BK Virus and Current Immunosuppressive Therapy

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BK virus reactivation and disease is emerging as an important cause of allograft dysfunction and loss, and the incidence of BK virus reactivation and disease appears to be rising. This has been attributed to the recent use of newer, highly potent immunosuppressive agents. Little is known about the biologic behavior of the virus after reactivation and its progression to disease. Also, no accepted approach to deal with the issue of BKV exists. The authors present results of an interim analysis of their ongoing prospective, randomized controlled trial of renal transplant recipients randomized to receive either tacrolimus or cyclosporine, to determine the incidence of BK virus reactivation and disease, as well as the preliminary results of a management strategy centered around preemptive reduction of immunosuppression upon detection of BK viremia in an attempt to prevent BK nephropathy. Their findings suggest that it is the overall intensity of immunosuppression, and not individual agents, that determines BKV reactivation. Active surveillance is useful because on detection of viremia, preemptive reduction of immunosuppression has led to clearance of viremia in almost 90% of cases with no progression to disease thus far.

Introduction

BK virus (BKV) is a nonenveloped DNA virus from the *Papovaviridae* family. It was discovered in the urine and ureter of a renal transplant patient with uretral stenosis in 1971.[1] The initials of the patient's name were B. K.—thus the name *BK virus*. It is a very successful pathogen of humans; a vast majority of the general population is exposed to it in childhood. Up to 90% of adults have serologic evidence of exposure.[2-5] Despite this wide exposure, the infection is of no consequence to the immune-competent host: if the immune system is compromised, though, then the BKV can cause disease.

Renal transplant patients are treated with immunosuppressive medicines and are at risk for BKV disease. Interestingly, for many decades after discovery, BKV disease of the renal transplant patient remained something of a novelty. In recent years, it has been seen more frequently and has been implicated as a cause of interstitial nephritis in up to 5% of renal transplant patients.[6] Unfortunately, once established, the disease results in allograft loss in 45% to 70% of patients.[6,7] Consequently, there is great interest in studying the behavior of the BKV and factors affecting its reactivation. As an immunosuppressed state is critical for this disease, our immunosuppressive drugs and strategies are under intense scrutiny as contributory factors. No established antiviral treatment is currently available, making prevention a key consideration. Skillful manipulation of immunosuppression to avoid rejection but prevent infection is probably the best option available for management at this time.

Life Cycle of the BKV

The Primary Infection

Almost everyone is exposed to the virus as a child.[2-5] The primary infection is either completely asymptomatic or takes the form of a mild respiratory illness. As depicted in Figure 1, the virus then

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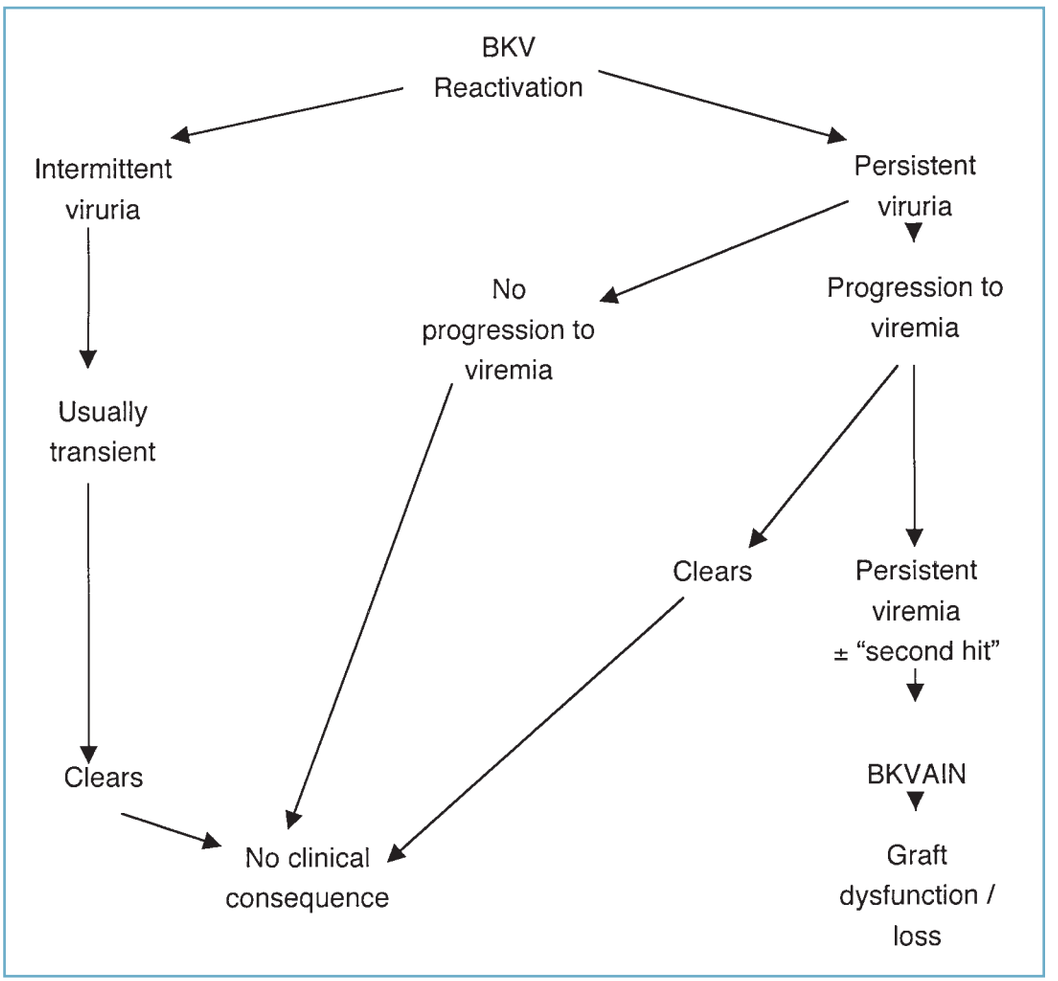

Figure 1. Life cycle of the BKV.

enters a latent state and resides primarily in the kidney: it has been shown to infect renal tubular cells, the parietal epithelial layer of Bowman's capsule, and the transitional epithelium.[8] There is also some evidence that it may hibernate in peripheral blood lymphocytes.[9-11]

Reactivation

"Reactivation" is a state of heightened viral replication in the urogenital tract with virus spillover in the urine.[12] BKV is nonenveloped, and thus its recovery in the urine requires lysis of the host cells.[13] The immune system is very effective in suppressing BKV replication, and the virus seldom reactivates in the immune-competent host. However, even subtle changes in the status of the immune system may be enough to allow reactivation. For instance, BKV reactivation has been documented with pregnancy,[14-16] diabetes,[17] and old age.[18] Nevertheless, clinically important reactivation (and progression to disease) is seen only in states of severe immune deficiency due to hereditary or acquired causes.[19-26]

Reactivation to Disease

Following reactivation, if a high level of replication continues, lysis of tubular cells releases BKV into tubules with bare basement membranes (because of virus damage). It is thought that this re-

sults in leakage of virus particles into the medullary interstitium, from where the virus gains access to the blood.[27] Viremia signifies a state of extremely high virus activity (and therefore failure of immune surveillance), with imminent danger of end organ damage. If allowed to proceed unchecked, this results in virus DNA replication in target cells, target cell destruction, and precipitation of BKV disease. Not only does the BKV have strong tropism for the urogenital tract, but it also displays a predictable disease pattern in different patient populations. For example, BKV-associated interstitial nephritis (BKVAIN) and uretral stenosis in the renal transplant patient and hemorrhagic cystitis in the bone marrow transplant (BMT) patient are well known. This provides an insight (i.e., the immunosuppressed state is critical) but is insufficient in itself to cause disease. A "second hit" is required, probably some form of tissue damage.[28,29] In the case of BMT patients, damage to the bladder by cyclophosphamide may explain BKV tropism for the bladder.[30] Surgical trauma sustained by the ureter may make it susceptible to BKV invasion;[31] repeated immunologic injury such as rejection (clinical or subclinical) may make the transplanted kidney vulnerable to BKVAIN.[29]

Impact of Immunosuppression

BKV infection has been seen more frequently in recent years. There certainly is a bias toward suspecting and diagnosing the disease early given heightened awareness. However, many centers have carried out extensive retrospective reviews of the transplant kidney biopsy archives. Nickeleit et al.[29] did not document a single case of BKVAIN in 616 patients transplanted from 1985 to 1995. They encountered 11 cases from 1996 to 1999. It is now generally accepted that there is a true rise in the incidence of BKVAIN.

A search for new variables that may have altered the course of this infection is therefore natural. Clearly, intensity of immunosuppression is an issue. Thus, although reactivation can occur in states of relative immune deficiency such as pregnancy, diabetes mellitus, or old age, the immune deficit is not critical enough to allow a complete breakthrough of the virus. Actual end organ damage resulting in BKV disease occurs only with more severe forms of immune suppression (such as with HIV or solid organ transplantation). The past decade has seen the introduction of potent immunosuppressive medicines. It is logical to think that these agents are responsible for increased incidence of the disease. A key clinical question, however, is that of asymmetric predisposition: do certain drugs/combinations predispose to BKV in particular, or is the disease related to the overall degree of effective immunosuppression being afforded?

Most case series reviewing BKVAIN have looked at possible roles of particular immunosuppressive agents: tacrolimus has been a prime suspect as a drug with higher potential to cause BKV reactivation. Of 11 patients with documented BKV reviewed by Nickeleit et al.,[27] 8 (73%) were on tacrolimus. Similarly, 20 of 22 (90.9%) patients retrospectively diagnosed in another study were on tacrolimus.[32] Other reviews have made similar assumptions.[33,34] A few factors confound these seemingly obvious associations. The use of tacrolimus probably reflects the prevalent practice in the respective centers; most of these patients were treated for rejection and therefore had received antibody therapy, high-dose steroids, and exposure to very high levels of tacrolimus > 15 ng/ml.[32,34] This indicates a milieu of very intense immunosuppression that may have been responsible for virus reactivation.

In similar fashion, a review of BKVAIN from 1995 to 1998 at the Duke University Medical Center identified 7 cases. It was noted that 6 of 7 patients were on mycophenolate mofetil (MMF) with cyclosporine and steroids.[35] A role of MMF in this infection was thus suggested.[34,35] Another report implicated sirolimus in a case of BKVAIN; the patient got better once the sirolimus was tapered down.[36]

Prospective Evaluation of BKV Infection in Renal Transplant Patients

Our current understanding of the interplay between immunosuppressive regimens and BKV disease is based on studies outlined above. These studies are remarkable in the insights they provide into this quite complex problem. However, they do have the disadvantage of being retrospective in their design.

To settle the issue of the role these newer and more potent immunosuppressive agents may play

in asymmetrically predisposing to BKV, we have been involved in a prospective, randomized trial of adult renal transplant patients. Patients receive either tacrolimus or cyclosporine A in addition to other immunosuppression, as per our protocol at the Washington University/Barnes-Jewish Hospital Transplant Center. BKV is detected using a modified light-cycler-based real-time polymerase chain reaction (PCR) technique. All patients are screened for the virus in urine and plasma prior to transplant. After transplant, the urine and plasma are screened weekly for 16 weeks and then at months 5, 6, 9, and 12. The results of the PCR are available in real time, allowing for therapeutic interventions. All positive specimens are quantified to establish viral loads in urine and blood.

This design allows us to prospectively follow the course of a patient after reactivation of the virus. Positive-urine PCR is judged to be evidence of viruria due to reactivation of virus in the urogenital tract. Previous observations have suggested a positive-blood BKV PCR to be suggestive of nephropathy. In one study, all 9 patients with evidence of BKVAIN on histopathology had positive-blood BKV PCR that had turned positive 16 to 33 weeks prior to clinical declaration of BKVAIN.[37] Similarly, in another retrospective study, all 4 patients with histologic evidence of BKVAIN had positive-blood BKV PCR, whereas none of the 16 controls had a positive test ($p < 0.0001$); blood BKV PCR turned positive at a median of 32 weeks before the diagnosis of BKAVIN. Interestingly, blood BKV titers decreased after reduction of immunosuppression in 3 of the 4 patients.[38] We therefore believe patients with positive-blood BKV PCR are in imminent danger of developing BKVAIN. Intensive surveillance with results available in real time allows preemptive reduction in the intensity of immunosuppression in an effort to prevent progression from viremia to viruria. At the first positive-blood PCR, the antimetabolite component of the immunosuppressive regimen (azathioprine or MMF) is withdrawn. If viremia fails to clear despite this, the calcineurin inhibitor is tapered to minimum acceptable levels (typically targeting CyA levels around 100–200 ng/ml and tacrolimus levels of 3–5 ng/ml). All patients who develop an unexplained elevation of the serum creatinine undergo a kidney biopsy interpreted by a pathologist blinded to the treatment arms. All biopsies are evaluated for the BKV with light and electron microscopy as well as immunoperoxidase stain employing the mc3 antibody to the large T antigen of the simian virus 40 (SV40).

Preliminary results of the study have been presented in abstract form at the American Transplant Congress 2002.[39] On interim analysis, 28% of patients reactivated the virus in urine at a mean follow-up of 43 weeks after transplant, with no difference in the incidence between the tacrolimus and cyclosporine groups. Patients followed three general trends after reactivation:

1. Intermittent viruria
2. Sustained viruria but no viremia
3. Progression to viremia

We defined *intermittent viruria* as viruria lasting less than 3 consecutive weeks. Patients with this pattern usually had low titers of the virus (average virus loads of 8.9×10^3 copies/ml) and did not progress to viremia. Patients with sustained viruria had higher initial urine virus loads (average first 2-week load of 9.31×10^7 copies/ml).

Of the viruric patients, 42% (12% of the total study population included in the analysis) progressed to viremia. Importantly, no patient developed viremia without having declared viruria first. Average time to develop viremia after viruria was noted was about 33 days. Patients developing viremia tended to show sustained high-level viruria (average urine virus load of 3.05×10^7 copies/ml at the time of detection of viremia). No difference was noted in the tacrolimus and cyclosporine arms. We have not seen an independent effect of MMF on the incidence of viruria. At this time, our data do not show an added risk of viruria or viremia even for the combination of tacrolimus and MMF. However, because the number of patients in this subgroup analysis was low, a type II error cannot be excluded.

Interestingly, we did not see any clinical or laboratory signs of BKV reactivation. No cases of BKVAIN have yet been diagnosed despite an average virus exposure of 38 days in the blood. Importantly, 88% of viremic patients cleared their blood with a decrease in immunosuppression, according to our protocol. Again, blood cleared before the urine, maintaining the rule that there cannot be viremia without concomitant viruria.

Reflections on the Biologic Behavior of the Virus

All observations reflect that the initial site of reactivation is the urinary tract, and the first manifestation of viral reactivation is asymptomatic excretion of the virus in the urine. This is reinforced by the observation that no patients developed a positive-blood PCR without first having a positive-urine PCR; also, after immune modulation, the blood always cleared first and then the urine.

After reactivation, the virus can potentially take three routes: it can clear spontaneously, persist in the urine, or progress to viremia, as depicted in Figure 2. At lower levels of replication, the virus is probably controlled and either is cleared from the urine or persists at low levels, not sufficient to break through into the blood. At higher levels of replication or presumably with higher immunosuppression, the immune system is unable to ward the virus off, and it gains access to the bloodstream. BK viremia thus may serve as a de facto in vivo bioassay system betraying a state of overimmunosuppression. Patients with high-titer persistent nephropathy are at prime risk to develop viremia.

Most retrospective studies have mentioned the importance of a positive-blood BKV PCR vis-à-vis diagnosis of BKVAIN.[36-38] We find that a positive-blood PCR is not synonymous with BKVAIN. It follows that a variable period of sustained viremia is required for BKVAIN to set in, as observed by Nickeleit et al. as well as Limaye et al.[37,38] However, a positive-blood PCR is predictive of a high risk for development of BKVAIN. If steps are taken as soon as viremia declares itself, then the degree of virus replication can be curtailed, minimizing the chances for histologic abnormality to set in. Thus, precipitation of BKVAIN may be avoided in a large majority of patients with positive-blood BKV PCR by timely modification in the intensity of immunosuppression.

Risks of Altering Immunosuppression

Our data support that the best approach for managing BKVAIN probably is an active surveillance for reactivation and a preemptive decrease in immunosuppression to prevent progression from viremia to disease. The obvious risk associated with this approach is underimmunosuppression and risk of rejection. This is of paramount concern and deserves extreme caution and close follow-up of patients. In our study population, despite active preemptive reduction of immunosuppression after detection of a positive-blood BKV PCR, the acute rejection rate remained < 5%.

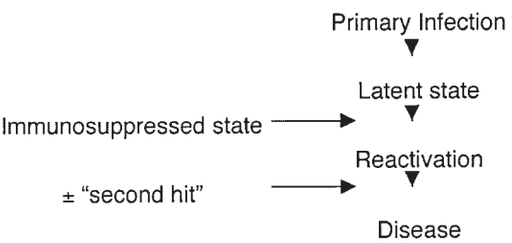

Figure 2. Possible outcomes of BKV reactivation.

Immunosuppression Management for BKV Infection

The most sensitive marker for BKV reactivation is viruria. Therefore, urine PCR or other reliably sensitive tests such as the decoy cell preparation, where available, are reasonable methods to screen for virus reactivation. Once reactivation is discovered, management of immunosuppression is outlined (Fig. 3).

1. Viruria alone is closely followed, but no change in immunosuppression is recommended.
2. Once a patient declares viremia, it is reasonable to suspend the adjuvant agent. If viremia persists, the calcineurin inhibitor should be decreased to minimally tolerable doses and levels.
3. If viremia fails to clear or if allograft dysfunction develops, allograft biopsy is recommended before decreasing immunosuppression further as it is important to establish histologic abnormalities consistent with BKVAIN and to exclude rejection.
4. If histologic evidence of BKVAIN is seen, tapering of the calcineurin inhibitor to lower levels is justified, given the likelihood of progressive renal dysfunction and high rates of graft loss associated with the disease.[6,7]
5. If no histologic evidence of BKVAIN is found, close follow-up is required after alternative diagnoses are entertained.

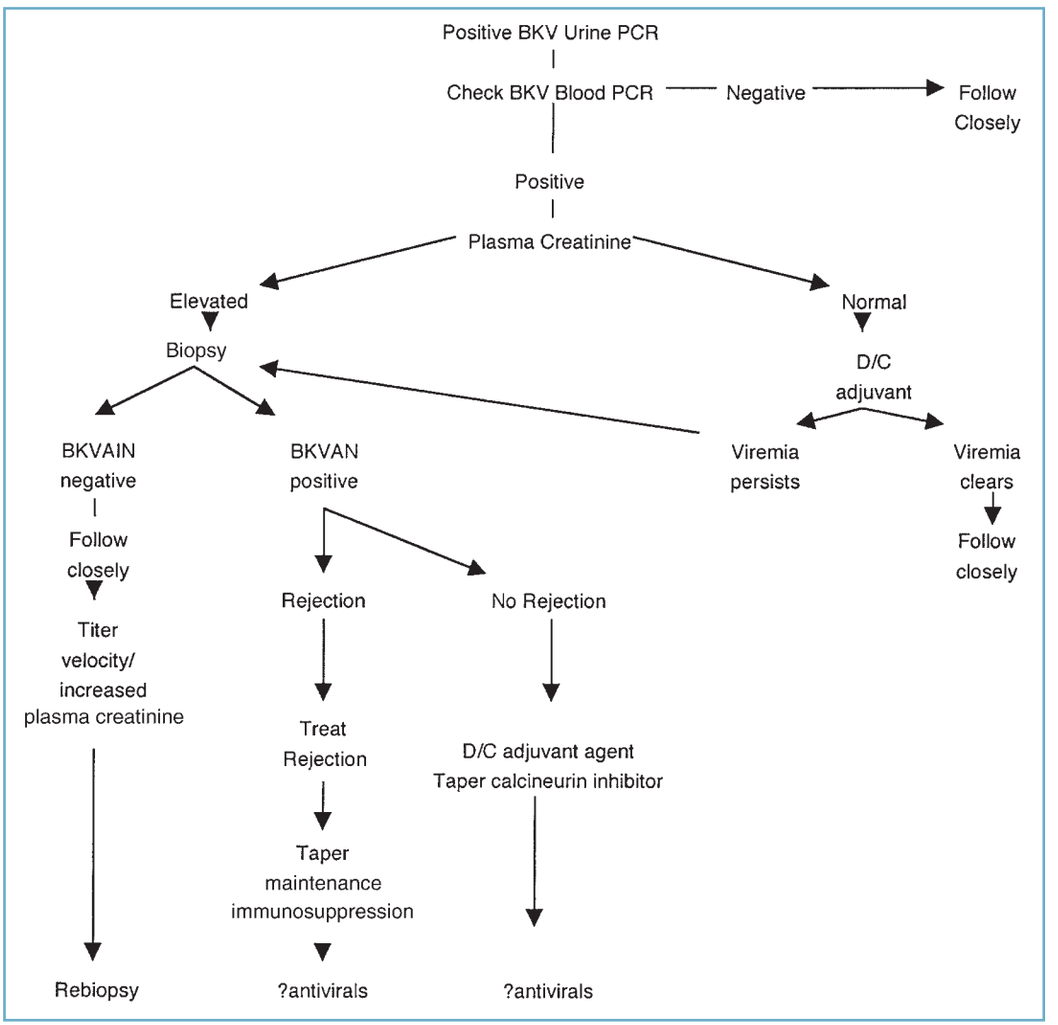

Figure 3. Algorithm for management of BKV infection in the renal transplant patient.

6. Should there be an abrupt rise in the virus titers or further deterioration in renal function, a renal biopsy may be indicated to exclude declaration of BKVAIN: if established, treat with appropriate reduction of immunosuppression as outlined.

The question of coexisting rejection and BKVAIN is perplexing, with the treatment of one potentially aggravating the other. Based on case reports sparingly available in the literature, the best course in this situation is treatment of the rejection first and then appropriate reduction in immunosuppression once the rejection is neutralized. The efficacy and role of antiviral agents are still not established.

Our data do not suggest an asymmetric predisposition to develop BKVAIN with the use of tacrolimus or MMF. These preliminary results also do not find a higher rate of reactivation or disease with the combination of tacrolimus-MMF, although the number of patients is too small to make this conclusion firmly. It appears that the intensity of overall immunosuppression is a key factor in pre-

disposing to BKVAIN. The choice of the immunosuppressive agents currently used probably does not play a determining role in the development of BK viruria or viremia. Rather, each patient appears to be his or her own "in vivo bioassay" for determining what could be considered overimmunosuppression, as manifested by the development of BK viruria and the progression to viremia. Our findings indicate that active surveillance and preemptive reduction in immunosuppression are effective management strategies to deal with BKV in renal transplant patients until suitable antiviral therapy becomes available.

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