blood cell subpopulations of HIV-1-infected individuals. *J Neurovirol* 1999;5:430-5.
4. Chatterjee M, Weyandt TB, Frisque RJ. Identification of archetype and

- rearranged forms of BK virus in leukocytes from healthy individuals. *J Med Virol* 2000;60:353-62.
5. Dorries K, Vogel E, Gunther S, Czub S. Infection of human polyomaviruses JC and BK in peripheral blood leukocytes from immunocompetent individuals. *Virology* 1994;198:59-70.
 6. Rosen S, Harmon W, Krensky AM, et al. Tubulo-interstitial nephritis associated with polyomavirus (BK type) infection. *N Engl J Med* 1983;308:1192-6.
 7. Mathur VS, Olson JL, Darragh TM, Yen TS. Polyomavirus-induced interstitial nephritis in two renal transplant recipients: case reports and review of the literature. *Am J Kidney Dis* 1997;29:754-8.
 8. Shah KV. Human polyomavirus BKV and renal disease. *Nephrol Dial Transplant* 2000;15:754-5.
 9. Sandler ES, Aquino VM, Goss-Shohet E, Hinrichs S, Krisher K. BK papova virus pneumonia following hematopoietic stem cell transplantation. *Bone Marrow Transplant* 1997;20:163-5.
 10. Cubukcu-Dimopulo O, Greco A, Kumar A, Karluk D, Mittal K, Jagirdar J. BK virus infection in AIDS. *Am J Surg Pathol* 2000;24:145-9.
 11. Bratt G, Hammarin AL, Grandien M, et al. BK virus as the cause of meningoencephalitis, retinitis and nephritis in a patient with AIDS. *AIDS* 1999;13:1071-5.
 12. Hedquist BG, Bratt G, Hammarin AL, et al. Identification of BK virus in a patient with acquired immune deficiency syndrome and bilateral atypical retinitis. *Ophthalmology* 1999;106:129-32.
 13. Vallbracht A, Lohler J, Gossmann J, et al. Disseminated BK type polyomavirus infection in an AIDS patient associated with central nervous system disease. *Am J Pathol* 1993;143:29-39.
 14. Itoyama Y, Webster HD, Sternberger NH, et al. Distribution of papovavirus, myelin-associated glycoprotein, and myelin basic protein in progressive multifocal leukoencephalopathy lesions. *Ann Neurol* 1982;11:396-407.
 15. Walker DL. Progressive multifocal leukoencephalopathy. In: Vinken PJ, Bruyn GW, Klawans HL, Koetsier JC, eds. *Demyelinating diseases*. Vol. 47 of *Handbook of clinical neurology*. Rev. series 3. Amsterdam: Elsevier Science, 1985:503-24.
 16. Dvorak AM. Procedural guide to specimen handling for the ultra-structural pathology service laboratory. *J Electron Microscop Tech* 1987;6:255-301.
 17. Arthur RR, Dagostin S, Shah KV. Detection of BK virus and JC virus in urine and brain tissue by the polymerase chain reaction. *J Clin Microbiol* 1989;27:1174-9.
 18. Hamilton RS, Gravel M, Major EO. Comparison of antibody titers determined by hemagglutination inhibition and enzyme immunoassay for JC virus and BK virus. *J Clin Microbiol* 2000;38:105-9.
 19. Parton RG, Lindsay M. Exploitation of major histocompatibility complex class I molecules and caveolae by simian virus 40. *Immunol Rev* 1999;168:23-31.
 20. Pho MT, Ashok A, Atwood WJ. JC virus enters human glial cells by clathrin-dependent receptor-mediated endocytosis. *J Virol* 2000;74:2288-92.
 21. Agostini HT, Ryschkewitsch CE, Mory R, Singer EJ, Stoner GL. JC virus (JCV) genotypes in brain tissue from patients with progressive multifocal leukoencephalopathy (PML) and in urine from controls without PML: increased frequency of JCV type 2 in PML. *J Infect Dis* 1997;176:1-8.
 22. Dorries K. Molecular biology and pathogenesis of human polyomavirus infections. *Dev Biol Stand* 1998;94:71-9.
 23. Moens U, Johansen T, Johnsen JI, Seternes OM, Traavik T. Noncoding control region of naturally occurring BK virus variants: sequence comparison and functional analysis. *Virus Genes* 1995;10:261-75.
 24. Johnsen JI, Seternes OM, Johansen T, Moens U, Mantjarvi R, Traavik T. Subpopulations of non-coding control region variants within a cell culture-passaged stock of BK virus: sequence comparisons and biological characteristics. *J Gen Virol* 1995;76:1571-81.
 25. Smith RD, Galla JH, Skahan K, et al. Tubulointerstitial nephritis due to a mutant polyomavirus BK virus strain, BKV(Cin), causing end-stage renal disease. *J Clin Microbiol* 1998;36:1660-5.

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