Polyomavirus (BK Virus) Nephropathy in Kidney Transplant Patients: A Pathologic Perspective

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Reactivation of polyoma virus (BK virus) is a significant cause of morbidity in kidney transplant patients. This seemingly insignificant viral infection that affects the majority of population at a young age, once reactivated by immunsuppression, is a major factor contributing to graft loss. Screening techniques have been developed for early prediction of BK virus reactivation. These include plasma and urine assays for detection of BK virus DNA by PCR, urine cytology for detection of "decoy cells" and electron microscopy. Combining urine cytology and serology screening can be more effective for early detection of BK virus reactivation. Immunohistochemistry can be utilized as an additional tool to support the diagnosis. Once screening tests reveal a suspicious BK virus reactivation, tissue biopsy should be performed to confirm the diagnosis, rule out acute cellular rejection and plan treatment approaches. Treatment normally includes decreasing immunosuppression and the use of antiviral drug therapy. Unfortunately, disease outcome is often unfavorable and can culminate with eventual graft loss. Renal retransplantation has been performed with mixed results. As new data emerges, we will gain a better understanding of the disease caused by BK virus and respond with improved early diagnosis and treatment to preserve graft function.

Key Words: BKV, polyoma, transplant, urine

INTRODUCTION

Polyomaviruses are ubiquitous infectious agents found in over 90% of the general population. The polyoma virus hominis, commonly known as BK virus (BKV), is a causative agent of human diseases, such as haemorrhagic cystitis, ureteric stenosis, pneumonitis, vasculopathy, and even multi-organ failure. Primary infection is usually asymptomatic, and reactivation of the virus results because of severe immunosuppression or altered cellular immunity, as occurs in kidney transplant patients. The first case of polyoma virus infection in a kidney transplant patient was reported in 1971. The virus was named BKV after the initials of the first patient who was diagnosed with the infection. Since then it has been recognized that reactivation of BKV is a significant cause of renal allograft dysfunction and subsequent graft loss. In this review, an overview of BKV infection in renal transplant patients is presented, with emphasis on the pathologic aspects of the resulting disease.

VIROLOGY

Polyomaviruses are a family of small, non-enveloped DNA viruses with icosahedral capsids of 40-44-nm in diameter. The viral genomes within the capsids are circular double stranded DNA of 5300 base pairs, coated by host cell histones, that encode the early (regulatory) and late (structural) proteins. BKV, along with JC virus (JCV) and simian virus SV40 are species of the genus polyoma virus and part of the family polyoma viridae. Although these viruses share similarities at the DNA and protein level, BKV and JCV are human pathogens with different infection outcomes - BKV causes nephritis and JCV causes progressive multifocal leukoencephalopathy (PML). The principal target organ for BKV infection is the kidney, however, primary infection can be followed by latency in the urinary tract epithelium as well as lymphoid cells and central nervous system. The homology between
the genomes of the two human polyomaviruses (JVC and BKV) and SV40 is approximately 70%. For viral replication to proceed, virions must attach to the host cell membrane and target their genome to the nucleus, where the uncoated viral DNA is transcribed (reviewed in ). In the host cell nucleus, electron microscopy shows a dense even crystal-like array of particles (Fig. 1). These correspond to nuclear inclusion structures seen by light microscopy. Early gene expression and replication of the BKV minichromosomes precedes late gene expression and virion assembly. Early genes encode the regulatory large tumor antigen (LT-ag) and the supporting small 2-ag. Transcription of the LT-ag induces quiescent to cells to resume cell cycling and synthesize DNA. Multiple domains in the LT-ag are responsible for the inactivation of proteins, such as retinoblastoma (Rb) and p53, which contributes to loss of cell cycle control and prevention of apoptosis. Polyomaviruses use host cell enzymes to replicate their genome and do not encode their own viral DNA polymerases. The LT-ag regulates DNA replication and late gene expression, utilizing the host cell’s transcription factors. Activation of the host cell by growth factors and other signals can facilitate viral replication. For example, regenerating and dividing cells, such as urothelia, are more permissive for BKV infection. Completion of viral DNA replication and expression of late genes that encode the structural viral capsid proteins, VP-1, VP-2, and VP-3, are the final steps prior to virion assembly. Viral capsomeres form around daughter minichromosomes in the host cell’s nucleus to produce stable viral particles.

The host receptors for BKV are unknown, but ubiquitous alpha 2-3 sialic acid modifications may be involved (reviewed in ). Studies show that there are four different BKV subtypes: I (prototype PT, Dunlop Dun, Gardner GS and MM), II (SB), III (AS) and IV (IV and MG), with subtype I being the most common. Antigenic determinants of these BKV subtypes have been mapped to amino acids 61-83 of VP-1, the viral component that may also be involved in attachment to host cells.

**EPIDEMIOLOGY**

Detection of BKV seropositivity is at its lowest at the age of 6 months and increases to 75% among adults worldwide, with seropositivity ranging from 46% to 94%. Initial infection with BKV usually occurs during childhood. In normal individuals, subclinical BKV primary infections have been associated with seropositivity in more than 98% of children by ages 7-9 years. After primary infection in renal tubular cells and urothelia, the principal reservoir site of BKV is the renal urinary tract. Reactivation of BKV infection can occur spontaneously in individuals with depressed immune responses, primarily as a result of immunosuppressive therapy in organ transplantation, but also as a result of pregnancy, chemotherapy for cancer treatment, human immunodeficiency virus (HIV), uncontrolled diabetes, and use of cytotoxic drugs (reviewed in ). Patients with higher blood levels of immunosuppressive agents, such as tacrolimus, are at an increased risk of viral reactivation. In addition to the kidney, lymphoid cells have been proposed as sites of viral latency, but a recent study suggested that the presence of BKV in peripheral blood monocellular cells is more indicative of reactivation or recent infection. BKV DNA has also been described in brain tissue.

BKV may contribute to allograft dysfunction in 2-5% of renal transplant patients, resulting in graft loss in 45% of cases. In one study, up to 9.3% of renal transplant recipients had reactivation of

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**Fig. 1.** Electron Microscopy: Dense viral particles of BKV (Courtesy of C. Drachenberg, M.D., University of Maryland, Baltimore, MD, USA).
BK Virus Nephropathy

BKV. Reactivation of BKV can cause three different lesions in renal transplant recipients, which include hemorrhagic cystitis, ureteral stenosis and interstitial nephritis. Ahuja et al. reported that reactivation may start as early as 4 months post transplant and run a course until graft failure, with a median time of diagnosis of 9.5 months. Patients with reactivation of BKV have shorter graft life than patients free of BKV infection.

BKV associated nephropathy is also an emerging complication in pediatric kidney transplantation. The incidence of BKV reactivation has been reported in up to 26% of pediatric transplant patients, with high viral DNA titers in the blood directly correlating with impairment of renal-allograft function and increased risk of developing BKV nephropathy.

MODE OF INFECTION

The mechanism by which BK virions enter host cells and target the nucleus remains poorly understood. For JCV it has been shown that viral entry proceeds through a typical endocytic pathway via clathrin-coated vesicles. For BKV, Drachenberg et al. recently reported the ultrastructural mechanism through which the virus gains cellular entry to eventually cause cell lysis. The authors observed that BKV attached randomly on the cell surface of tubular cells, forming vesicles. These vesicles then entered the cell through the process of endocytosis fusing with irregular tubulo-vesicular structures. These vesicles accumulated in the vicinity of the nucleus and subsequently fused with the membranes surrounding the virions and the perinuclear membranes. In the majority of cases, the virions found inside the nucleus were membrane bound. With the production of daughter viral particles, nuclei enlarged due to the formation of viral aggregates separated from the nuclear membrane by a rim of chromatin. This was followed by nuclear and cytoplasmic swelling with disruption of the cell membranes resulting in cell lysis. The same group also noted the presence of tubuloreticular inclusions similar to those inclusions present in patients with HIV-associated nephropathy, lupus nephritis or patients treated with interferon.

RISK FACTORS

Among risk factors that promote BKV nephropathy, immunosuppression is the most significant. Specific immnosuppressive agents, especially tacrolimus and mycophenolate mofetil (MMF), have been implicated in BKV infection. Mengel et al. found that use of tacrolimus in combination with MMF increased the risk of BKV nephropathy. However, other reports have shown that it is the total level of immunosuppression that is associated with BKV nephritis rather than the level of a specific agent. Other factors could contribute to the development of BKV nephropathy, such as male gender and increased age, as recently noted in a four year retrospective study, though the significance of these findings remains unclear.

TRANSMISSION

The natural route of transmission of BK virus is not known. Oral transmission through contaminated food or water has been suggested. Other potential routes include semen, blood products and organ transplantation, particularly renal allografts. BKV may also be transmitted through the placenta.

SEROLOGY

Testing for the presence of BKV in plasma is a sensitive and specific approach for identifying viral nephropathy. Rahamov et al. suggested that kidney transplant recipients who experienced deterioration of their graft function several months after transplantation should have a full clinical work-up, including assay of serum and urine for detection of BKV viral load. Assessment of BKV infection by viral load is, therefore, an important tool for diagnosis and monitoring of BKV reactivation.

In the early 1980's, the measurement of viral hemagglutination antibodies was used to detect BKV infection. It was a relatively fast and easy method, with no risk to the patient. In recent years, an even more sensitive method is being
used for the measurement of BKV viral load in the plasma and urine, employing the polymerase chain reaction (PCR) assay. Measurement of viral load in the plasma is a reliable method of predicting BKV infection. Hirsch et al. reported that BKV viremia PCR assays had a diagnostic sensitivity of 100%, with a specificity of 88%. The mean viral load in plasma was higher in patients with biopsy-proven BKV than in patients without histologic evidence of disease (28,000 copies per milliliter vs. 2000 copies per milliliter). The authors concluded that BKV nephropathy can be monitored by measuring the viral load in plasma. Attempts were made to diagnose BKV nephritis by the non-invasive method of measuring mRNA for the BKV capsid protein, VP-1, in urine, by real time quantitative PCR. Ding et al. found that the level of BKV VP-1 mRNA, but not levels of control 18S rRNA, predicted BKV nephritis. The authors demonstrated that with this method BKV nephritis could be predicted with a sensitivity of 93.8% and specificity of 93.9%. Hence, various screening methods have been tested to reliably predict BKV infection in patient serum and urine samples.

The significance of seropositivity of both donors and recipients is still controversial. A serological investigation reported that the prevalence of BKV reactivation increased from 7.3% to 33.7% when the kidney donors were seropositive rather than seronegative. Some authors have suggested that BKV might be transmitted by the allografts, in that two recipients developed BKV infection that was traced back to the same donor (reviewed in ). However, with the high incidence of BKV seropositivity in the population, it is doubtful whether the serological status of donors has any impact on development of BKV infection. For example, a study by Hirsch et al. showed that most recipients that developed BKV infection were seropositive before receiving transplants.

In a pediatric population, Gineri et al. reported that the antibody status of the recipient was the most important predictor of reactivation of BKV infection, whereas seropositivity of the donor was not a risk factor per se but only in association with recipient seronegativity. However, in adult populations, seropositive donors increased the rate of primary and reactivated BKV infections in transplant patients.

CYTOLOGY

The use of urine cytology for diagnosis of BKV shedding has been documented since the 1970s. It is a rapid, convenient, and inexpensive method of detecting shedded tubular epithelial cells infected with BKV. In urine, the infected cells, known as ‘decoy cells’, show rounded nuclei that are generally larger than the average transitional and tubular cells (Fig. 2). The nuclei contain viral inclusions appearing as dense granular basophilic cytoplasm with no surrounding halo. An important observation in cases with abundant decoy cells is the presence of “dirty background” which contains transitional cells, tubular cells, and inflammatory cells with clumps of amorphous basophilic material. However, in our experience, there is a significant decrease or even absence of background when urine samples are prepared with the thin-layer method. Hirsch et al. reported that the sensitivity of decoy cell shedding for the diagnosis of BKV was 100%, with a specificity of 71% when concurrent allograft-biopsy samples were used as the diagnostic standard.

However, the presence of decoy cells in urine should be evaluated quantitatively because this does not always indicate reactivation of BKV infection. For example, Drachenberg et al. suggested that BKV infection could be common and harmless to the graft, since, in their study of one hundred urine samples, BKV excretion was detected in nearly 20% of renal transplant recipients that had stable graft function. These investigators showed that the presence of decoy cells in urine sediment could be divided into three groups, (1) rare cells, (2) up to 4 decoy cells per cytospin, and (3) more than 10 decoy cells per cytospin. The authors observed that urine specimens with rare viral inclusions and no inflammatory background were less likely to have a positive biopsy showing BKV infection. Patients with rare cells had creatinine slopes similar to those of negative controls. Therefore, urine cytology can be used as a screening tool to anticipate and follow up patients with BKV infection. It is important that patients with rare decoy cells
and clean background that show no clinical picture of BKV reactivation be closely monitored for the possibility of developing BKV infection.

Ancillary studies such as electron microscopy, plasma and urine BKA-DNA and in situ hybridization may be used for definitive diagnosis. De Las Casas et al. added that electron microscopic studies increased the sensitivity and specificity of urine cytology.45

Several authors have advocated the use of the combination of serology and cytology for effective diagnosis of BKV reactivation. Semiquantitative PCR assay for BKV detection in urine is more sensitive than urine cytology alone.46 Merlino et al. described a protocol for a quantitative assay to evaluate viral load in renal transplant patients. In this report, the authors concluded that viral load in urine was dissociated from viral load in the blood and that both parameters should be investigated when evaluating BKV reactivation in renal transplant patients.57 The presence of decoy cells in the urine and viremia, as measured by plasma PCR assays, may serve as noninvasive markers of BKV replication.38 Mayr et al. stressed the importance of urine cytology and detection of BKV DNA in the plasma as markers for diagnosis and treatment of infection with concurrent acute cellular rejection.48 On the other hand, Maiza et al. discussed the importance of urine cytology screening with confirmation by in situ hybridization before reducing immunosuppressive therapy in transplant recipients.49 Such diverse work suggests the need for measurement of more than one parameter for detection of BKV activation.

The differential diagnosis of decoy cells in urine includes high-grade urothelial carcinoma (Table 1). Occasionally, a homogenous central clearing is not evident and there is associated prominent chromatin network. In such cases, performing immunohistochemistry for detection of BKV infected cells with cross-reacting antibodies against the large T-antigen of the related simian polyoma virus SV 40 (Fig. 3) can be useful.50 Wojcik et al. reported that BKV infected urothelial cells show a unique DNA content pattern with mildly elevated proliferative activity and a dispersed hyperdiploid DNA.51 DNA studies such as these can help to differentiate BKV infected cells from high-grade urothelial carcinoma.

JCV infection can occur with or without concurrent BKV infection. Randhawa et al. reported co-infection of BKV and JCV.52 While, Kazory et al. reported the first case of JCV nephropathy with no co-infection with BKV.53 Diagnostic observation of JCV infection is similar to BKV, both in urine cytology and by histologic examination. Analysis of a biopsy by in situ hybridization is needed to confirm JCV infection.

**HISTOLOGY**

The rise in BKV diagnosis over the last decade can be attributed to the increased use of biopsies and the use additional tools such as immunohistochemistry and electron microscopy, for diagnosis.51 Histological evaluation of biopsy specimens is necessary to confirm the presence of BKV

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<th>Table 1. Differential Diagnosis between BK Virus and High-grade Urothelial Carcinoma in Urine</th>
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<td><strong>Hyperchromasia</strong></td>
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IHC, immunohistochemistry; HGUC, high grade urothelial carcinoma.
reactivation in renal transplant recipients. In conjunction with the use of screening techniques - urine cytology or BKV-DNA PCR assay in urine and plasma - tissue biopsy for histological assessment is recommended, especially upon detection of any significant increases in decoy cells, increases in viral DNA levels, or in the presence of clinical symptoms.

Tissue biopsy is considered the gold standard for documentation of BKV reactivation. However, this procedure does have drawbacks. Tissue biopsy is invasive and therefore relatively expensive. There is associated risk of complications, including infection, bleeding, and hematuria. Patient compliance to undergo the procedure repeatedly can also be an issue. Buehrig et al. and Drachenberg et al. advocated the need for biopsy and routine screening for early diagnosis of BKV in transplant recipients. Both studies concluded that patients with early diagnosis had better graft outcome with lower interstitial and tubular injury.

The histologic pattern of BKV reactivation ranges from a mild form where only rare cytopathic changes in the medulla are seen, a cytopathic form with abundant cytopathic changes and significant interstitial inflammation, and, eventually, an advanced form with graft sclerosis. In early infection with BKV, scattered tubular cells show viral cytopathic changes that are seen in the collecting ducts of the medulla. As the infection with BKV advances, more tubules in the cortex begin to show numerous cytopathic changes, including anisonucleosis of the nuclei with hyperchromasia and smudging or clumping or peripheral margination of the chromatin. Infected cells have nuclei that are enlarged by 2-5 times, with associated high N/C ratio. The most characteristic sign is the presence of basophilic intranuclear inclusions with no prominent surrounding halo and occasional ground glass appearance. Tubular cell apoptosis, cell dropout desquamation, and flattening and sloughing of the epithelial lining are also observed (Fig. 4).

The interstitium usually shows mixed tubulointerstitial infiltrates with focal tubulitis. Occasional tubules may show no significant inflammatory response. As the infection progresses, or with repeated episodes of rejection, extensive graft sclerosis will develop terminating in eventual loss of kidney function. Hirsch et al. stated that immunohistochemistry using antibodies against the large T-antigen of SV 40 increased the sensitivity and specificity of the diagnosis. Another reported observation was the presence of viral cytopathic changes in the glomeruli, commonly infecting the parietal layer of the Bowman's capsular epithelium. The visceral layer was also affected. In addition, other changes included crescent formation in 12% of cases, increased mesangial matrix, ischemic glomerulopathy and chronic transplant glomerulopathy.
Differential diagnosis for BKV can be challenging. Symptoms produced by BKV reactivation include fever, persistent hematuria, and increased serum creatinine levels. These symptoms are similar to that of acute cellular rejection, which makes controlling immunosuppressive therapy more difficult. Interstitial inflammatory cell infiltrates may represent virally induced interstitial nephritis or can also indicate acute cellular rejection. Differences between BKV and acute cellular rejection are summarized in Table 2. What makes the differentiation even more difficult is the discovery of a recent BKV mutant that causes widespread endothelial infection with extensive vasculopathy, which can be confused with acute vascular rejection.50

Microscopically, tubulointerstitial infiltrates with associated tubulitis and reactive or degenerative changes can be seen in acute cellular rejection. However, a careful search of cytopathic changes could fail to recognize the diagnostic intranuclear inclusions of BKV. Ancillary studies, such as electron microscopy and immunohistochemistry are helpful in reaching the correct diagnosis.43

If a biopsy specimen from a transplanted organ shows abundant cortical mononuclear cell infiltrates and typical tubulitis with no associated observable virally infected cells, rejection is the most likely diagnosis.50 Jeong et al. reported that the degree of tubulitis and interstitial inflam-
information was higher in biopsies with acute cellular rejection. Several ancillary studies have been used to differentiate acute cellular rejection from BKV infection. For example, the evaluation of B and T lymphocytes in tissue biopsies can distinguish between the two. Marked increases in B cell lymphocytes (21%) and decreases in cytotoxic T cells lymphocytes (7%) were detected in BKV infected tissue biopsies, while in specimens from patients with ACR, B cell lymphocyte numbers (6%) were decreased and cytotoxic T cell numbers (24%) were increased. Another marker to distinguish between BKV reactivation and acute cellular rejection is the level of MHC class II molecules. In one study, the upregulation of MHC class II (HLA-DR) and ICAM-1 on tubular epithelial cells was a finding in graft biopsies with acute cellular rejection. In contrast, in BKV infection, tubular epithelial cells were found negative for HLA-DR expression and positive for ICAM-1.

TREATMENT

Although discussion of treatment for BKV infection is beyond the scope of this review, it can be stated that drug therapy has so far had limited success in the control of BKV infection. The presence of numerous virally infected cells with significant tubular damage, with insignificant to mild tubulitis, and the absence of vascular rejection should point to an infectious process and decreasing immunosuppression is indicated. Reduction of immunosuppression has long been a cornerstone in controlling BKV infection. However, successful intervention has also been achieved with the potentially nephrotoxic drug, cidofovir (reviewed in 7). Bjorang et al. reported that treatment with cidofovir was successful along with a concurrent reduction in immunotherapy. Both measures managed to decrease viral load. However, a reported case has surfaced that implicated topical application of cidofovir with acute renal failure.

Trofe et al. described a protocol for management of polyomavirus nephropathy. They proposed a decrease in immunosuppression with possible careful administration of the antiviral drugs while monitoring for nephrotoxicity. Continuous histologic monitoring was recommended for detection of acute cellular rejection.

OUTCOME OF INFECTION

Unfortunately, many patients may undergo a vicious cycle of alternating BKV nephropathy and acute cellular rejection following increasing or decreasing immunosuppressive therapy. The ultimate outcome of this cycle is, in many cases, graft loss. Graft loss due to BKV reactivation varies in many reports, ranging from 45%63 to 67%. The problem is complicated by the lack of an effective nonnephrotoxic therapy.

A problem may arise with a patient who loses a graft due to BKV infection. With retransplantation, reactivation of BKV is very common. Contradictory reports have surfaced in the last few years regarding the role of retransplantation after graft loss due to BKV nephropathy. Retransplantation in two patients showed recurrence of BKV nephropathy after 8 and 28 months. Ginevri et al. reported a case of a graft that was lost due to BKV nephropathy. The patient was retransplanted without a nephrectomy of the original graft and remained BKV-DNA negative in both urine and plasma. Ramos et al. reported a study of 10 patients who underwent retransplantation after losing the original graft to BKV nephropathy. This retrospective study, which included a mean post-transplantation follow up time of 34.6 months, concluded that neither nephrectomy nor changing immunosuppressive drugs seemed to alter the risk of recurrence of infection. All retransplantations were performed at a time of negative urine cytology for decoy cells. The authors also recommended that viral load in the plasma be absent or low at the time of retransplantation. Al-Jedai et al. reported successful retransplantation of renal allograft in 2 patients with simultaneous kidney-pancreas transplant, who lost the original graft to BKV nephritis, with no evidence of recurrent BKV reactivation after 22 and 37 months. In another study, BKV infection reoccurred six months after retransplantation, after the original graft failed due to BKV.

Boucek et al. recommended that retransplantation should be done in patients with no evidence...
of active infection. Quantitative or qualitative plasma or urine PCR should be used to evaluate the patients before retransplantation. However, the reported cases to date are insufficient to determine if nephrectomy of the original graft is necessary or if there is a need to adjust the level of immunosuppression to avoid BKV recurrence.

CONCLUSION

BKV infection of renal allografts is a major cause of morbidity for transplant patients and a source of frustration for physicians. With the rise in BKV infection in the last decade, current data is derived from a few studies with small number of patients and limited follow-up time. The need for a non-nephrotoxic treatment is of paramount importance. As more findings are published, we may better understand the disease caused by BKV reactivation and develop improved approaches for treatment with better outcomes. Until newer approaches are found, current strategies of early screening and detection and early aggressive management should minimize the risk or altogether prevent early graft loss.

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