# BK Virus: Discovery, Epidemiology, and Biology

Parmjeet Randhawa, Abhay Vats, Ron Shapiro, Karen Weck and Velma Scantlebury

*Graft* 2002; 5; 19

# BK Virus: Discovery, Epidemiology, and Biology

*Parmjeet Randhawa, Abhay Vats, Ron Shapiro, Karen Weck, and Velma Scantlebury*

This article describes the discovery of BK virus in 1970 and chronicles the growing understanding of its epidemiology from a historical perspective. The biology of the virus is summarized with particular reference to mechanisms of host cell entry, maintenance of viral latency, and mechanisms of subsequent reactivation leading to clinical disease. The rudimentary nature of the current understanding of BKV is highlighted, and areas worthy of future investigation are identified.

ABBREVIATIONS:

| | |
|---|---|
| AIDS | Acquired immunodeficiency syndrome |
| BKV | BK virus |
| BKVAN | BKV nephropathy |
| CMV | Cytomegalovirus |
| ELISA | Enzyme-linked immunosorbent assay |
| JCV | JC virus |
| MHC | Major histocompatibility |
| NCCR | Noncoding control region |
| PCR | Polymerase chain reaction |
| SV40 | Simian virus 40 |

Parmjeet Randhawa, C903
1 PUH.UPMC-Presbyterian
200 Lothrop St
Pittsburgh, PA 15213
email: randhawapa@msx.UPMC.edu
DOI: 10.1177/1522162802238454

## Discovery of BKV

BK virus (BKV) was discovered in 1970 at the Virus Research Laboratory, London, England. This laboratory was a part of the Central Public Health Laboratories set up to combat infectious diseases that were rampant in the post–World War II period. The discovery occurred during a study initiated by a virologist, Dr. Sylvia Gardner, who was interested in understanding the epidemiology of cytomegalovirus infection in kidney transplant recipients. Urine collected from a Sudanese patient 3.5 months after transplantation was found to contain numerous cells bearing viral inclusions. Electron microscopy performed by Dr. Anne Field demonstrated viral particles that resembled the common wart virus (papillomavirus).[1-3] It was questioned whether the patient had genital warts because a report earlier that year had documented a high incidence of such warts after kidney transplantation. However, the most important element in this patient's clinical history was a ureteric stricture, which required surgical correction. Inoculation of the urine into rhesus monkey kidney cells and human embryonic kidney cells produced a viral cytopathic effect, confirming that the virus was different from papillomavirus. Hence, this microbe was identified as a new virus and named BKV after the initials of the patient from whom it was isolated.[4] The original viral isolate is known as the Gardner strain in honor of Dr. Sylvia Gardner. BKV differs from the simian polyomavirus SV40, which was discovered in 1960 as a contaminant of polio vaccines by its ability to cause hemagglutination of human red cells. Subsequently, BKV was shown to be serologically and genetically distinct from polyomavirus JC (JCV), a virus cultured from a patient with progressive multifocal encephalopathy. The clinical syndrome of progressive multifocal leukoencephalopathy was first described in 1958, and electron microscopic evidence suggesting that it is a viral disease was provided by two independent groups of investigators in 1964.[1,2] However, prominent virologists, including Dr. Albert Sabin, remained unconvinced until isolation of JCV and BKV was reported in back-to-back articles in the same year in *Lancet*.[1,4,5]

Following the discovery of BKV in 1970, extensive epidemiological studies were conducted at the Virus Research Laboratories in the 1970s and 1980s. Although the discovery of progressive multifocal leukoencephalopathy preceded discovery of its causative virus, JCV, BKV was discovered before the clinical consequences of infection could be appreciated. Serologic studies showed that up to 90% of some human populations became exposed to BKV by adulthood. Following transplantation, 10% to 60% of renal allograft recipients were found to excrete virus in the urine. However, viral infection was usually asymptomatic or associated with only transient graft dysfunction. Rare reports of viral inclusions were, however, recorded at nephrectomy or at autopsy. Sporadic cases of virus-induced kidney damage were also observed in the

**Key Points**

- BKV was discovered in 1971 from a renal transplant patient with ureteric stenosis.
- Serologic studies indicate that most adults have become exposed to the virus by adulthood.
- The virus remains latent primarily in the urogenital tract and lymphoid tissue.
- Following reactivation under conditions of immunosuppression, the virus can be detected in respiratory secretions, urine, blood, and other body fluids.
- BKV nephropathy develops only in a small percentage of immunosuppressed patients who develop primary or reactivation infection. The viral and host immune interactions that lead to this complication are not yet completely understood.

setting of congenital immunodeficiency[6] and human immunodeficiency virus infection.[7]

A new era in the study of BKV in renal transplant recipients began in 1993 at the University of Pittsburgh, with the first ever case of BKV interstitial nephritis diagnosed by a needle biopsy of the renal allograft.[8] This patient was a 60-year-old African American female who presented 14 months after kidney transplantation with a rise in the serum creatinine, which prompted a biopsy to exclude acute rejection. Histologic examination showed renal tubules with intranuclear inclusion bodies highly suspicious of viral infection, but immunostains for cytomegalovirus, herpes simplex, varicella, and adenovirus were negative. Electron microscopy performed on formalin-fixed paraffin-embedded tissue demonstrated viral particles having a virion diameter consistent with polyomavirus. Further confirmation was obtained by immunohistochemistry using a commercially available antiserum to polyomavirus SV40. Subsequently, BKV infection was documented by polymerase chain reaction (PCR) using primers directed to the VP1 region, and the true identity of the PCR product was confirmed by DNA sequencing.[9] Recently, using quantitative PCR, the tissue viral load was determined to be extraordinarily high, with up to 21,400 copies of viral DNA per cell present in the renal allograft biopsies derived from this patient.[10]

Following the first case at Pittsburgh, cases of BKV-associated interstitial nephritis were reported from most major kidney transplant centers around the world.[11-13] This emergence of BKV nephropathy in the 1990s seems to coincide with the widespread use of potent immunosuppressive drugs such as tacrolimus, mycophenolate mofetil, and sirolimus. It is unlikely that the apparent increase in the incidence of BKV nephropathy (BKVAN) is simply the result of increased awareness. Thus, pathologists at the University of Basel, Switzerland, retrospectively reviewed all renal allograft biopsies performed between 1985 and 1995, but could not document any cases occurring during that time period.[14]

## Taxonomy and Genomic Organization

Polyomaviruses were initially grouped with the papillomaviruses under the family *Papovaviridae*. The International Committee for Taxonomy of Viruses has now dropped the term *papovavirus* and recommends that *polyomavirus* be considered a distinctive genus. The polyomavirus species most relevant to human disease are BKV, JCV, and simian virus 40 (SV40). Veterinary species of interest include murine polyoma virus, hamster papovavirus, lymphotropic papovavirus, bovine polyomavirus, and rabbit kidney vacuolating virus.[15]

A detailed discussion of the molecular biology of the polyomaviruses is presented elsewhere in this issue. Briefly, this genus comprises nonenveloped icosahedral DNA viruses that measure about 45 nm.[16,17] The viral genome averages 5 kilobases in length. It is composed of double-stranded, circular, supercoiled DNA, arranged in three general regions: the noncoding control region (NCCR), the early coding region, and the late coding region. A brief discussion of the functional significance of these genomic regions follows.

The NCCR contains (a) the origin of replication (ori) and (b) regulatory regions for early and late transcription.[18] The tandem repeats found in the regulatory regions usually represent enhancer elements that are important activators of viral transcription. Naturally occurring SV40, BKV, and JCV strains in the kidney and urine usually have an

archetypal regulatory region,[19-23] although this is not an invariable rule.[24,25] By contrast, JCV found in the brain tissue of patients with progressive multifocal leukoencephalopathy (PML) usually shows a variety of point mutations, deletions, and duplications on the late side of ori.[23,26,27] There is in vitro evidence that NCCR variants determine host cell permissivity and the rate of viral replication.[28,29] Hence, genetic arrangements are believed to be critical in permitting viral transcription in the brain, which ultimately culminates in the pathologic lesions of progressive multifocal leukoencephalopathy. It is currently not known if similar genetic alterations are required for the pathogenesis of polyomavirus interstitial nephritis in the allograft kidney. A mutant BKV (Cin) strain with deletions and duplications in the regulatory region has, however, been reported from a HIV–infected individual with interstitial nephritis.[7]

The early coding region of BKV encodes t and T antigens, which can bind to tumor suppressor proteins Rb and p53 and stimulate host cell entry into the cell cycle.[30,31] This is an important event because polyomavirus is dependent on host cellular factors for replication, and the required cellular factors may not be present in quiescent host cells. A secondary consequence of the ability of polyomavirus to stimulate the cell cycle is its tendency to cause transformation of host cells. Carcinogenicity of the polyomavirus family is well established in experimental animals, and there is increasing evidence for participation of these viruses in human tumors, discussed elsewhere in this issue.

The late coding region of the BKV genome codes viral capsid proteins VP1, VP2, VP3, and agnoprotein. VP1, VP2, and VP3 are structural proteins required for the assembly of complete virions. Because viral replication and assembly occur in the host cell nucleus, these proteins localize primarily to the intranuclear compartment of host cells. Agnoprotein differs from all other early and late proteins in that it localizes primarily to the cytoplasmic and perinuclear regions of infected cells. Unlike viral capsid proteins, it is not detectable in the virion itself. This pattern of intracellular distribution has led to the suggestion that agnoprotein may promote virion release from cell.[32] Other proposed roles for agnoprotein include participation in host cell lysis, enhanced nuclear localization of viral capsid protein VP1, and help in viral capsid assembly.[33] Cultured cells infected with agnogene mutants show a 17- to 100-fold reduction in virion burst size.[32,34] It is not known if agnogene mutations participate in the pathogenesis of BKV nephropathy.

## Epidemiology

Serologic studies have been the primary tool used to assess the prevalence of BKV in human populations. The most widely used test has been the hemagglutination inhibition assay,[34,35] which detects IgG and IgM antibodies to viral capsid antigens. This assay requires availability of large amounts of viral antigen in the laboratory. Nonspecific inhibitors of hemagglutination present in human samples have to be removed by dilution of serum or treatment with KIO4 or neuraminidase.[36,37] A number of enzyme-linked immunosorbent assays (ELISA) have also been described.[38-41] These can measure specific immunoglobulin subclasses produced in response to defined viral antigens.

As mentioned earlier, BKV infection occurs primarily in early childhood. Maternal antibody is present in neonatal blood at birth but is lost during the first few months of life. Adult levels of seroprevalence, on the order of 65% to 90% in most studies, are reached between 5 and 10 years of age. Some studies show a tendency to falling antibody positivity rates after the age of 40 to 50 years.[42] If BKV-specific IgM antibodies are measured by ELISA, fewer subjects test positive: 0% of infants, 11% of healthy children,[43] 21.1% of unwell children,[44] and 0% to 7% of healthy adults or blood donors.[43,45] IgM antibody production can represent recent primary or reactivation infection. It also appears that some healthy adults can show persistent IgM production for several years following primary infection.[46] The incidence of BKV antibodies in human populations is independent of sex and shows no consistent relationship with socioeconomic status, family size, and rural versus urban location.[42] Geographically isolated populations can, however, show extremely low rates of BKV infection. Thus, the seroprevalence of BKV in three indigenous Brazilian tribes was found to vary from 2.5% to 6.1%.

The high incidence of BKV infection in childhood raises obvious questions about the mode of

transmission from one individual to another. Given the known latency of the virus in the kidney, urine would appear to be a natural vehicle for spread within and between families. A variety of laboratory techniques have accordingly been used to assess the prevalence of viruria in the pediatric age group. Urine cytology investigations show viral inclusions in 0% to 1.2% of children but cannot distinguish between BKV and JCV, unless specific immunofluorescence staining is performed.[42] Viral cultures give a similarly low yield, varying from 0% to 1%, in part because samples can test false negative if viral particles are coated with antibodies present in urine, and because cell substrates used for viral culture are not always well suited to isolate BKV with an archetypal NCCR. Higher rates of BKV viruria can be detected using PCR, but the results vary in different studies ranging from 4% to 26.7%, with all but one study in children reporting values < 5%.[42] In adults, BKV DNA has been amplified from 0% to 40% of urine samples, with a tendency to higher values in older subjects. The viral DNA concentrations reported have varied from < 3 fg/ml to 5 pg/ml.

Other body fluids are potentially involved in transmission of BKV infections. BKV DNA has been amplified in 1% of nasopharyngeal aspirates obtained from hospitalized infants with serious respiratory infections.[47] The occurrence of respiratory symptoms during primary BKV infection is quite consistent with this portal of entry. The possibility of feco-oral transmission has been recently raised by the demonstration of BKV DNA in urban sewage.[48] Detection of virus in healthy blood donors suggests contaminated blood as yet another potential source of infection.[49] Semen, genital tissues, and normal skin biopsies have also been shown to contain BKV in one study.[50] Finally, given the frequent detection of viral genomes in healthy kidneys, the donor organ itself might be an important vehicle of viral transmission after renal transplantation. Primary BKV infections attributed to the use of infected donor kidneys are documented in the literature.[51-54]

Transplacental transmission of BKV from mother to fetus is a controversial issue. BKV-specific IgM antibodies were found in cord blood samples from 3 of 6 infants whose mothers seroconverted during pregnancy.[55,56] On the other hand, Shah et al.[57] could not detect anti-BKV IgM antibodies in the cord blood of 387 infants, although only 3 of the mothers studied during this investigation had anti-BKV IgM antibodies in the serum. Coleman et al.[58] studied 309 mothers, 39 of whom excreted viral inclusion-bearing cells in the urine during pregnancy. Neonatal and cord blood samples drawn from the offspring tested negative for BKV-specific IgM. Transplacental transmission of polyomavirus has been demonstrated in mice,[59] and it is likely to occur in humans from time to time. Gross fetal pathology directly linked to primary BKV infection in pregnancy has not yet been documented.

## Mechanism of Viral Entry in Host Cells

The entry of BKV into the host cell is believed to be mediated by receptors comprising α(2-3)–linked sialic acids.[60] The detailed biochemical characteristics of these receptors have not been defined. More work has been done for JCV, in which an N-linked glycoprotein containing α(2-6)–linked sialic acids has been described on the surface of glial cells and B cells. JCV enters the cell by clathrin-dependent endocytosis, which can be blocked by chlorpromazine.[61] In contrast, SV40-specific receptors comprise MHC class 1 proteins and O-linked glycans, and viral entry is a caveolae-dependent endocytosis susceptible to nystatin.[62] Sialic acid receptors of BKV and JCV mediate hemagglutination of red cells, and this property has been used in seroepidemiologic surveys described earlier.

The primary receptor-binding determinant on all polyomaviruses is the VP1 molecule, which is arranged in the form of icosahedrally symmetric pentamers.[63] Indeed, VP1 molecules can self-assemble into virus-like particles, which are currently being developed as gene delivery vehicles. Receptor-mediated viral entry into the cell is not a sufficient condition for viral replication. Additional interactions between host-specific permissive factors and viral genetic regulatory elements are also needed. Viral entry is believed to be targeted initially to the endoplasmic reticulum. By a process that is not well understood, the virus is then released into the cytoplasm, goes through the nuclear pores, and enters the nuclear compartment, where it starts replicating. In permissive and semipermissive cells, production

of progeny virions can result in a lytic cycle, leading to cell death. Infection of nonpermissive cells can result in abortive infection, with subsequent loss of the viral genome from the target cell.

## Primary BKV Infection in Humans

Relatively few clinical observations have been made in individuals undergoing BKV seroconversion, but it is believed that that most primary infections are subclinical. When reported, the most frequent clinical finding is an upper respiratory infection, but some of the reported cases had documented infections with known respiratory viruses, whereas other individuals were not specifically tested along these lines.[64] The occurrence of tonsillitis suggests that mucosa-associated lymphoid tissue in the oropharyngeal region (tonsils and Waldeyer's ring) is another potential site of primary infection.[47,65] Several children presenting with cystitis, with or without hematuria, are also on record,[66-68] but in one of these patients, a rising titer to adenovirus was also demonstrated. Unusual clinical manifestations associated with BKV seroconversion include Guillane-Barre syndrome and encephalitis.[69,70]

## Latent Phase of Viral Infection

After primary infection has resolved, serology and PCR continue to show evidence of BKV infection in apparently healthy subjects. The virus in these individuals is believed to be in a clinically latent state, but this does not exclude low-level expression of a restricted set of viral genes at the molecular level. There is evidence that this presumed viral latency can be maintained in a number of different organ systems in humans.

The best-known site of BKV latency in humans is the urogenital tract, and viral sequences can be detected in up to 50% of human kidneys.[71] A comparable rate of infection is found in the urinary bladder and prostate tissues procured from asymptomatic individuals. Viral DNA has also been demonstrated in 70% of cervical or vulvar tissues and 95% of sperm samples.[50,54,72,73]

Peripheral blood mononuclear cells are a second important potential site of BKV latency. In healthy individuals, rates of detection vary from 0% to 94%.[54] This wide variability in incidence in different studies is, in part, due to the extremely low viral load present in healthy subjects. Successful detection requires sensitive assays, typically a nested PCR, with great care taken to avoid contamination. Other potential variables include the presence of plasma inhibitors, the transient nature of infection, the short life span of infected mononuclear cells, and differences in susceptibility of different mononuclear fractions. Limited low-level BKV infection has been shown in both T cells and B cells maintained in culture. In one study, monocytes showed viral attachment and penetration but no viral replication, unless the cells were treated with antimacrophage antiserum.[74] BKV mRNA has been detected in circulating mononuclear cells of healthy donors using RT-PCR and in situ hybridization.[24,75]

Mucosa-associated lymphoid tissue is a potential site of latency because genome-length BKV DNA has been demonstrated in 5 of 12 tonsils obtained from children with recurrent respiratory infections.[65] Likewise, detection of virus in throat washings has been interpreted as evidence for the virus being present in the Waldeyer's ring.[47]

Other sites of viral latency have also been proposed. Thus, BKV DNA has been amplified from the brain tissue of individuals without neurologic disease,[76] normal bone, and bone tumors.[77] BKV has also been cloned from liver tissue.[78]

## Mechanisms of Viral Reactivation

Activation of virus lying latent in human tissues has been reported in a number of circumstances:

1. Sporadic activation seems to occur in some subjects for no recognizable reason. One can assume that this is due to subtle changes in the hormonal milieu or functional state of the immune system (vide infra).
2. Hormonal as well as immunologic changes may be related to viral activation occurring in old age, pregnancy, and diabetes mellitus. The incidence of BKV antibodies in pregnant women has varied from 3% to 7.5%.[43,55,57] Urinary decoy cells typically appear in the second and third trimesters and sometimes persist in the postpartum period.
3. Excessive immunosuppression is clearly important. Compared to healthy subjects, there is an increased incidence of viruria in HIV-

infected individuals, ranging from 20% to 44% in most studies. The level of BKV, but not JCV viruria, seems to show an inverse correlation with the circulating T cell count.[54] Viruria is detected in 22% to 100% of patients following bone marrow transplantation, 10% to 60% of kidney transplant recipients, and 50% of heart transplant recipients.[54] The duration of viruria is variable, and cases with intermittent as well as persistent viral excretion are described. A small proportion of viruric patients goes on to develop BKV nephropathy in the setting of AIDS, congenital immunodeficiency, and solid organ as well as bone marrow transplantation. In some kidney transplant recipients, the occurrence of BKVAN is preceded by biopsies showing histologic evidence of tacrolimus toxicity, again pointing to the role of excessive immunosuppression.

4. There is experimental evidence that tissue injury can in itself precipitate polyomavirus replication.[79] The clinical counterpart of this observation may be the reactivation of BKV infection in patients who suffer from recurrent acute rejection, although augmented immunosuppression could also be an important contributory factor in these cases.
5. JCV infections occurring concurrently with BKV infection are well documented. We have been able to amplify JCV DNA from allograft renal biopsies only in the setting of BKVAN, raising the possibility that JCV and BKV replication are interlinked.[80] This issue could be further addressed by measuring comparative tissue BKV loads in patients with and without JCV coinfection. Another approach would be to study potential molecular-level interactions between JCV and BKV using cell lines, such as fetal glial cells, which are susceptible to both viruses. The clinical implications, if any, of these biologic interactions also need to be defined by future studies.
6. Cytomegalovirus (CMV) infections are widely prevalent in kidney transplant recipients, and patients showing simultaneous evidence of BKV infection are recorded.[13] Tubular epithelial cells are a common target for both viruses, and we have recently seen a case with both CMV and polyomavirus viral inclusions in the same biopsy sample. BKV T antigen can induce CMV immediate early and early gene expression in culture.[54,81] However, culture systems infected with both viruses do not show activation of BKV infection.[82] In patients with acquired immunodeficiency syndrome (AIDS), there is no correlation between CMV viruria and polyo- mavirus viruria.[83] Acyclovir treatment has been shown to reduce CMV activation in bone marrow transplant patients but does not affect the incidence of BKV-associated cystitis. In vivo interactions between CMV and BKV at the clinical level have not yet been investigated in solid organ transplant recipients.
7. It has been proposed that polyomavirus JCV reactivation can be triggered by infection with herpes virus 6 (HHV6). HHV6 and JCV have both been colocalized to oligodendroglial cells in brain tissue with progressive multifocal encephalopathy.[84] The relevance of this observation to BKV infection is unclear, but it should be noted that both BKV and HHV6 can infect renal parenchyma.
8. Human immunodeficiency virus encoded HIV-1 Tat protein can transactivate polyomavirus JCV by induction of the JCV promoter.[85,86] A similar effect on the BKV promoter could potentially explain the observation of an increased incidence of BKV DNA in lesions with AIDS-associated Kaposi's sarcoma.[50] It is of interest that BKV/Tat-transgenic mice develop Kaposi's sarcoma-like lesions.[87]

## Immune Response to BKV

Immunologic studies pertaining specifically to human BKV infection are limited. However, some generalizations can be made based on work done in experimental models of polyomavirus-induced carcinogenesis and investigations that have sought to define the immune response to SV40 infection in rhesus monkeys.[88]

Acute infection with BKV is self-limited. Presumably, resolution of the acute infection eliminates in-

fected cells expressing antigens toward which the immune response is primarily directed. On the other hand, cells that retain latent or relatively inactive virus must be somehow capable of escaping the host immune response. The mechanism by which viral latency is maintained is not understood. The immune system is presumably involved because the severity of BKV viruria correlates well with the degree of immunosuppression.[83,89,90] It is generally held that constant surveillance by virus-specific T cells is the key factor preventing viral reactivation. CD4+ T cell responses to BKV have been demonstrated in healthy volunteers using lymphocyte proliferation assays.[91] Antibody-mediated immune mechanisms are not considered important in mediating latency because viral-neutralizing antibodies are typically present at the time viral reactivation is demonstrated clinically.

No work has been done on immune responses to specific viral antigens in the context of BKVAN. Major histocompatibility (MHC) class II antigens are demonstrable in tissues with BKVAN when significant interstitial inflammation and tubulitis are present.[92] MHC class I antigens, although not specifically investigated in the preceding study, are widely expressed in allograft kidneys.[93,94] Hence, classical MHC-restricted mechanisms of T cell–mediated immunity are presumably operating in patients with BKV nephropathy. This notion is supported by a recent report describing polyomavirus JC-specific T cytotoxic cells in the circulation of long-term survivors of progressive multifocal leukoencephalopathy.[95]

BKV is unique among primate polyomaviruses with regard to the marked antigenic variability that it shows in the viral capsid protein VP1 region. This variability has been used to classify BKV into well-defined antigenic subtypes.[96] Sequencing of the VP1 region in biopsies of patients with BKV nephropathy has suggested that the viral genome is unstable, and changes in nucleotide sequence occur as patients are followed over time.[97] Thus, in 1 of our patients with serial biopsies available for evaluation, type I virus was detected initially but was replaced by type IV virus in subsequent specimens. It is conceivable that a constantly changing genome might represent a strategy adopted by the virus to evade host immunity.

## Conclusion

Our knowledge of the epidemiology, biology, and immunology of BKV has come a long way since the initial discovery of the virus in 1970. However, most of the work in the literature has focused on healthy individuals, patients with human immunodeficiency virus infection, and recipients of kidney or bone marrow transplants. It will be of great clinical interest to determine the prevalence of BKV infection in liver, pancreas, lung, and heart transplant recipients. When these individuals develop renal dysfunction, it is customary to blame it on calcineurin inhibitor toxicity, without any further investigation for polyomavirus viruria. Another area in critical need of additional investigation is delineation of the human immune response to specific viral antigens. As our knowledge of anti-BKV immunity becomes more refined, it might become possible to treat BKV nephropathy with immunotherapy using appropriately targeted virus-specific T cells. The feasibility of controlling polyomavirus infection by immune manipulation has been shown by the successful use of a virus-inactivated vaccine in birds.[98]

### References


1. Astrom KE. Progressive multifocal leukoencephalopathy: the discovery of a neurologic disease. In: Khalili K, Stoner GL, editors. Human polyomaviruses: molecular and clinical perspectives. **New York: Wiley-Liss; 2001. p. 1-10**.
2. ZuRhein GM. Papova virions in progressive multifocal leukoencephalopathy: a discovery at the interface of neuropathology, virology, and oncology. In: Khalili K, Stoner GL, editors. Human polyomaviruses: molecular and clinical perspectives. **New York: Wiley-Liss; 2001. p. 11-24**.
3. Walker DL. Progressive multifocal leukoencephalopathy: cultivation and characterization of the etiologic agent. In: Khalili K, Stoner GL, editors. Human polyomaviruses: molecular and clinical perspectives. **New York: Wiley-Liss; 2001. p. 25-44**.
4. Gardner SD, Field AM, Coleman DV, Hulme B. New human papovavirus (B.K.) isolated from urine after renal transplantation. **Lancet 1971;1: 1253-7**.
5. Padgett BL, Walker DL, ZuRhein GM, Eckroade RJ, Dessel BH. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. **Lancet 1971;1:1257-60**.
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. 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**.
8. Pappo O, Demetris AJ, Raikow RB, Randhawa PS. Human polyoma virus infection of renal allografts: histopathologic diagnosis, clinical significance, and literature review. **Mod Pathol 1996;9:105-9**.
9. Baksh F, Finkelstein SD, Swalsky PA, Stoner G, Ryschkewitsch CF, Randhawa PS. Molecular genotyping of BK and JC viruses in human poly-

omavirus associated interstitial nephritis after renal transplantation. **Am J Kid Dis 2001;38:354-65**.

10. Randhawa PS, Vats A, Zygmunt D, et al. Quantitation of viral DNA in renal allograft tissue from patients with BK virus nephropathy. **Transplantation 2002;74:485-8**.
11. Drachenberg CB, Beskow CO, Cangro CB, et al. Human polyoma virus in renal allograft biopsies: morphological findings and correlation with urine cytology. **Human Pathology 1999;30:970-7**.
12. Nickeleit V, Hirsch HH, Binet IF, et al. Polyomavirus infection of renal allograft recipients: from latent infection to manifest disease. **J Am Soc Nephrol 1999;10:1080-9**.
13. Howell DN, Smith SR, Butterly DW, et al. Diagnosis and management of BK polyomavirus interstitial nephritis in renal transplant recipients. **Transplantation 1999;68:1279-88**.
14. Binet I, Nickeleit V, Hirsch HH, et al. Polyomavirus disease under new immunosuppressive drugs: a cause of renal graft dysfunction and graft loss. **Transplantation 1999;67:918-22**.
15. Lednicky JA, Butel JS. Tissue culture adaptation of natural isolates of simian virus 40: changes occur in viral regulatory region but not in carboxy-terminal domain of large T-antigen. **J Gen Virol 1997;78:1697-705**.
16. Shah KV. Polyomaviruses. In: Fields BN, Knipe KM, Howley PM, eds. Virology. **Philadelphia: Lippincott-Raven; 1995. p. 2027-43**.
17. Demeter LM. JC, BK, and other polyomaviruses: progressive multifocal leukoencephalopathy. In: Mandel GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases. **New York: Churchill Livingstone; 1995. p. 1400-6**.
18. 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**.
19. Rubinstein R, Harley EH. BK virus DNA cloned directly from human urine confirms an archetypal structure for the transcriptional control region. **Virus Genes 1989;2:157-65**.
20. Flaegstad T, Sundsfjord A, Arthur RR, Pedersen M, Traavik T, Subramani S. Amplification and sequencing of the control regions of BK and JC virus from human urine by polymerase chain reaction. **Virol 1991;180:553-60**.
21. Tominaga T, Yogo Y, Kitamura T, Aso Y. Persistence of archetypal JC virus DNA in normal renal tissue derived from tumor-bearing patients. **Virol 1992;186:736-41**.
22. Negrini M, Sabbioni S, Arthur RR, Castagnoli A, Barbanti-Brodano G. Prevalence of the archetypal regulatory region and sequence polymorphisms in nonpassaged BK virus variants. **J Virol 1991;65:5092-5**.
23. Newman JT, Frisque RJ. Detection of archetype and rearranged variants of JC virus in multiple tissues from a pediatric PML patient. **J Med Virol 1997;52:243-52**.
24. Dorries K, Vogel E, Gunther S, Czub S. Infection of human polyomaviruses JC and BK in peripheral blood leukocytes from immunocompetent individuals. **Virol 1994;198:59-70**.
25. Sundsfjord A, Johansen T, Flaegstad T, et al. At least two types of control regions can be found among naturally occurring BK virus strains. **J Virol 1990;64:3864-71**.
26. Loeber G, Dorries K. DNA rearrangements in organ-specific variants of polyomavirus JC strain GS. **J Virol 1988;62:1730-5**.
27. Ault GS, Stoner GL. Brain and kidney of progressive multifocal leukoencephalopathy patients contain identical rearrangements of the JC virus promoter/enhancer. **J Med Virol 1994;44:298-304**.
28. Johnsen JI, Seternes OM, Johansen T, Moens U, Mantyjarvi 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**.
29. Daniel AM, Swenson JJ, Mayreddy RP, Khalili K, Frisque RJ. Sequences within the early and late promoters of archetype JC virus restrict viral DNA replication and infectivity. **Virol 1996;216:90-101**.
30. Carbone M, Rizzo P, Grimley PM, et al. Simian virus-40 large-T antigen binds p53 in human mesotheliomas. **Nat Med 1997;3:908-12**.
31. De Luca A, Baldi A, Esposito V, et al. The retinoblastoma gene family pRb/p105, p107, pRb2/p130 and simian virus-40 large T-antigen in human mesotheliomas. **Nat Med 1997;3:913-6**.
32. Resnick J, Shenk T. Simian virus 40 agnoprotein facilitates normal nuclear location of the major capsid polypeptide and cell-to-cell spread of virus. **J Virol 1986;60:1098-106**.
33. Carswell S, Alwine JC. Simian virus 40 agnoprotein facilitates perinuclear-nuclear localization of VP1, the major capsid protein. **J Virol 1986;60:1055-61**.
34. Ng SC, Mertz JE, Sanden-Will S, Bina M. Simian virus 40 maturation in cells harboring mutants deleted in the agnogene. **J Biol Chem 1985;260:1127-32**.
35. Gardner SD, MacKenzie EF, Smith C, Porter AA. Prospective study of the human polyomaviruses BK and JC and cytomegalovirus in renal transplant recipients. **J Clin Pathol 1984;37:578-86**.
36. De Stasio A, Orsi N, Pana A, Seganti L, Sinibaldi S, Valenti P. The importance of pre-treatment of human sera for the titration of hemagglutination-inhibiting antibodies towards BK virus. **Ann Sclavo 1979;21:249-57**.
37. De Stasio A, Mastromarino P, Pana A, et al. Characterization of a glycoprotein inhibitor present in human serum and active towards BK virus hemagglutination. **Microbiologica 1980;3:293-305**.
38. Burguiere AM, Fortier B, Bricout F, Huraux JM. Control of BK virus antibodies in contacts of patients under chronic hemodialysis or after renal transplantation (by an enzyme linked immunosorbent assay). **Pathol Biol (Paris) 1980;28:541-4**.
39. Iltis JP, Cleghorn CS, Madden DL, Sever JL. Detection of antibody to BK virus by enzyme-linked immunosorbent assay compared to hemagglutination inhibition and immunofluorescent antibody staining. **Prog Clin Biol Res 1983;105:157-68**.
40. Flaegstad T, Traavik T. Detection of BK virus antibodies measured by enzyme-linked immunosorbent assay (ELISA) and two haemagglutination inhibition methods: a comparative study. **J Med Virol 1985;16:351-6**.
41. Hamilton RS, Gravell 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**.
42. Knowles WA. The epidemiology of BK virus and the occurrence of antigenic and genomic subtypes. In: Khalili K, Stoner GL, editors. Human polyomaviruses: molecular and clinical perspectives. **New York: Wiley-Liss; 2001. p. 527-60**.
43. Flaegstad T, Traavik T, Kristiansen BE. Age-dependent prevalence of BK virus IgG and IgM antibodies measured by enzyme-linked immunosorbent assays (ELISA). **J Hyg (Lond) 1986;96:523-8**.
44. Brown DW, Gardner SD, Gibson PE, Field AM. BK virus specific IgM responses in cord sera, young children and healthy adults detected by RIA. **Arch Virol 1984;82:149-60**.
45. Jung M, Krech U, Price PC, Pyndiah MN. Evidence of chronic persistent infections with polyomaviruses (BK type) in renal transplant recipients. **Arch Virol 1975;47:39-46**.
46. Flower AJ, Banatvala JE, Chrystie IL. BK antibody and virus-specific IgM responses in renal transplant recipients, patients with malignant disease, and healthy people. **Br Med J 1977;2:220-3**.
47. Sundsfjord A, Spein AR, Lucht E, Flaegstad T, Seternes OM, Traavik T. Detection of BK virus DNA in nasopharyngeal aspirates from children with respiratory infections but not in saliva from immunodeficient and immunocompetent adult patients. **J Clin Microbiol 1994;32:1390-4**.
48. Bofill-Mas S, Pina S, Girones R. Documenting the epidemiologic patterns of polyomaviruses in human populations by studying their presence in urban sewage. **Appl Environ Microbiol 2000;66:238-45**.
49. 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**.
50. Monini P, Rotola A, de Lellis L, et al. Latent BK virus infection and Kaposi's sarcoma pathogenesis. **Int J Cancer 1996; 66:717-22**.
51. 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**.

52. Andrews CA, Daniel RW, Shah KV. Serologic studies of papovavirus infections in pregnant women and renal transplant recipients. **Prog Clin Biol Res 1983;105:133-41**.
53. Andrews CA, Shah KV, Daniel RW, Hirsch MS, Rubin RH. A serological investigation of BK virus and JC virus infections in recipients of renal allografts. **J Infect Dis 1988;158:176-81**.
54. Dorries K. Latent and persistent polyomavirus infection. In: Khalili K, Stoner GL, editors. Human polyomaviruses: molecular and clinical perspectives. **New York: Wiley-Liss; 2001. p. 197-236**.
55. Taguchi F, Nagaki D, Saito M, Haruyama C, Iwasaki K. Transplacental transmission of BK virus in human. **Jpn J Microbiol 1975;19:395-8**.
56. Pietropaolo V, Ditaranto C, Degener AM, et al. Transplacental transmission of human polyomavirus BK. **J Med Virol 1998;56:372-6**.
57. Shah K, Daniel R, Madden D, Stagno S. Serological investigation of BK papovavirus infection in pregnant women and their offspring. **Infect Immun 1980;30:29**.
58. Coleman DV, Wolfendale MR, Daniel RA, et al. A prospective study of human polyomavirus infection in pregnancy. **J Infect Dis 1980;142:1-8**.
59. McCance DJ, Mims CA. Transplacental transmission of polyoma virus in mice. **Infect Immun 1977;18:196-202**.
60. Atwood WJ. Cellular receptors for the polyomaviruses. In: Khalili K, Stoner GL, editors. Human polyomaviruses: molecular and clinical perspectives. **New York: Wiley-Liss; 2001. p. 179-96**.
61. Liu CK, Wei G, Atwood WJ. Infection of glial cells by the human polyomavirus JC is mediated by an N-linked glycoprotein containing terminal alpha(2-6)-linked sialic acids. **J Virol 1998;72:4643-9**.
62. Atwood WJ, Norkin LC. Class I major histocompatibility proteins as cell surface receptors for simian virus 40. **J Virol 1989;63:4474-7**.
63. Salunke DM, Caspar DL, Garcea RL. Self-assembly of purified polyomavirus capsid protein VP1. **Cell 1986;46:895-904**.
64. Mantyjarvi RA, Meurman OH, Vihma L, Berglund B. A human papovavirus (B.K.), biological properties and seroepidemiology. **Ann Clin Res 1973;5:283-7**.
65. Goudsmit J, Wertheim-van Dillen P, van Strien A, van der Noordaa J. The role of BK virus in acute respiratory tract disease and the presence of BKV DNA in tonsils. **J Med Virol 1982;10:91-9**.
66. Hashida Y, Gaffney PC, Yunis EJ. Acute hemorrhagic cystitis of childhood and papovavirus-like particles. **J Pediatr 1976;89:85-7**.
67. Saitoh K, Sugae N, Koike N, Akiyama Y, Iwamura Y, Kimura H. Diagnosis of childhood BK virus cystitis by electron microscopy and PCR. **J Clin Pathol 1993;46:773-5**.
68. Mininberg DT, Watson C, Desquitado M. Viral cystitis with transient secondary vesicoureteral reflux. **J Urol 1982;127:983-5**.
69. van der Noordaa J, Sol CJ, Schuurman R. Bovine polyomavirus, a frequent contaminant of calf sera. **Developments in Biological Standardization 1999;99:45-7**.
70. Voltz R, Jager G, Seelos K, Fuhry L, Hohlfeld R. BK virus encephalitis in an immunocompetent patient. **Arch Neurol 1996;53:101-3**.
71. Heritage J, Chesters PM, McCance DJ. The persistence of papovavirus BK DNA sequences in normal human renal tissue. **J Med Virol 1981;8:143-50**.
72. Monini P, Rotola A, Di Luca D, et al. DNA rearrangements impairing BK virus productive infection in urinary tract tumors. **Virol 1995;214:273-9**.
73. Shinohara T, Matsuda M, Cheng SH, Marshall J, Fujita M, Nagashima K. BK virus infection of the human urinary tract. **J Med Virol 1993;41:301-5**.
74. Traavik T, Uhlin-Hansen L, Flaegstad T, Christie KE. Antibody-mediated enhancement of BK virus infection in human monocytes and a human macrophage-like cell line. **J Med Virol 1988;24:283-97**.
75. De Mattei M, Martini F, Corallini A, et al. High incidence of BK virus large-T-antigen-coding sequences in normal human tissues and tumors of different histotypes. **Int J Cancer 1995;61:756-60**.
76. Elsner C, Dorries K. Evidence of human polyomavirus BK and JC infection in normal brain tissue. **Virol 1992;191:72-80**.
77. De Mattei M, Martini F, Tognon M, Serra M, Baldini N, Barbanti-Brodano G. Polyomavirus latency and human tumors. **J Infect Dis 1994;169:1175-6**.
78. Knepper JE, diMayorca G. Cloning and characterization of BK virus-related DNA sequences from normal and neoplastic human tissues. **J Med Virol 1987;21:289-99**.
79. Atencio IA, Shadan FF, Zhou XJ, Vaziri ND, Villarreal LP. Adult mouse kidneys become permissive to acute polyomavirus infection and reactivate persistent infections in response to cellular damage and regeneration. **J Virol 1993;67:1424-32**.
80. Randhawa P, Baksh F, Aoki N, Tschirhart D, Finkelstein S. JC virus infection in allograft kidneys: analysis by polymerase chain reaction and immunohistochemistry. **Transplantation 2001;71:1300-3**.
81. Kristoffersen AK, Johnsen JI, Seternes OM, Rollag H, Degre M, Traavik T. The human polyomavirus BK T antigen induces gene expression in human cytomegalovirus. **Virus Res 1997;52:61-71**.
82. Goldstein SC, Tralka TS, Rabson AS. Mixed infection with human cytomegalovirus and human polyomavirus (BKV). **J Med Virol 1984;13:33-40**.
83. Sundsfjord A, Flaegstad T, Flo R, et al. BK and JC viruses in human immunodeficiency virus type 1-infected persons: prevalence, excretion, viremia, and viral regulatory regions. **J Infect Dis 1994;169:485-90**.
84. Blumberg BM, Mock DJ, Powers JM, et al. The HHV6 paradox: ubiquitous commensal or insidious pathogen? A two-step in situ PCR approach. **J Clin Virol 2000;16:159-78**.
85. Remenick J, Radonovich MF, Brady JN. Human immunodeficiency virus Tat transactivation: induction of a tissue-specific enhancer in a nonpermissive cell line. **J Virol 1991;65:5641-6**.
86. Valle LD, Croul S, Morgello S, Amini S, Rappaport J, Khalili K. Detection of HIV-1 Tat and JCV capsid protein, VP1, in AIDS brain with progressive multifocal leukoencephalopathy. **J Neurovirol 2000;6:221-8**.
87. Cavallaro U, Gasparini G, Soria MR, Maier JA. Spindle cells isolated from Kaposi's sarcoma-like lesions of BKV/tat-transgenic mice co-express markers of different cell types. **AIDS 1996;10:1211-9**.
88. Tevethia SS, Schell TD. The immune response to SV40, JC and BKV. In: Khalili K, Stoner GL, editors. Human polyomaviruses: molecular and clinical perspectives. **New York: Wiley-Liss; 2001. p. 585-611**.
89. Knowles WA, Pillay D, Johnson MA, Hand JF, Brown DW. Prevalence of long-term BK and JC excretion in HIV-infected adults and lack of correlation with serological markers. **J Med Virol 1999;59:474-9**.
90. Markowitz RB, Thompson HC, Mueller JF, Cohen JA, Dynan WS. Incidence of BK virus and JC virus viruria in human immunodeficiency virus-infected and -uninfected subjects. **J Infect Dis 1993;167:13-20**.
91. Drummond JE, Shah KV, Donnenberg AD. Cell-mediated immune responses to BK virus in normal individuals. **J Med Virol 1985;17:237-47**.
92. Nickeleit V, Hirsch HH, Zeiler M, et al. BK-virus nephropathy in renal transplants-tubular necrosis, MHC-class II expression and rejection in a puzzling game. **Nephrol Dial Transplant 2000;15:324-32**.
93. Wood KJ, Hopley A, Dallman MJ, Morris PJ. Lack of correlation between the induction of donor class I and class II major histocompatibility complex antigens and graft rejection. **Transplantation 1988;45:759-67**.
94. Milton AD, Spencer SC, Fabre JW. The effects of cyclosporine on the induction of donor class I and class II MHC antigens in heart and kidney allografts in the rat. **Transplantation 1986;42:337-47**.
95. Koralnik IJ, Du Pasquier RA, Letvin NL. JC virus-specific cytotoxic T lymphocytes in individuals with progressive multifocal leukoencephalopathy. **J Virol 2001;75:3483-7**.
96. Knowles WA, Gibson PE, Gardner SD. Serological typing scheme for BK-like isolates of human polyomavirus. **J Med Virol 1989;28:118-23**.
97. Randhawa PS, Khaleel-Ur-Rehman K, Swalsky PA, et al. DNA sequencing of viral capsid protein VP-1 region in patients with BK virus interstitial nephritis. **Transplantation 2002;73:1090-4**.
98. Ritchie BW, Vaughn SB, Leger JS, et al. Use of an inactivated virus vaccine to control polyomavirus outbreaks in nine flocks of psittacine birds. **J Am Vet Med Assoc 1998;212:685-90**.