The individuality of (virus-specific) CD8 T cells
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BK VIRUS INFECTION IN TRANSPLANT RECIPIENTS: 
CLINICAL MANIFESTATIONS, TREATMENT OPTIONS 
AND THE IMMUNE RESPONSE

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ABSTRACT

Polyomavirus BK (BKV) is ubiquitously present amongst the general population establishing a latent, seemingly asymptomatic infection in immunocompetent individuals. In transplant recipients, however, BKV reactivation is common and can lead to distinctive pathological entities in different patient groups: in renal transplant (RT) recipients, it is associated with nephropathy (BKVN) and ureteral stenosis, and in haematopoietic stem cell transplant (HSCT) recipients to haemorrhagic cystitis (HC). Furthermore, BKV employs several potentially oncogenic mechanisms to promote its replication in cells and has been inconsistently linked to the development of malignancies. BKVN is currently a major cause of allograft failure in RT recipients. HC causes prolonged hospital stay and increased mortality in HSCT recipients. Despite its discovery more than forty years ago, little advances have been made with regard to therapeutic strategies. Current therapies aim to restore the impaired immune response, e.g. by lowering immunosuppressive agents in RT recipients. However, this is a double-edged sword since it also increases the chance on rejection. Therefore, more specific- and effective treatment strategies are urgently needed. Here, we will review the current knowledge on the structure and lifecycle of BKV, characteristics of the BKV-specific immune response, its clinical manifestations and the strengths and limitations of available treatments methods.
INTRODUCTION

In 1971, Gardner and co-workers were the first to isolate polyomavirus BK (BKV) from both urine and ureteral epithelial cells of a Sudanese renal transplant (RT) recipient who presented with renal failure and ureteral stenosis. They named the virus after the initials of this patient. Since then numerous publications on various aspect of this virus have been published.

BKV seems to be ubiquitously present amongst the general population and up to 100% of tested individuals may be seropositive, with peak seroprevalences reported to occur in children and young adults. Up to now, BKV has not definitively been associated with disease in immunocompetent individuals. However, in immunocompromised individuals, the virus frequently reactivates and currently poses a major challenge to transplantation medicine. In this review, several aspects of this virus, such as structure and life cycle, BKV-directed immunity, as well as clinical manifestations and therapeutic strategies are discussed.

STRUCTURE AND LIFE CYCLE

BKV is a small, ~45nm in diameter, non-enveloped DNA virus with a double-stranded circular genome that comprises ~5000 base pairs. BKV shows 70-75% sequence homology to other polyomaviruses such as JC virus (JCV), and Simian vacuolating virus 40 (SV40). The viral capsid is composed of the structural virion proteins (VP) 1, 2 and 3, and accommodates the viral minichromosome. Pentameres of VP1, arranged in an icosahedral lattice form the outer capsid. On the inside, the VP1 pentameres have a central groove in which VP2 or VP3 is inserted. All three structural proteins contain DNA binding motifs.

A simplified visualisation of the virus is presented in Figure 1.

The life cycle of BKV, visualized in Figure 2, is initiated by the binding of VP1 to certain sialic acid motifs on N-linked glycoproteins and/or to gangliosides GD1b and GT1b on the cell membrane. After attachment, BKV traverses the cell membrane by caveolae-mediated endocytosis. Caveolae arise from lipid rafts; plasma membrane regions enriched in cholesterol and the afore mentioned gangliosides. Next, BKV is transported towards the endoplasmic reticulum via microtubules. Disassembly of the outer capsid is essential for the exposure of VP2 and VP3 which mediate entry into the nucleus via importins. The precise mechanism of capsid disassembly is not known but seems to involve an early acidification step and ultimately leads to cleavage of VP1 molecules and capsid rearrangement. The viral minichromosome consists of circular double stranded DNA wound around histones. The BKV genome can roughly be divided into three regions as depicted schematically in Figure 3: the non-coding control region (NCCR), which contains the origin of replication, a bidirectional promoter-enhancer region and binding sites for host transcription factors; the early region, containing genes coding for the Tumour Antigen (TAg) proteins; and the late region, which contains genes coding for agnoprotein and VP1, 2 and 3.
Counter-clockwise transcription of the early region is the first step of replication and leads to the production of the TAg proteins. Three T antigen proteins are produced; large TAg (LTAg), truncated TAg (TruncTAg), and the small T antigen (stAg). Multiple LTAg molecules form a dodecameric complex that binds to the viral origin of DNA replication. Here it acts like a helicase by opening up and unwinding the DNA to initiate clockwise transcription of the late regions. LTAg was also demonstrated to bind to heat shock proteins, members of the retinoblastoma protein family, and p53, as such driving the cell into the S phase and preventing cell cycle arrest. Knowledge on the function of BKV stAg is limited. In mice, stAg of murine polyomavirus (mPyv) complements LTAg in driving cell cycle progression by several mechanisms such as activation of the promoter of the proto-oncogene c-myc. TruncTAg results from alternative splicing of the LTAg transcript and its functions remain to be elucidated. Transcription of the late region genes leads to the production of the structural VP1, VP2 and VP3 proteins, as well as the non-structural agnoprotein. Little is known on the functions of agnoprotein but it seems to mediate assembly of BKV virions. JCV agnoprotein was found to inhibit double-stranded DNA-repair and may as such increase the production rate of more virulent mutant viruses. Intranuclear accumulation of daughter virions ultimately results in rupture of cell membranes, thereby releasing virus progeny into the extracellular space.

**Figure 1. Polyomavirus BK.** The BKV virion consists of the structural viral proteins (VP), VP1, VP2 and VP3. VP1 complexes into pentameres and forms the outside of the capsid. VP2 and VP3, both originating from the same gene, insert into a groove on the inside of the VP1 pentamere. Together with VP1, they bind the viral minichromosome, which consists of circular DNA wrapped around histones.

**IMMUNE RESPONSE**

The precise route of transmission of BKV is still unclear but may involve salivary, faecal and possibly even trans placental transmission. Recently, it was demonstrated that certain defensins, small cationic molecules involved in neutralizing a broad spectrum of
pathogenic microbes, are able to inhibit BKV infection in an early stage of the virus life cycle. The human α-defensin 5 (HD5), which is present in the small intestine and in the urogenital tract, reduces the binding of BKV to the cell surface probably via electrostatic binding to the BKV capsid. Moreover, HD5 aggregates virions, thereby sequestering them away from cells.

Immune and non-immune cells express various receptors that recognize viral nuclear acids and/or viral proteins. Triggering of these sensors induces the production of pro-inflammatory, chemotactic, anti-viral and pro-apoptotic mediators that are crucial for the activation of innate and adaptive immune responses aimed at restricting viral replication. The receptors that recognize proteins and/or nucleic acids of BKV have not been identified thus far, but a recent study showed that BKV infection enhanced expression of the double-stranded RNA sensor, Toll-like receptor 3, the cytokine IL-6 and the chemokine IL-8/CXCL8 in renal collecting duct cells. We observed that expression of TLR3 and the cytosolic dsRNA sensors MDA5 and RIG-I in kidney transplant biopsies was enhanced during BKV infection. These findings raise the question to what extent cytosolic RNA and DNA receptors mediate anti-viral immune responses against BKV.

Using microarrays, the global effects of BKV infection on gene expression have been addressed in human proximal tubular epithelial cells and endothelial cells. In both studies, BKV infection was found to activate genes involved in cell division, DNA replication and apoptosis. Surprisingly, the virus did not promote transcription of pro-inflammatory cytokines, type I interferons or chemokines. In tubular epithelial cells only two inflammatory genes, PTX3 and MICB, were upregulated after infection with BKV. In BKV infected endothelial cells, immunological defence genes, amongst which IL-15, tended to be down-regulated or unaffected. One could therefore hypothesize that BKV employs immunosuppressive strategies by inhibiting the expression of genes involved in the anti-viral response. In line with this hypothesis, induction of IL-6 and IL-8 transcription occurred only within the first 6 hours after BKV infection in collecting duct cells, but was not maintained.

The adaptive immune response and in particular T cells play a crucial role in the clearance of most viral infections. Given the occurrence of BKV-associated disease in transplant recipients and to a lesser degree in HIV-infected patients, T cells are likely to be important. Indeed, BKV-specific T cells are detectable in the peripheral blood of both healthy individuals and transplant recipients. T cells are directed against epitopes from all viral proteins, except for agnoprotein which does not seem to be immunogenic. T cells specific for the structural proteins VP1, VP2 and VP3 appear prior to T cells that recognize TAg proteins. The latter seem to play a leading role in the control of BKV infection and correlate with a drop in viral load. In vitro analysis of T cell reactivity against overlapping peptide pools suggests that CD4+ T cells dominate over CD8+ T cells. However, this finding might have been biased by the use of peptide pools that favour presentation in MHC class II molecules. BKV-specific T cells can be polyfunctional with regard to production of IFNγ, IL-2, and TNFα. Interestingly, polyfunctional T cells directed against LTAg were less frequently detectable in patients...
with high viral loads and/or BKVN, compared to patients with a rapid viral clearance or no reactivation. In vitro expanded BKV-specific CD4+ and CD8+ T cells appeared to express cytotoxic mediators and could eliminate peptide-loaded targets. Direct ex vivo analysis of the cytotoxic potential of BKV-specific T cells is hampered by their low frequency in peripheral blood. Nevertheless, the availability of BKV-peptide-HLA multimers enables examination of the phenotype of BKV-specific T cells in the future. Notably, several studies revealed a significant cross-reactivity between BKV and JCV multimers.

Development of both cellular and humoral immunological memory is important in the immune response against BKV. As mentioned previously, BKV-specific antibodies are ubiquitously present amongst the population. However, their presence alone does not protect against BKV reactivation, BKVN or HC. Nevertheless, negative or low antibody titers prior to transplantation have been proposed as risk factors for BKV viraemia and BKVN. Also, the report on BKVN in a patient with hyper-IgM syndrome suggests that immunoglobulin class-switching and affinity maturation may be important in the control of BKV infection. BKV reactivation is associated with significantly increased levels of BKV-specific IgG and IgM. Antibodies were neither quantitatively nor qualitatively related to viral load or to recovery from BKVN, indicating the importance of other, and probably cellular immune responses.

BKV persists latently in healthy individuals, thus having developed ways to evade the immune system. One study of particular interest showed that BKV and JCV produce a microRNA (miRNA) that suppresses the expression of ULBP3, a protein recognized by the activating receptor NKG2D present on natural killer cells and CD8+ T cells. Expression of this miRNA reduced the effector function of NK cells in vitro. In the case of SV40, this virus encodes another viral miRNA that accumulates during infection and paradoxically reduces the expression of TAg proteins, apparently without affecting viral replication. This downregulation of TAg proteins was proposed to reduce immunogenicity. Lastly, BKV replication leads to the emergence of ‘quasispecies’; virions with mutated NCCRs and/or structural proteins of which several may be found in a given individual. Selective pressure might lead to the rise of mutants that are capable of evading immunological surveillance.

**CLINICAL MANIFESTATIONS AND THERAPEUTIC STRATEGIES**

The cellular reservoir of latent BKV infection in immunocompetent individuals seems to comprise numerous cell types amongst which cervical squamous epithelial cells, peripheral blood leukocytes, salivary gland cells, prostate glandular epithelial cells, and urothelial cells. BKV was also found in the urine in 7% of healthy individuals, but never in plasma. In immunocompromised patients BKV has been associated with several clinical manifestations amongst which most prominently BKVN, ureteral stenosis and late-onset HC. Also, the association of human polyomaviruses with malignancies remains a topic of ongoing discussion. Other less apparent associations include: encephalitis, retinitis, respiratory tract infections and vasculopathy. In the next paragraphs, we will discuss...
Figure 2. Polyomavirus BK: process of infection. A. BKV attaches to sialic acid motives on N-linked glycoproteins and/or to gangliosides on the cell membrane. B. By caveolae-mediated endocytosis, BKV enters the cell and is transported to the endoplasmic reticulum (ER) via microtubules. C. After internalisation, BKV capsid rearrangement occurs and leads to exposure of VP2 and VP3 D) BKV enters the nucleus via importins where it pirates the cell’s replication machinery. Ultimately, BKV progeny accumulates inducing rupture of the cell and release of daughter virions.

the main clinical manifestations of BKV infection and its possible role in malignancies in more detail. Table I gives an overview of therapeutic strategies available for BKVN and HC.

Nephropathy

BKVN occurs in about 5% of RT recipients, mostly within one year after transplantation. Patients generally do not present with any symptoms other than a decrease in renal function. BKVN is also observed in native kidneys of HSCT recipients, lung and heart transplant recipients, as well as in immunocompromised HIV-infected patients. Even though BKVN is not specifically monitored in these patients, its prevalence seems to be lower.

Development of BKVN has been associated to specific immunosuppressive agents such as the calcineurin inhibitor tacrolimus (TAC), the ionosine monophosphate dehydrogenase
<table>
<thead>
<tr>
<th>Intervention</th>
<th>BKVN</th>
<th>HC</th>
<th>Proposed mechanism of action</th>
<th>Reported adverse events</th>
<th>Effectiveness</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tapering immune suppression</td>
<td>Yes</td>
<td>No</td>
<td>Reconstitution of immune responses directed against BKV</td>
<td>Rejection of the allograft kidney</td>
<td>Effective</td>
<td></td>
</tr>
<tr>
<td>Cidofovir</td>
<td>Yes</td>
<td>Yes</td>
<td>Inhibitor of viral replication, mechanism unknown</td>
<td>Severe uveitis anterior, potentially nephrotoxic</td>
<td>Doubtful</td>
<td></td>
</tr>
<tr>
<td>Leflunomide</td>
<td>Yes</td>
<td>Yes</td>
<td>Pyrimidine depletion</td>
<td>Thrombocytopenia, (haemolytic) anaemia and thrombotic microangiopathy</td>
<td>Doubtful</td>
<td></td>
</tr>
<tr>
<td>IVIg</td>
<td>Yes</td>
<td>Yes</td>
<td>Antibody-mediated neutralisation of BKV</td>
<td>Paradoxical increase in viral load</td>
<td>Doubtful</td>
<td></td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>Yes</td>
<td>Yes</td>
<td>Inhibition of large T antigen helicase activity</td>
<td>None</td>
<td>Doubtful</td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>No</td>
<td>No</td>
<td>Prevention of caveolae-mediated endocytosis</td>
<td>None</td>
<td>Not effective</td>
<td></td>
</tr>
</tbody>
</table>
inhibitor mycophenolate mofetil (MMF), therapy with polyclonal anti-T cell antibodies, and number of corticosteroid pulses given for the treatment of rejection $^{56,81-84}$. Other studies suggest that the cumulative intensity of the immunosuppressive regimen rather than one specific agent increases the risk for BKVN $^{85,86}$. Altogether, it remains unclear whether development of BKVN is attributable to qualitative and/or quantitative differences in immune suppression.

Given the strong association with renal allografts, kidney damage may be involved in the development of BKVN. Indeed, mPyv was found to reactivate and replicate to a significantly higher degree in damaged mouse kidneys $^{87}$. However, transplantation factors leading to graft damage, such as cold ischaemia duration and donor origin (living or non-living) have been inconsistently associated with BKVN in humans $^{88,89}$. The immune system is pivotal in keeping BKV at bay but may also contribute to the pathogenesis of BKVN. In one study, detectable circulating BKV-specific CD8+ T cells were observed in two out of 15 RT recipients with particularly high plasma BKV viral loads. Interestingly, those two patients were the only ones who lost their grafts $^{90}$. Other immunological factors involved in the development of BKVN might be allo-HLA-reactivity and heterologous immunity, the latter concerning T cells that cross-react to both BKV- and allo-antigens. Furthermore, one could reason that allo-HLA molecules presenting BKV-peptides are not recognized by host BKV-specific effector-memory T cells, thereby at least temporarily allowing BKV to escape immunological surveillance. In this regard, murine renal allografts were indeed found to be more susceptible to mPyv infection than isografts $^{91}$. Murine Pyv infection led to an increase in allo-reactive T cells that, however, lacked cross-reactivity to the virus. The authors propose that virus-induced allograft inflammation and a subsequent increase in donor antigen presentation might explain this finding $^{91}$. On the other hand, CD4+ T cells with cross-reactivity against BKV VP1 and allo-HLA antigens have been observed in the human situation $^{90}$. The number of HLA-mismatches and rejection episodes are also reported inconsistently as risk factors for the development of BKVN in human RT recipients $^{56,81-83,88,89,92-99}$.

Lastly, viral factors have been proposed as the cause of BKVN. NCCR and/or capsid mutants may enhance BKV virulence $^{65,100,101}$. Indeed, in RT recipients with overt viral activity, i.e. viraemia and BKVN, more mutants were detected $^{65,102}$. However, extensive virus replication would logically lead to the emergence of more mutants, therewith confounding associations with clinical disease severity. Of specific interest is the report on more cytopathology in kidneys infected with multiple NCCR mutants $^{102}$.

The renal disease spectrum seems to begin with viruria and ends with extensive irreversible kidney damage and graft failure. It is therefore of paramount importance to intervene in an early phase to prevent graft loss. Screening for active BKV replication may involve the detection of viral DNA by quantitative PCR in urine and in blood. Monitoring of the urine may also comprise the detection of BKV-infected ‘decoy-cells’ or aggregates of BKV virions, the so called ‘haufen’. Solitary point prevalence measurements of urinary BKV viral load, positive in 20-57% of RT recipients $^{89,103}$, and/or decoy-cells, positive in 13-42% of renal RT recipients $^{76,104}$, were found to have low positive predictive value for
the development of BKVN\(^{56,76}\). However, sustained viruria as well as the presence of haufen were found to have higher predictive value\(^{94,104,105}\). Viraemia appears to occur only in immunocompromised patients, with 7-29\% of RT recipients showing BKV-viraemia at least once after transplantation\(^{84,89,94}\). Moreover, viruria always precedes viraemia\(^{94}\). As such viraemia seems to reflect a state of more elaborate infection. Indeed, high plasma viral loads and sustained viraemia were found to be even better predictors for the development of BKVN than the presence of viruria\(^{76,94,106}\).

Ultimately, BKVN is a histopathological diagnosis. Histopathological grades of severity have been defined ranging from stage A: viral cytopathic changes of near-normal renal parenchyma and no or minimal tubular atrophy, interstitial fibrosis or inflammation, to stage C: Diffusely scarred renal tissue with extensive tubular atrophy, interstitial fibrosis and inflammation\(^{107}\). Since BKV affects the kidney in a random, multifocal manner, false-negative biopsy results may occur, especially in an early stage of disease.

Currently, reducing immunosuppression is the only established mode of therapy and aims to restore the anti-viral immune response. Graft loss due to BKVN is significantly higher in RT recipients with BKVN than in control RT recipients, and may be especially high when tapering of immunosuppression is not applied\(^{92,108}\). The combination of regular screening for BKV replication and subsequent pre-emptive adjustment of immunosuppressive therapy seems to be particularly effective\(^{75,94,104,106,109,110}\). Given the lack of an evident link between one specific immunosuppressive agent and the development of BKVN, there is no standard strategy for adjusting immunosuppressive therapy. Ex vivo and in vitro analyses with different immunosuppressive agents revealed that BKV-specific T cells were particularly inhibited by TAC and not so much by MMF or prednisone\(^{111}\), indicating TAC as a first target of modification. Upon in vitro infection, BKV activates the intracellular PI3K/Akt/mTOR pathway. Subsequent titration with sirolimus reduced LTAg expression in a dose-dependent manner\(^{112}\). Another option may therefore involve the use of the mTOR-inhibitors sirolimus or everolimus, which also did not inhibit interferon-\(\gamma\) (IFN\(\gamma\)) production by BKV-specific T cells in vitro\(^{111}\). However, so far few and conflicting reports on the clinical efficacy of mTOR inhibitors in treating BKVN have been published\(^{113-116}\).

Beyond tapering and/or altering immunosuppression, other anti-viral agents have been proposed. Cidofovir, known to be nephrotoxic, showed in vitro inhibitory activity against polyomaviruses\(^{117}\). However, since polyomaviruses do not express the known target of cidofovir, viral DNA polymerase, its mechanism of action is unknown. Several studies proposed to administrate cidofovir simultaneously to reducing immunosuppressive agents\(^{118,119}\), or when the latter alone proved ineffective\(^{120,121}\). Unfortunately, randomized controlled trials are lacking and several confounders, amongst which most importantly the concomitant tapering of immunosuppression, complicate the interpretation of the effectiveness of cidofovir. Although long lasting nephrotoxic effects have not been reported, severe anterior uveitis occurred in up to 7\% of patients, sometimes leading to permanent visual impairment\(^{119}\). Treatment with intravenous immunoglobulins (IVIg) might help to neutralize BKV particles\(^{122,123}\). Nevertheless, IVIg was recently associated with a paradoxical increase in BKV viral load rather than a decrease\(^{124}\). The pyrimidine synthesis
inhibitor leflunomide may also be effective by inhibiting tyrosine kinase activity and by
inducing pyrimidin depletion\textsuperscript{112,125-129}. Apart from a doubtful clinical effect, leflunomide
also has a high rate of side-effects such as (haemolytic) anaemia, thrombocytopenia and
possibly also thrombotic microangiopathy\textsuperscript{127,128}. Fluoroquinolones have been described to
inhibit LTAg helicase activity. Also here, the reports on clinical efficacy are contradicting
each other\textsuperscript{130-132}. Lastly, one study reported that statins inhibit the formation of caveolae
and as such may block virus cell entry\textsuperscript{133}.

Ureteral stenosis

The original patient B.K. presented with a stenosis of his graft ureter. On further examination,
a segment of the ureter appeared to be ischaemic and fibrotic, and large numbers of
virions were observed in epithelial cells lining the ureter lumen\textsuperscript{[1]}. Other publications
on supposedly BKV-related ureteral stenosis in RT recipients followed, reporting
a prevalence of 2-6\textsuperscript{%}\textsuperscript{84,134-136}.

There has been discussion on the association of BKV with ureteral stenosis. However, its
prevalence was found to be significantly higher in RT recipients who developed viraemia
than in patients who did not\textsuperscript{84}. Apart from a proposed role for BKV in a reversible form of
ureteral stenosis in HSCT recipients with haemorrhagic cystitis\textsuperscript{137}, to our knowledge BKV
as the cause of irreversible ureteral stenosis has not been reported in non-renal transplant
patients. Treatment generally consists of temporary percutaneous nephrostomy and
the concomitant lowering of immunosuppressive agents.

HAEMORRHAGIC CYSTITIS

BKV-reactivation is common in HSCT recipients; viruria and viraemia, occurring at least
once during follow-up in respectively 47-94\% and in 23-53\% of recipients\textsuperscript{138-140}. HC occurs
mainly in HSCT patients and can be caused by conditioning, e.g. with cyclophosphamide
and total body irradiation, but also by several viruses amongst which CMV, adenovirus
and indeed BKV. The virally induced form of HC usually occurs after engraftment and is
therefore referred to as late-onset HC, which occurs in 6-29\% of HSCT patients, generally
within the first two months after transplantation\textsuperscript{140,141}.

Patients present with haematuria, painful voiding, bladder cramps, and/or flank pain.
Four degrees of disease severity are currently recognized: grade I: microscopic haematuria,
grade II: macroscopic haematuria, grade III: haematuria and clots, grade IV: haematuria
with clots, clot retention and renal failure due to obstructive nephropathy. More often,
late-onset HC is of higher severity grade than early-onset HC\textsuperscript{140,142}. Bleeding may be so
severe that patients require red blood cell and/or platelet transfusion, ultimately even
necessitating cystectomy in some refractory cases\textsuperscript{143,144}. Not only was HC reported to
prolong hospital stay\textsuperscript{145}, it also seems to have a significant negative effect on overall
patient survival\textsuperscript{146,147}.

Also here, the connection of BKV to late-onset HC remains a topic of discussion. Various
groups found an association with solely BKV reactivation, defined as detectable virus in
Figure 3. Genomic structure of polyomavirus BK. The non-coding control region (NCCR) contains a bidirectional promoter/enhancer site to which host transcription factors can bind. First, the early region is transcribed to produce the tumour antigen (TAg) proteins. Twelve large TAg proteins then complex into a helicase-like structure that attaches to the NCCR and subsequently initiates transcription of the late region which contains genes encoding agnoprotein and the structural viral proteins (VP) 1, 2 and 3.

urine and/or blood \(^{146,148-150}\), whereas others could relate HC only to very high urinary BKV viral loads \(^{140-142,151}\). Of specific interest is one study reporting on a positive correlation between the degrees of viruria and haematuria \(^{139}\). Lastly, with 81-100% of late-onset HC patients having viruria and 75% viraemia \(^{138,140,142,146}\), BKV viral replication seems to occur more frequently in these patients than in HSCT patients in general. Together these studies suggest that BKV reactivation contributes to the pathogenesis of late-onset HC.

The pathogenesis of HC has been proposed to involve two steps: I. Severe immune suppression together with urothelial damage due to conditioning and irradiation, creating an environment favourable for viral replication, as well as leading to an increase in immunological danger signals and antigen presentation; II. Attack of virus-infected host urothelial cells by donor T cells \(^{152}\). In support of this theory, BKV-associated HC patients showed more signs of immune hyperactivity such as graft versus host disease (GVHD) than patients with adenovirus-associated HC \(^{140,150,152,153}\). Other inconsistently reported risk factors include donor origin, i.e. cord blood or haploidentical graft, NCCR mutants, treatment with antithymocyte globulins, full conditioning instead of reduced intensity conditioning, and conditioning with busulphan \(^{140,145,148,149,152,154}\). Taken together, the pathogenesis of HC is complex but may very well involve immune reconstitution rather than immune suppression.
Due to the risk of GVHD and to the possibility that HC is caused by immune reconstitution, tapering of immunosuppressive therapy is an unattractive treatment option in this clinical context. In many patients, symptoms can be relieved by (intravenous) hydration. Cidofovir has been proposed in the treatment of HC, especially since it may also be given locally by bladder instillation, thereby reducing (cumulative) nephrotoxicity of cidofovir alone or in addition to the several other nephrotoxic agents often used in treating HSCT patients. Interestingly, both patients treated with cidofovir and patients treated only with supportive care achieved remission. The self-limiting nature of late-onset HC, apparently occurring in a significant part of patients, has been confirmed by other studies. Remission of symptoms varied from two to seven weeks after haematuria, and did not differ significantly in duration between cidofovir- or supportively treated patients. With regard to other virus-targeting strategies, prophylactic treatment with ciprofloxacin led to a significant reduction in the occurrence of HC in one retrospective analysis, but not in another. To our knowledge, no publications have addressed the use of leflunomide or IVIg in the treatment of BKV-associated HC. Taken together, it seems that treatment of BKV-associated HC should mainly be supportive. Supportive treatment strategies, not directly targeting the virus, are beyond the scope of this review and have been reviewed in detail by Harkensee and co-workers.

**BKV AND MALIGNANCY**

BKV has been associated with several human neoplasms, amongst which bladder- and prostate carcinoma, brain tumours, tumours of pancreatic islets, Kaposi sarcoma, Ewing sarcoma and osteogenic sarcoma. Of specific interest are the cases of a simultaneous pancreas en kidney transplant recipient with BKVN and a metastasized bladder carcinoma, and a kidney transplant recipient without BKVN who developed a renal cell carcinoma. In each of them, the primary tumours as well as the metastases contained BKV DNA and expressed high amounts of LTAg. Nevertheless, numerous other studies did not find BKV to be related to malignancy.

In rodent cells, BKV drives malignant transformation in vitro, giving rise to full blown tumours after subsequent inoculation back into the animals. Furthermore, the majority of transgenic mice expressing a single copy of the BKV early region developed renal and lymphoid malignancies. Human embryo fibroblast- and foreskin cells, however, were not inclined to such transformation, possibly owing to the presence of specific human tumour suppressor genes.

The previously mentioned actions of LTAg and of agnoprotein would render an infected cell less capable of arresting the cell cycle for DNA repair and may drive a cell towards a continuously dividing state. Not only does BKV benefit from the ensuing increase in host-derived transcription factors, it can thereby also contribute to malignant transformation. In permissive cells, BKV infection results in either cell lysis, leading to release of viral particles, or latency, which is characterized by low expression of viral genes and immune evasion. Infection of non-permissive cells may lead to an aberrant form of replication with continuous expression of only the early region of the BKV genome. In these cells,
TAg proteins accumulate, which may ultimately result in malignant transformation. Interestingly, BKV species with mutated NCCRs were found to possess altered replicative and/or transforming capabilities.\textsuperscript{167,186-189}

In conclusion, BKV possesses oncogenic potency and is theoretically able to at the least contribute to malignant transformation of cells. A definitive association with specific human malignancies remains to be proven. For a more in-depth review on this topic, we refer to the publication of Abend and co-workers.\textsuperscript{190}

CONCLUSION

BKV is ubiquitously present amongst the general population. When immunological surveillance is hampered, BKV reactivates and causes BKVN and/or ureteral stenosis in RT recipients, and late-onset HC in HSCT recipients. Treatment options targeting viral replication are still limited. The most effective therapy in RT patients is improvement of the host immunological defence by lowering immunosuppressive drugs. In refractory cases of BKV-associated disease, antiviral agents such as cidofovir, leflunomide, IVIg and fluorochinolones may be applied. The effectiveness of these agents is, however, doubtful and some of them can cause severe side-effects. BKV has also been implied to be involved in human malignancies, yet its precise role remains to be elucidated. All compartments of the immune system seem to be involved in keeping BKV at bay, virus-specific T cells being of particular importance. In order to develop novel effective treatment strategies and vaccines, more research towards the characteristics of the BKV-specific immune response is necessary.

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