BK Virus in Kidney Transplant: Current Concepts, Recent Advances, and Future Directions

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Abstract

BK virus nephropathy is a challenging clinical problem in kidney transplant recipients with wide range of surveillance and management practices, based on individual experience. BK virus reactivation in kidney transplant recipients can result in BK virus nephropathy and graft loss. The most effective strategy for early diagnosis and treatment of BK virus nephropathy is regular monitoring for BK virus, currently achieved by quantification of viral DNA in blood by quantitative polymerase chain reaction. Immunosuppression reduction remains the mainstay of treatment; however, viral clearance is often followed by acute rejection, likely secondary to a delay between immune reconstitution and viral clearance. Impaired cell-mediated immune response to BK virus has been shown to correlate with progression to BK virus nephropathy, while reconstitution of this response correlates with resolution of nephropathy. There is recent research to support monitoring BK virus-specific cell-mediated immune response as a predictor of disease progression and resolution. In this article, we review the current concepts and recent developments in understanding BK virus-associated disease in the context of kidney transplant and outline areas for future research.

Key words: BK virus nephropathy, Cell-mediated immune response, Immunosuppression, Transplant

Introduction

BK polyomavirus continues to remain a challenging problem in kidney transplant recipients, with a wide range of surveillance and treatment practices, which are based on individual experience. We review the recent developments in understanding BK virus (BKV)-associated disease in the context of kidney transplant.

The first Polyomavirus (mouse polyomavirus) was identified in 1953 as filterable tumor-causing agent in mice, followed by Simian vacuolating virus (SV40) isolated from rhesus monkey kidney cells that had been used for poliovirus vaccine preparation in 1960. The discovery of the first human polyomaviruses was in 1971 independently from each other; one was BKV isolated from a urine sample of a renal transplant patient and the other was JC virus isolated from the brain tissue of a patient with progressive multifocal leukoencephalopathy, with both named after the patients’ initials. BK and JC viruses were the only well-known human polyomaviruses for 36 years; however, a dramatic increase in the number of newly identified human polyomaviruses was recorded in the past 8 years due to the use of sophisticated molecular methods and new generation sequencing technologies.1

BK virus is a nonenveloped virus with a genome of double-stranded circular DNA. Functionally, the genome consists of an early region that codes for the large and small T antigens involved in viral replication, a late region that codes for viral capsid proteins 1-3 and agnoprotein involved in viral assembly, and a noncoding control region that is required for viral gene expression and replication. BK virus strains are classified into genotypes 1 to 6 based on the polymorphisms in the viral capsid protein 1 region and the noncoding control region.3

Pathogenesis

Primary BK virus infection is acquired during childhood, most likely from person-to-person contact from respiratory secretions or urine; it usually
manifests as mild respiratory infection or fever. The virus multiplies in the respiratory tract and then spreads to other organs through the bloodstream. BK virus remains clinically silent, in the kidneys, in immunocompetent hosts. It is usually reactivated by immunosuppression. Impairment of cell-mediated immune response has been associated with reactivation, which begins with active viral replication in the graft, followed by viral shedding in the urine, and finally viremia. Tubular epithelial cells are common targets, resulting in tubulointerstitial nephritis. Approximately 80% of the general population has detectable antibody to BKV, which appears early in life and persists throughout life. BK virus reactivation in kidney transplant recipients is first evident by viral shedding in urine, which can be seen in 20% to 60% of patients. Incidence of BK viremia in kidney transplant recipients is about 13%, whereas BKV nephropathy is seen in nearly 8% of kidney transplant recipients and can result in allograft loss. Data from the United Network for Organ Sharing have shown that graft loss attributable to BK virus-associated nephropathy was 7.5% (70/938) in 2009 and 5.7% (36/632) in 2010.

Immunosurveillance and potential applications
It is likely that humoral immune response alone does not play a significant role in preventing progression to BKV nephropathy, as patients with levels of anti-BKV antibodies similar to those of healthy individuals still develop BKV nephropathy. Furthermore, high levels of antibodies in patients with BKV nephropathy correlate with high levels of viremia and low CD8-positive T-cell responses. A cellular immune response to BKV was demonstrated in normal individuals by Drummond and associates, who found that the cell-mediated immune response was important in preventing viral reactivation in healthy volunteers. This was further supported by a study of cellular immune response to JC virus in patients with progressive multifocal leukoencephalopathy, in which Koralnik and associates found that JC virus-specific cytotoxic T lymphocytes were a key factor in containment of progressive multifocal leukoencephalopathy. Low levels of BKV-specific interferon gamma-producing T cells correlate with progression to BKV nephropathy, whereas reconstitution of these cells correlates with resolution of nephropathy. Batal and associates measured global cell-mediated immune response in kidney transplant recipients with BKV infection using the Cylex ImmuKnow Test (Cylex, Columbia, MD, USA) and found that the mean ± standard deviation amounts of ATP released in recipients with BK viremia was 102.9 ± 58.6 ng/mL, versus 227.2 ± 146.4 ng/mL in those with viruria and 231.8 ± 150.8 ng/mL in those with no viremia or viruria ($P = .002$, viremia vs all other samples), showing that a decreased immune cell function test result correlates with active viral replication.

Impaired BKV-specific cell-mediated immune response is associated with BKV nephropathy, and BKV-specific immune monitoring has been suggested as a prognostic tool to identify patients who are at risk of BKV nephropathy. Monitoring pretransplant and posttransplant BKV-specific T cells was suggested by Schachtner and associates as a sensitive marker to identify kidney transplant recipients at increased risk of BKV reactivation. They studied 24 recipients with BKV replication and compared these patients with a control group of 127 recipients without BKV replication. BK virus-specific and alloreactive T cells were measured using an interferon gamma Elispot assay. The extent of immunosuppression was quantified by lymphocyte subpopulations and interferon gamma levels. Recipients with a loss of BKV-specific T cells directed to large T-antigen from pretransplant to posttransplant were found to be at increased risk of BKV replication ($P < .001$), whereas recipients with stable or rising BKV-specific T cells were more likely to not develop BKV reactivation ($P < .05$). Recipients with BKV reactivation showed significantly lower CD3-positive, CD4-positive, and CD8-positive T cells and interferon gamma levels posttransplant, but with significantly higher alloreactive T cell levels ($P < .05$), thereby making a case for immunosurveillance as a predictor of disease progression and resolution. However, this has not been studied in the context of an anti-BKV treatment strategy.

A potentially major breakthrough in the prevention and treatment of BKV nephropathy was suggested by a recent longitudinal serologic study of kidney transplant recipients that used sensitive new methodologies to demonstrate that BKV subtype 1 and subtype 4 are serologically distinct. In particular, the authors relied on BKV reporter vectors (pseudovirions) to evaluate serotype-specific neutralizing antibodies rather than more traditional recombinant virus-like particle enzyme-linked
immunosorbent assays, which crucially detect both neutralizing and nonneutralizing antibodies in the latter case. With these antibody-mediated neutralization assays, the studies found that 5% and 49% of kidney transplant recipients were BKV subtypes 1 and 4 naïve before transplant, with 100% of the BKV subtype 1 and 43% of the BKV subtype 4 seronegative patients pretransplant seroconverted in a type-specific manner. A model was presented showing that BKV nephropathy can arise from a de novo infection arising from a BKV subtype 4-infected kidney leading to replication in immunocompromised patients without prior exposure to this more rare BKV subtype. Interestingly, previous studies have reported higher seroprevalence of BKV subtype 4 in patients with interstitial nephritis. Pastrana and associates argued that induction of a neutralizing antibody response to BKV subtype 4, or all subtypes, by vaccination of kidney transplant patients immunologically naïve for certain subtypes before transplant may prevent replication and BKV nephropathy associated with virus present in the transplanted organ. In vitro enrichment of BKV-specific T cells and subsequent adoptive T-cell transfer may improve the restoration of immunocompetence in kidney transplant recipients with BKV infection. Vaccination of potential transplant recipients with BKV antigens in conjunction with an adjuvant that preferentially induces cell-mediated immune response is a potential area for future research.

**Diagnosis**

More recently, the emphasis has been on surveillance for earlier diagnosis and treatment. Quantitative estimation of viral DNA in urine by polymerase chain reaction is the most frequently used screening test, but it does not provide any additional information other than the fact that the virus is present in urothelium. Persistently high BKV DNA in plasma > 4 log10 gEq/mL by quantitative polymerase chain reaction for more than 4 weeks in kidney transplant patients defines presumptive BKV nephropathy. BK virus-specific cell-mediated immune response has been a recent area of research. From available literature, BKV-specific immune monitoring has been suggested as a prognostic tool to identify patients who are at risk of BKV nephropathy.

There has been recent interest in investigating the role of the BKV micro-RNA (miRNA) in regulating virus replication. The function of the BKV miRNA was investigated in archetype BKV, which is the transmissible form of the virus and thought to establish a persistent infection in the host urinary tract. Broekema and associates showed that the BKV miRNA targets early mRNAs and that the miRNA plays a significant role in limiting archetype BKV replication in a natural host cell model of infection. This occurs through a balance of regulatory elements located within the noncoding control region that controls early gene expression and miRNA expression before genome replication. Li and associates demonstrated that circulating BKV-encoded miRNA (BKV-miRNA-B1) is detectable in the plasma of patients with biopsy-proven BK virus-associated nephropathy and those with significant BKV DNA in the blood. The group also showed that BKV-miRNA-B1-5p levels are highest in patients with biopsy-proven BKV nephropathy, suggesting that circulating virally encoded miRNAs may act as indicators of severity of virus replication. Other diagnostic tools include quantitative estimation of polyomavirus-Haufen in voided urine. It has been suggested as a specific biomarker for intrarenal viral disease, with positive and negative predictive values of greater than 90%. However, allograft biopsy and histology (confirmed by immunohistochemistry or in situ hybridization) still remain the criterion standard for diagnosing BK virus-associated nephropathy. Because of the focal nature of the disease and possibility of sampling error, a minimum of 2 cores are recommended, preferentially containing medullary tissue. Typical findings are focal interstitial mononuclear inflammatory cell infiltrates, presence of plasma cells, necrotic tubular epithelium, and presence of homogenous intranuclear inclusion bodies. Histologic findings have been classified into types A, B, and C for standardized assessment and reporting. The Banff working group on polyoma virus nephropathy classified the disease into 3 grades based on histology to allow comparative analyses and improvement in predicting clinical presentation and outcome. Tubular atrophy and interstitial fibrosis remain the most important predictors of poor outcome.

**Risk factors**

In addition to immunosuppression, several risk factors have been proposed for BKV reactivation.
after kidney transplant. Grafts from BKV-seropositive donors to BKV-seronegative recipients are believed to increase the risk.39,40 The number of HLA mismatches, ischemic injury, and prior acute rejection are associated with increased risk of BKV reactivation.32,41,42 Recipient old age, male sex, and prior graft loss due to BKV nephropathy have also been reported to increase the risk.43 Viral capsid serotype and rearrangement of control region are also believed to increase the risk for BKV reactivation.44 It is widely agreed that, with more potent immunosuppression, there is a greater likelihood for viral replication.32,45 However, there are conflicting reports on the effect of individual agents on BKV nephropathy.46 It has been reported that induction with thymoglobulin (Genzyme Corporation, Framingham, MA, USA) is associated with a higher incidence of treatment for BKV compared with no induction or induction with interleukin 2 receptor blockers. Alemtuzumab does not increase rates of BK viruria, BK viremia, or BKV nephropathy compared with that shown with non-lymphocyte-depleting therapy.47–49 Cyclosporine is believed to be associated with a lower incidence of BKV nephropathy than tacrolimus, and mycophenolate mofetil is associated with a higher incidence of treatment for BKV compared with no antimetabolite therapy or azathioprine.9,50–52

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Abbreviations: BKV AN, BK virus-associated nephropathy; MMF, mycophenolate mofetil; OPTN, Organ Procurement and Transplantation Network; SRTR, Scientific Registry of Transplant Recipients
data on mammalian target of rapamycin inhibitors, sirolimus and everolimus, are limited, it would be safe to state that they are not associated with an increased risk of BKV nephropathy.\textsuperscript{50,53-55} It is well established that the combination of tacrolimus and mycophenolate mofetil is associated with a significantly higher risk for BKV nephropathy\textsuperscript{56,57} (Table 1).

**Treatment**

Treatment is indicated for BKV nephropathy, defined by persistently high BKV in plasma $> 4 \log_{10}$ gEq/mL for more than 4 weeks in kidney transplant recipients, even in the absence of elevated serum creatinine levels. BK virus nephropathy usually implies excessive immunosuppression, and immunosuppression reduction forms the mainstay of treatment. However, acute rejection is frequently observed after virus clearance.\textsuperscript{58} The most effective strategy for early diagnosis and treatment remains regular monitoring for BKV, which is currently achieved by quantification of viral DNA in blood by quantitative polymerase chain reaction. This method of monitoring is suboptimal because there is an apparent delay between immune reconstitution and viral clearance as measured by quantitative polymerase chain reaction, often resulting in acute rejection before resumption of baseline immunosuppression after viral clearance.

There is no perfect way to approach immunosuppression reduction, and several strategies have been suggested. A common initial approach is to reduce the calcineurin inhibitor dose by 25% to 50% with target tacrolimus trough levels $< 6$ ng/mL or cyclosporine levels $< 150$ ng/mL. This would be followed by dose reduction of the antiproliferative agent (eg, mycophenolate mofetil) by 50% followed by its discontinuation, depending on the response, as monitored with serial quantitative polymerase chain reaction analyses in serum. An alternative approach is to first reduce the dose of the antiproliferative agent and then reduce the calcineurin inhibitor dose. Successful outcomes have also been reported with other strategies, including switching from tacrolimus to cyclosporine, calcineurin inhibitor to sirolimus, or mycophenolate mofetil to leflunomide. The BKV treatment protocol at our center is presented in Figure 1. Acute rejection is frequently observed after immunosuppression reduction to treat BKV nephropathy\textsuperscript{58} and usually follows virus clearance, further complicating treatment options. Other antiviral treatment options include those detailed below.

**Leflunomide**

Leflunomide is an anti-inflammatory agent approved for use in rheumatoid arthritis. It has unique antiviral and immunosuppressive properties, making it useful in treatment of BKV nephropathy.\textsuperscript{59-61} Mycophenolate mofetil is usually switched to leflunomide. A loading dose of 100 mg daily for 3 to 5 days followed by a maintenance dose of 20 to 60 mg daily with target trough levels of 50 to 100 μg/mL is recommended.\textsuperscript{58} Significant toxic effects have been described, including hepatitis, hemolysis, thrombotic microangiopathy, bone marrow suppression, and fungal pneumonia.

**Cidofovir**

Cidofovir is a nucleoside analog approved for use in cytomegalovirus retinitis. Its mechanism of action against BKV is poorly understood. It is usually reserved as a last resort for refractory disease. It is primarily excreted by the kidneys but accumulates in tubular epithelial cells with potential for substantial nephrotoxicity. Concomitant administration of probenecid to reduce renal clearance can reduce toxicity. It is usually administered intravenously in doses ranging from 0.25 to 1.0 mg/kg at 1 to 3 weekly intervals. Favorable outcomes have been reported in several cases.\textsuperscript{62-64}

**Brincidofovir (CMX001)**

Brincidofovir (CMX001), an experimental prodrug of cidofovir, has shown antiviral efficacy and a better
adverse effect profile in isolated reports of kidney transplant and hematopoietic stem cell transplant recipients.\(^{65,66}\)

**Intravenous immunoglobulin**

Intravenous immunoglobulin preparations have high titers of potent BKV-neutralizing antibodies and act by direct neutralizing activity and indirect immunomodulatory effects, resulting in successful resolution of disease.\(^{67}\) It is generally used in doses ranging from 0.2 to 2.0 g/kg.

**Fluoroquinolones**

Fluoroquinolones exhibit modest antiviral activity by inhibiting the helicase activity of virus-encoded large T antigen, although the selectivity index is low. Ciprofloxacin has demonstrated prophylactic efficacy in both hematopoietic stem cell and kidney transplant recipients.\(^{68,69}\) However, a recent double-blind, placebo-controlled randomized trial by Knoll and associates failed to demonstrate efficacy of a 3-month course of levofloxacin to prevent BK viruria.\(^{70}\)

**Summary and Future Directions**

BK virus continues to remain a challenging clinical problem in kidney transplant with a fairly significant rate of graft loss secondary to BKV nephropathy. Better surveillance with early diagnosis and treatment can prevent potential graft loss.

Recent advances in studies of BKV-related disease in kidney transplant have focused on monitoring BKV-specific cell-mediated immune response and its potential clinical applications, including predicting disease progression and resolution, differentiating between rejection and BKV infection, vaccine development, and providing a guide for treatment. Recently, interest has been directed at investigating the role of BKV miRNA in regulating virus replication and as a marker for disease severity. Newer antiviral drugs like brincidofovir have shown promise in BKV treatment in isolated case reports. Fluoroquinolones, once thought to be effective prophylactic agents, have failed to demonstrate efficacy to prevent BK viruria in a recent randomized controlled trial.

Current treatment recommendations are based on immunosuppression reduction; however, there is no consensus on the perfect strategy, thereby providing an opportunity for a multicenter randomized controlled trial of the common treatment strategies.

**References**


