BK polyomavirus: virus-cell interactions, host immune response, and viral pathogenesis

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Abstract. The BK polyomavirus (BKPyV) is one of the main human polyomaviruses. After primary infection, it establishes a persistent infection, and acts as an opportunistic pathogen, innocuous in immunocompetent hosts, but causing potentially serious pathology in the context of immunosuppression, in particular in kidney and hematopoietic stem cell graft recipients. Much progress has been made in recent years in the description of virus-cell interactions, but many aspects of viral physiopathology remain mysterious, principally due to the asymptomatic nature of infection in immunocompetent individuals and the lack of an animal model. The characteristics of the antiviral immune response are beginning to become more clearly understood, particularly in kidney transplant patients. Work in these areas is important in order to identify patients at high risk of developing a severe infection. Indeed, in the absence of an effective antiviral therapy few therapeutic options are available, and patient management remains based on modulation of immunosuppressive therapy.

Key words: BK polyomavirus, immune response, renal transplantation

Introduction

BK polyomavirus (BKPyV) was, along with the JC polyomavirus (JCPyV), one of the first two human polyomaviruses to be identified. It was isolated in 1970 from the urine of a kidney transplant recipient. The patient’s initials were used to name the virus, while the isolate itself was named the Gardner strain, after the lead investigator [1]. BKPyV is a member of the Polyomaviridae family, which has recently been expanded to include 12 new human viruses, discovered since 2007 (table 1). BKPyV is a typical example of an opportunistic virus, which provokes overt pathology almost exclusively in the context of immunosuppression. It causes tubulo-interstitial nephropathies referred to as polyomavirus-associated nephropathy (PVAN), particularly in kidney transplant recipients, and is associated with hemorrhagic cystitis in hematopoietic stem cell recipients. As the number of graft recipients continues to expand, aided by the development of new immunosuppressive treatments, BKPyV is set to remain an important source of morbidity in these patient populations, since no specific antiviral treatment is available for BKPyV infection. Work over the last decade has significantly advanced our understanding of mechanisms by which this virus interacts with its host. However, due to the lack of a suitable animal model, many gaps remain, particularly in relation to pathogenesis at the level of the whole organism.

The aim of the current review is to summarize recent advances in BKPyV research, with particular attention to host cell-virus interactions and the antiviral immune response. Current recommendations concerning the management of BKPyV infections in renal transplantation will also be covered.

Structure and genome organization

The Polyomaviridae are small, non-enveloped viruses with a diameter of 40-45 nm. The viral genome is double-stranded super-coiled circular DNA that, together with the host-cell histone proteins H2A, H2B, H3 and H4, makes up a mini-chromosome. Like all small virus genomes of, it is organized so as to maximize the coding capacity in a minimum of space (5 kb), using alternative splicing and alternative translation start codons to express six distinct viral proteins from two primary RNA transcripts. The genome is divided into three functional regions: the
Table 1 Human polyomaviruses.

<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviation</th>
<th>Pathology</th>
<th>Biological compartment for virus detection</th>
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<tr>
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<td>BKPyV</td>
<td>Tubulo-interstitial nephropathy, hemorrhagic cystitis, ureteric stenosis</td>
<td>Kidney, urine</td>
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<td>KIPyV</td>
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<td>Respiratory secretions</td>
<td>[131]</td>
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<tr>
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<td>WUPyV</td>
<td>?</td>
<td>Respiratory secretions</td>
<td>[132]</td>
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<td>Merkel cell carcinoma</td>
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<td>Urine</td>
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<td>Faeces</td>
<td>[137]</td>
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<td>Faeces</td>
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<td>?</td>
<td>Digestive tract</td>
<td>[139]</td>
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<td>NJPyV</td>
<td>Myositis</td>
<td>Endothelial cells (muscle biopsy)</td>
<td>[140]</td>
</tr>
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</table>

non-coding control region (NCCR), and two protein coding regions known as the early (coding for the T-antigens), and late regions (coding for the agnoprotein, VP1, VP2 and VP3). The early and late regions are transcribed in opposing directions from the NCCR (figure 1).

The non-coding control region (NCCR)
The NCCR is defined as the sequence between the initiation codon of the large T antigen (TAg) of the early region, and the initiation codon of the agnoprotein in the late region. It is one of the principal determinants of host specificity in the Polyomaviridae. In the case of BKPyV, the NCCR is divided into five sequence blocks: O (142 bp), P (68 pb), Q (39 pb), R (63 pb) et S (63 pb) [2]. The O sequence contains the virus genome’s origin of replication and the transcription initiation site for the early genes, symmetrical sequences which are specifically recognized by the TAg, and an NF-kB binding site. This part of the NCCR is highly conserved. The P-Q-R blocks constitute an enhancer region, and carry consensus motifs for the binding of host-cell transcription factors. Many putative transcription factor-binding sites have been identified by bioinformatics analysis, not all of which have been confirmed experimentally. In particular, the P-block contains a cAMP responsive element (CRE); the P-Q junction carries a GC-rich region and Sp1 binding sites; while several binding sites for the NF1 and Ets1 transcription factors are found throughout the P, Q, and R blocks. Sp1 and Ets-1 could play key role in the regulation of viral gene transcription [3]. Finally, the distal region of the NCCR, the S-block contains an estrogen response element, and the initiation site of late gene transcription. The NCCR can accumulate insertion-deletion mutations that distinguish wild-type, or “archetype” BKPyV strains (NCCRww) from rearranged strains (NCCRrr). The archetype NCCRww is found in the urine of both healthy individuals and patients with active BKPyV replication, and is generally considered to be the form that is transmitted between hosts. In patients with prolonged viremia, characteristic of an uncontrolled infection, the frequency at which NCCRrr sequences are detected increases over time, and can eventually dominate the viral quasi-species [4]. NCCRrr sequences have been found in kidney biopsies, urine and blood cells [5-7]. Different NCCRrr strains have been detected in urine or biopsies from kidney transplant recipients, and different forms can coexist in the same patient [8]. In vitro, repeated passage of archetype strains isolated from urine samples resulted in the emergence of NCCRrr variants [9], indicating that NCCRrr viruses have an increased replicative capacity in cell culture. According to Gosert et al., NCCRrr viruses are also associated with higher plasma viral loads and more severe histological lesions than archetype viruses [4]. The same group
developed an experimental model to determine the relative levels of early and late transcription from a given NCCR sequence, leading them to conclude that different NCCRrr sequences have different phenotypes: certain NCCRrr are associated with stronger early transcription, and weaker late transcription, others direct moderate levels of both early and late transcription, while a third group of NCCRrr sequences are characterized by weak early transcription and moderate late transcription [3]. The absence of host control of infection in immunosuppressed patients could therefore favour the emergence of NCCRrr forms which replicate more rapidly, thus leading to a positive-feedback loop that amplifies viral pathogenicity.

The early region

The early region codes for the Large T- and small t-antigens (respectively, TAg and tAg), as well as a truncated T-antigen (trucTAg). They are produced by alternative splicing of the early transcript, and share the same 81 N-terminal amino acids. The T-antigens, particularly TAg, are multifunctional proteins that play a major role in the viral replication cycle. They are involved in replication of the viral genome, late gene transcription, and interact with many cellular factors that regulate the cell cycle and apoptosis. Their roles will be explained more precisely in the following paragraphs.

The late region

The late region codes for the capsid proteins VP1, VP2 and VP3, which are also products of alternative splicing of the same primary transcript. VP1 is the major capsid protein, which constitutes the external face of the BKPyV capsid, while the minor capsid proteins VP2 and VP3 are situated on the internal face of the capsid. VP1 forms pentamers, and one copy of either VP2 or VP3 associates with a VP1 pentamer to form a capsomere, 72 of which form the T = 7 icosahedral BKPyV capsid. Polymorphism in the VP1 gene is used to classify BKPyV into genotypes (I-IV) and, for certain genotypes, into subgroups. The hypervariable sequence is situated in the N-terminal region of the protein, between amino acids...
61-82 that correspond to the BC-loop on the exterior of the capsid that is directly involved in interactions with BKPyV’s ganglioside receptor. The four genotypes also correspond to distinct neutralizing serotypes. Genotypes I and IV are the most frequent in the population, although their relative frequencies vary with their geographical distribution [10, 11]. The robustness of phylogenetic analysis of BKPyV strains can be improved by considering both TAg and VP1 sequences [10].

The late region also codes for the non-structural agnoprotein, large quantities of which are produced during the later stages of the replication cycle (reviewed in [12]). The agnoprotein is a phosphoprotein found in three PyV: SV40, BKPyV and JCPyV, but it is absent from the more recently identified human *Polyomaviridae*. In infected cells, it is localised in the cytoplasm with a perinuclear distribution, and is also associated with the endoplasmic reticulum (ER). It can also be found on the internal face of the plasma membrane. The agnoprotein is phosphorylated on serine-11, and viruses carrying mutations at this position show reduced propagation in cell culture, as do mutant viruses that do not express the agnoprotein [13].

**Viral miRNA**

Like other polyomaviruses, BKPyV codes for a viral miRNA in the 3′ region of the late transcript which is complementary to the mRNAs coding for TAg and tAg [14]. Since the miRNA sequence is exactly complementary to its targets, its production leads to degradation of the early mRNAs. Because the miRNA sequence is identical in JCPyV and BKPyV, this implies that these two viruses would be able to regulate each other’s expression, in the case of co-infection. Both the 3p and 5p miRNAs derived from the pre-miRNA hairpin appear to have specific functions [15]. Recent work has shown that the expression of BKPyV miRNAs is under the control of the sequences in the NCCR, and is correlated with the level of expression of the viral late genes. The same study found that BKPyV miRNAs regulate expression of the early genes before the initiation of genome replication, and therefore act as a brake on viral replication. Interestingly, the reduction of viral replication was only observed in viruses with archetypal, not those with rearranged NCCR. Since the archetypal forms are those that are transmitted in the population, the authors suggest that miRNAs may have a role in viral persistence [16].

**Replication cycle of BK polyomavirus**

The principal steps in the replication cycle of BKPyV are shown in figure 2.

**Host cell receptors, entry and decapsidation**

The gangliosides GD1b and GT1b are required for the attachment and entry of BKPyV into human renal proximal tubular epithelial cells (hRPTECs) and expression of these gangliosides renders cells susceptible to infection [17]. More recently, supplementation of Vero cells with the GD2 and GD3 gangliosides was shown to increase the efficacy with which these cells can be infected with BKPyV. The common structural feature shared by the GD1b, GT1b, GD2 and GD3 gangliosides is an α 2-8 di-sialic acid motif. Crystallographic analysis of VP1 pentamers complexed with GD3 confirmed that the interaction between VP1 and the GD3 ganglioside directly involved the α 2-8 sialic acid dimer [18].

Unlike JCPyV, which is internalised by clathrin-mediated endocytosis, BKPyV enters into Vero cells and hRPTECs via caveosomes [19, 20]. This pathway involves cholesterol-rich membrane microdomains and the formation of endocytic vesicles by the action of caveolin proteins, notably caveolin-1. Electron microscopic analysis of PVAN biopsies showed the presence of virus particles inside vesicles with a morphology consistent with that of caveosomes, indicating that this pathway is also involved in infections in vivo.

After internalisation, BKPyV particles are transported along the microtubule network to the ER [21-23]. Transit via the ER is specific to polyomaviruses – no other nuclear replicating DNA virus uses this route to the nucleus. The interactions between ER proteins and polyomavirus capsids have been studied most extensively in SV40 and murine PyV (MPyV), and it is assumed that similar mechanisms are involved during BKPyV entry. Polyomavirus decapsidation is initiated in the ER, notably by the action of protein disulphide-isomerase, which unlinks the disulphide bridges that form covalent bonds between VP1 pentamers in the capsid [24, 25]. Chaperone proteins (ERP29, BiP, BAP31, DNAJ) allow the minor capsid proteins VP2 and VP3 to become accessible, and this is important for subsequent steps in virus entry. Polyomavirus capsids are then transported from the ER to the cytosol via the ER-associated degradation (ERAD) pathway, involving transmembrane ER proteins of the Derlin family [23, 24]. The ERAD pathway is linked to the degradation of misfolded proteins by the proteasome, and proteasome activity is also required for BKPyV entry. The end result of the interaction between polyomaviruses and the ERAD-proteasome machinery is the retrograde transport of a partially uncoated virus particle into the cytosol. Nuclear localisation signals in VP2/VP3 [26-28] then guide the particle to the nucleus. Further disassembly of the particle may accompany transport through nuclear entry pores [29].
Figure 2. BK polyomavirus (BKPvV) replication cycle.
1. Attachment to host-cell ganglioside receptors. 2. Internalisation by caveolin-mediated endocytosis. 3. Transport of caveosomes to the endoplasmic reticulum. 4. Partial decapsidation and retrograde transport from ER to cytoplasm. 5. Nuclear import of virus genomes through nuclear pores. In the nucleus, replication occurs in structures associating viral genomes and elements of the host cell (PML bodies, DDR). 6. First wave of transcription: early genes. TAg and tAg return to the nucleus, associate with the viral genome and cellular factors involved in viral DNA replication. 7. Viral DNA replication. 8. Second wave of transcription: late genes. 9. Capsid assembly around newly synthesized copies of the viral genome.

Early gene expression

Viral gene expression is controlled by the early and late promoters situated in the NCCR. These promoters are oriented in opposite directions, so that transcription of the early and late regions progresses outwards from the NCCR, using opposite strands of the virus genome as a template.

The early region is transcribed in the hours following the entry of the viral genome into the nucleus. The most abundant early protein, TAg, is a multifunctional protein that interacts both with the viral genome to initiate replication, and with numerous cellular proteins. The structure and function of TAg have been extensively described in the SV40 model, which is highly relevant to BKPvV, since SV40 TAg shares 76% homology with BKPvV TAg. The TAg of all Polyomavirusae contain four conserved regions: the J region, an origin binding domain (OBD), a zinc binding domain, and an ATPase domain. The OBD region recognizes the sequence GAGGC, which is repeated four times in the O region of the NCCR. After TAg binding, the O region also binds replication protein A (RPA). The zinc binding domain and the ATPase domain both contribute...
to the helicase activity of TAg: the zinc binding domain is required for the formation of TAg hexamers, while the ATPase domain provides the energy necessary for the helicase activity [30].

Modulation of the cell cycle and activation of DNA repair mechanisms

TAg forces cells to advance into the S phase of the cell cycle, and activates the DNA damage response (DDR) in order to enhance viral genome replication. The J-domain cooperates with a fifth region of TAg, the LXCXE domain, to bind pRB/p130/p107 and disrupt interactions between pRB and the E2F transcription factors. Release of E2F from pRB permits the formation of E2F-E2F dimers, which can then drive the expression of genes required for progress into the S-phase [30]. Viral genome replication co-localizes with nuclear PML bodies, which are involved in the DDR and gene transcription [31, 32]. BKPyV infection of renal epithelial cells activates protein kinases essential for the DDR: the ataxia telangiectasia, mutated (ATM) kinase, and to a lesser extent, the ATM and Rad-3 related (ATR) kinase. In cells deficient for ATM and ATR, BKPyV provokes major alterations in cellular DNA [33, 34]. Cellular DNA repair mechanisms therefore appear to be important for the replication of viral DNA, and for the integrity of the host genome during infection. Although ATR seems to be responsible for phosphorylation of the SV40 TAg, the precise mechanisms by which BKPyV proteins interact with DDR proteins are not currently known.

The specific functions of tAg include the inactivation of the PP2A protein, which leads to activation of the D1 and A cyclins, and hence to progression into the G1 and S phases of the cell cycle. In contrast, the precise role of the truncated TAg is not known.

Viral DNA replication

Since polyomaviruses do not code for a viral DNA polymerase, TAg orchestrates viral DNA replication by recruiting the multiple cellular proteins that are required for viral DNA replication. After binding to the origin of replication, TAg monomers assemble into two hexamers. After this first step, DNA strand separation is initiated by the helicase activity of TAg then completed by the cellular RPA protein. Next, the cellular DNA polymerase α primase complex synthesizes an RNA primer that is used by DNA polymerase λ. Topoisomerase I is also required in order to maintain a relaxed conformation of viral DNA during its replication (for review, see [30, 35]).

Late gene expression, encapsidation, and release

It is generally considered that late gene expression begins after initiation of genome replication. However, the precise relationship between early gene expression, genome replication and late gene expression in BKPyV appears to be influenced by viral miRNA expression and the nature of the NCCR. Capsid proteins expressed from late mRNAs contain nuclear localisation sequences, and are therefore imported into the nucleus, where they assemble infectious particles. The mechanisms by which BKPyV virions are released from infected cells have not been described in detail, but may involve the viroporin activity of the agnoprotein.

Natural history of BK polyomavirus infection

Transmission

The most frequent mode of transmission of BKPyV is likely to be via the oropharyngeal route. This is supported by the young age at seroconversion (65 to 90% seropositivity before age 10) and the detection of viral DNA in the saliva and oropharyngeal lymphoid tissues [36-38]. The presence of the virus in urine and in the environment is also compatible with oral contamination from these sources [39]. The detection of BKPyV DNA in mononuclear cells indicates that blood transmission is also a possibility. Finally, isolated cases of nosocomial transmission during hematopoietic stem cell (HSC) transplant in a pediatric patient [40] and vertical transplacental transmission [41] have also been reported.

Primary infection, persistence, and reactivation

Primary infection is asymptomatic, or may be associated with signs of non-specific respiratory infection. The virus is assumed to reach its target organs after dissemination via the bloodstream, but there are very few data available during the early stages of infection, due to the rather non-specific nature, or indeed absence, of clinical symptoms. In adults, virus reactivation from sites of persistent infection in the renal-urinary tract is clearly indicated by the detection of BKPyV in the urine both in immunosuppressed patients and, albeit at lower viral loads, in healthy individuals [42, 43]. However, the mechanisms of viral persistence are not clearly understood: does BKPyV exhibit “true” latency, without replication or expression of viral genes, or is a low level of chronic virus replication always present?
Cellular tropism

In vitro, BKPyV can productively infect a number of different cell types including human and simian renal epithelial cells, human fibroblasts, fetal neuronal cell lines, endothelial cells, and epithelial cells from sub-mandibular and parotid salivary glands [36]. In vivo, BKPyV genomic DNA has been detected in many different tissues (renal epithelia, the urothelium, prostate gland, salivary glands, brain...) although renal epithelial cells appear to constitute the main reservoir of persistently infected cells. Viral DNA has also been found in lymphocyte populations in healthy subjects and patients with active BKPyV replication [44, 45], and this is consistent with earlier experiments indicating the presence of BKPyV receptors on the surface of lymphocytes by the formation of rosettes [46]. The role of lymphocytes in the natural history of BKPyV infection has not been clearly described. In the case of JCPyV, the virus can enter B-lymphocytes, and although it does not actively replicate in these cells, virus can then be transmitted to glial cells. This suggests a model in which B-lymphocytes transport the virus across the blood-brain barrier [47]. A similar mechanism in BKPyV primary infection could explain viral dissemination from the initial site of infection to distant target tissues such as the urothelium and the renal epithelia. However, the lymphocyte sub-population carrying viral DNA have not been identified, and there is currently no convincing data to show that BKPyV can replicate actively in lymphocytes, nor has cell-to-cell transmission involving lymphocytes been demonstrated.

Pathogenicity

Hemorrhagic cystitis

BKPyV is one of the infectious agents associated with hemorrhagic cystitis (HC), particularly in the context of HSC graft. HC is characterized by the association of dysuria, pain, and varying degrees of hematuria. In the most severe cases, hematuria can lead to clotting and obstruction of the urinary vessels, hemorrhage, and kidney failure. HC associated with BKPyV is observed in 5-15% of patients within two months after HSC graft [48]. It should be distinguished from other causes of HC in this patient population: drug toxicity in the case of chemotherapy, or other viral infection (human cytomegalovirus HCMV, adenovirus). The pathogenesis of BKPyV associated HC has not been completely elucidated. It appears to be the result of several contributing factors: lesions in the urothelial mucosa secondary to induction therapies; viral reactivation favoured by immuno-suppression during aplasia, and contributing to urothelial lesions; and finally, an inflammatory reaction caused by a lymphocytic infiltrate and destruction of infected cells by cytotoxic T-lymphocytes (CTL) during engraftment [49]. Diagnosis of BKPyV associated HC in the context of HSC graft is based on the detection of BKPyV in the urine. Patients with a high urine viral load, or with viremia are at higher risk for development of HC [50, 51]. Newer graft protocols (cord blood graft, and the use of haploidentical HSC) seem to be associated with higher levels of BKPyV reactivation [52].

BK polyomavirus associated tubulo-interstitial nephropathy

PVAN is a tubulo-interstitial nephropathy that usually occurs within 12-24 months following a kidney transplant. The first stage of this disease is characterized by prolonged and intense viral replication in epithelial cells of the kidney and urinary tract. As viral replication continues unchecked, it induces a marked cytopathic effect involving lysis of epithelial cells and release of virus into the blood due to lesions in the basal membrane. Secondly, inflammatory cells migrate into the interstitium, and this leads to tubular atrophy and interstitial fibrosis [53]. This is accompanied by progressive graft dysfunction, which can be irreversible in later stages of the disease. Urothelial cells are also the site of intense viral replication and are the principal source of virus found in the urine [54].

Although small amounts of BKPyV can be found in circulating lymphocytes, in the majority of patients, viremia is a reflection of intense viral replication in the renal/urinary tissue. The absence of viremia is a strong predictor that PVAN will not occur, and the risk that BKPyV replication will evolve into PVAN seems to increase with higher and more prolonged viremia. A level of 10^4 copies BKPyV DNA copies per mL is an internationally recognized threshold, above which immunosuppression should be reduced [55]. As with many post-transplant viral pathologies, the risk factors for PVAN include factors related to the donor, the receiver, the graft, and the virus itself (figure 3). In the case of BKPyV, therapeutic immunosuppression, which disrupts the equilibrium between virus replication and the host immune response, is the principal factor favouring viral pathogenesis. The rise in frequency of PVAN after kidney transplant coincided with the introduction of more effective immunosuppressive regimens at the end of the 1990s (corticotherapy + antiproliferative agent + calcineurin inhibitor), which highlighted the importance of cellular immunity in the control of BKPyV replication [56, 57]. However, the tissue environment is certainly an important co-factor in BKPyV reactivation, since PVAN is observed almost exclusively in the context of kidney transplantation, and only very rarely in patients receiving other solid organ transplants, or HSC grafts [58]. The specific therapies that have been frequently associated with post-graft viremia...
Ureteric stenosis

BKPyV was initially isolated from a renal transplant patient suffering from a ureteric stenosis, and the virus has been repeatedly implicated in this pathology. It occurs in adult and pediatric kidney recipients and has also been reported following HSC graft.

Other pathologies associated with BK polyomavirus

Isolated clinical cases associating BKPyV with a variety of pathologies have been described in strongly immunosuppressed patients. These include pneumopathies, meningo-encephalitis, and disseminated infections with vascular involvement [59-61].

Recently, BKPyV has been implicated in a salivary gland pathology in human immunodeficiency virus (HIV) seropositive patients characterized by inflammation with lymphocytic infiltration and fibrosis of the salivary glands. The arguments in favour of BKPyV involvement are the detection of higher viral loads in the saliva of patients with this pathology compared to either healthy controls or other HIV-positive individuals, and the capacity of BKPyV to replicate in salivary gland epithelial cells [62, 63].

Oncogenicity

The well-characterized interactions between polyomavirus early proteins and tumor suppressor genes, such as pRB, suggest that BKPyV could potentially be oncogenic. Consequently, several studies have analyzed cancer tissues for the presence of BKPyV, either by PCR or by in situ hybridization. Although the virus genome has been found in tumors, the results remain controversial [64]. For example, BKPyV has been reproducibly detected in prostate tissue, and at a higher frequency in prostate tumors compared to healthy tissue. This observation has led some authors to propose that BKPyV, through the action of TAg and tAg, could be
a factor in the early stages of prostate cancer (for review see [65]). The transforming potential of the viral early proteins has been demonstrated in vitro in non-permissive cells in which an abortive infection occurs, stopping at the early phase of the virus replication cycle. In addition, infection of rodents with BKPyV induces various types of tumor [66]. On the other hand, the arguments in favour of an oncogenic role of BKPyV in its natural host are circumstantial in nature, and there is no formal proof that BKPyV causes cancer in humans. Taking all the existing data into account, the International Agency for Research on Cancer has classified BKPyV and JCPyV as “possibly carcinogenic to humans” (group 2B, [67]).

**Antiviral immune response**

**Innate immunity**

Among the innate effector cells of the immune system, natural killer (NK) cells play an important role in the early control of viral infections [68]. Their functions are controlled by a complex balance between signals transmitted by activating and inhibitory receptors on the NK cell surface. The highly polymorphic Killer cell immunoglobulin-like receptors (KIR receptors), which bind to HLA class I molecules are one such group of NK cell receptors. One recent retrospective study of kidney transplant recipients found a higher number of KIR activating alleles, in particular the activating receptor KIR3DS1, in control patients compared to those who developed PVAN [69]. However, in a previous study of transplant recipients, a protective role for KIR3DS1 was shown for HCMV, but not for BKPyV infection [70]. These results therefore need to be confirmed in a larger patient cohort. Furthermore, results from our group show that polymorphism in the gene coding for the MHC class I polypeptide-related sequence A (MICA) protein, which is a ligand of the activating receptor natural-killer group 2, member D (NKG2D), are associated with susceptibility to BKPyV infection. The underlying mechanism behind this association could involve changes in the level of expression of MICA on the surface of infected cells, and hence to different levels of NK activation according to the MICA genotype [71]. Very few data exist concerning the type I interferon response to BKPyV, either in vitro or in vivo. Some recent results suggest that the inflammation seen in PVAN lesions could be related to activation of intracellular innate immune receptors, such as Toll-like receptor 3 (TLR3) or Retinoic acid-inducible gene-I (RIG-I) [72]. However, the mechanism by which these molecules, that are known to specifically recognize RNA, become activated during PyV infection remains to be clarified. In addition, very few studies have addressed the role of dendritic cells in BKPyV infection. One publication found that the number of circulating myeloid dendritic cells in kidney transplant recipients before graft was lower in patients who developed BKPyV viremia than in those who did not [73].

**Humoral response**

Following the discovery of BKPyV, epidemiological studies employing a variety of serological techniques (inhibition of hemagglutination, neutralization, complement fixation and indirect immunofluorescence) found very high seroprevalence rates for BKPyV infection on every country studied. Indeed, one study of sera from diverse ethnic groups found that BKPyV had a worldwide distribution, with only a few isolated populations living in the Amazon rain forest with seroprevalence rates < 20% [74]. Studies from the 1970s using inhibition of hemagglutination to detect BKPyV-specific antibodies found that seroconversion occurs in developed countries during early childhood, with seroprevalence rates from 35-55% in children 1-5 years old, which rise after school entry. Peak seroprevalence was found in adolescents and young adults, followed by a progressive reduction with age. Similar results have also been found in studies using enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) techniques [42].

More recently, Buck’s group has developed pseudotype viruses composed of BKPyV capsids carrying a reporter gene in place of the viral genome. These biological tools were used to study the neutralizing response against different BKPyV genotypes [75, 76]. In a cohort of healthy adults from North America, more than 80% of sera had neutralizing activity against BKPyV genotype I capsids, while fewer than 50% of sera had detectable neutralizing activity against genotypes III or IV. Different BKPyV genotypes therefore constitute different neutralizing serotypes. Furthermore, the sera of certain individuals neutralized subtype Ib1 capsids, but not the closely related subtype Ib2. Using pseudotype viruses with chimeric Ib1/Ib2 VPI proteins, it was possible to show that differences in the BC-loop were responsible for the extreme specificity of this neutralizing antibody response. As previously noted, sequence differences between BKPyV genotypes are concentrated in the BC-loop, and this explains why the neutralizing antibody response is genotype-specific.

In the case of HSC grafts, the principal source of virus is the reactivation of latent infection in the receiver, which of course occurs predominantly in seropositive recipients. In one study, the titre of BKPyV-specific antibodies in the receiver was positively correlated with viruria [77], suggesting firstly, that the antibody titre may be an indication of some degree of “occult” virus replication, and secondly, that the presence of antibodies does not prevent reactivation. On
the other hand, a second study of pediatric patients found an inverse correlation between anti-BKPyV titre before HSC graft, and BKPyV viremia after graft [78], which indicates a protective effect of the adaptive antiviral immune response. These two conclusions are not necessarily contradictory, however, since viremia was not correlated with viruria in the study published by Wong et al.

In kidney transplant, the situation is more complex, since the virus may be present in both the graft and the receiver. BKPyV infections post-transplant may therefore represent either primary infections (seropositive donor to seronegative receiver), reactivations (seronegative donor to seropositive receiver), or superinfections and/or reactivations (donor and receiver both seropositive). Each of these situations implies a distinct immunological context, and this perhaps explains why the role of the humoral response has not yet been clearly established, despite research on this topic stretching over more than 30 years.

The first study addressing this question, conducted by Andrews et al., found that the combination of seropositive donor to seronegative recipient carried the highest risk of BKPyV infection after kidney transplant [79]. In these first studies, BKPyV replication was not directly detected, but inferred by an increase in the titre of BKPyV-specific antibodies following transplant. In more recent studies, seronegativity of the receiver has been confirmed as a risk factor for the occurrence of viruria, viremia, or PVAN in the context of pediatric kidney transplant [80, 81]. In adult kidney recipients, however, the data are more equivocal.

In one study of donor-receiver pairs the serostatus of the donor, but not that of the receiver was associated with post-transplant BKPyV viruria [82]. On the other hand, a more recent study of 192 donor-receiver pairs found the same association between the seropositive donor/seronegative receiver pairing and increased risk of BKPyV infection – in this case defined as post-graft viremia – as that reported by Andrews et al. thirty years previously [83]. Furthermore, in adult kidney transplant recipients seropositive for BKPyV, two studies found an inverse correlation between the titre of anti-BKPyV antibodies at the time of transplant, and post-transplant virus replication [84, 85]. The majority of case-control studies are therefore consistent with the idea that pre-existing immunity to the virus has a protective role against BKPyV reactivation and super-infection in the context of adult and pediatric kidney transplant.

Another way to assess the impact of the humoral response on viral replication in vivo is to follow its evolution longitudinally following kidney transplant, in parallel with viremia or viruria. For some authors, the increase in anti-BKPyV titres that accompany the resolution of PVAN suggest that the humoral response contributes to resolution of the infection [86], whereas others consider that increases in antibody titres should be considered simply as markers of viral replication [84]. Furthermore, investigators who studied both the T-lymphocyte and antibody responses to BKPyV antigens concluded that the T-cell response, and not the humoral response, was associated with resolution of the infection [87, 88].

**CD4+ and CD8+ T-lymphocyte responses**

Although the Polyomaviridae express at least five immunogenic proteins (TAg, tAg, VP1, VP2 and VP3), the study of the T-cell response has generally been restricted to TAg and VP1. The BKPyV and JCPyV genomes share 70% sequence identity, and there is significant antigenic cross-reactivity between the two viruses with respect to both VP1 and TAg [89, 90]. The minor capsid proteins, in particular VP3 [91], can also be targeted by the T-cell response. Stimulating PBMC with pools of peptides from five BKPyV proteins (tAg, TAg VP1, VP2 and VP3), rather than only VP1 and TAg, significantly increases the probability of detecting a positive response [92].

Overall, the frequency of circulating BKPyV-specific T-cells appears to be lower than the frequency of T-cells specific for other persistent viruses that cause opportunistic infection, such as HCMV or EBV. The low level of the BKPyV-specific response may be explained by the restriction of viral reactivation to the urinary tract, with – unlike HCMV or EBV – little or no dissemination into the bloodstream. In a study of 25 healthy subjects, a positive CD8+ T-cell response was only detected in 60% of cases, despite in vitro expansion of specific T-cells by stimulation with VP1 or TAg peptides and IL-2. VP1-specific T-cells were more frequently detected than TAg-specific T-cells. These CD8+ T-cells were found to have an effector-memory (CD45RA-CCR7-) phenotype and were capable of secreting several cytokines after stimulation [93]. More recently, the relationship between the CD4+ T-cell response against BKPyV and age was studied cross-sectionally in a cohort of 122 healthy subjects. Overall, 34.5% of the study population had a detectable CD4+ T-cell response, with the highest frequency of positive responses (68.4%) observed in young adults 20-30 years old. This age group also showed the highest titres of BKPyV-specific IgG [94].

**Role of BK polyomavirus-specific T-lymphocytes in kidney transplantation**

Over the first months after kidney transplant, in the absence of viral replication, the BKPyV-specific T-cell response remains stable at a low level [95]. However, if BKPyV infection occurs, case-control studies have shown that the BKPyV-specific T-cell response is significantly increased in
patients in the control phase of the infection (that is, when viremia is decreasing), compared to patients with prolonged viremia or histological signs of PV AN \[96, 97\]. The T-cell response therefore seems to mirror viremia, with decreasing viremia coinciding with an increase in the frequency of circulating virus-specific T-cells \[98\]. As is the case in other viral infections, polyfunctional T-cells appear to play an important role in the control of viral replication: in a study from Berlin, polyfunctional (IL-2+/TNFα+/IFNγ+ or IL-2+/TNFγ+) T-cells were more frequently detected in patients who rapidly controlled viral replication, compared to those who experienced prolonged viral infection \[92\].

The level of T-cell responses before transplant does not appear to predict the subsequent course of BKPyV infection. For example, in a cohort of 151 kidney transplant patients, BKPyV-specific IFNγ Elispot responses before transplant did not differ between the 24 patients who experienced subsequent BKPyV viremia, and the 127 patients who did not. However, there was a significant reduction in the frequency of BKPyV-specific T-cells after transplant in patients who subsequently became viremic, which was not observed in the non-viremic group. Therefore, the critical factor seems not to be the level of the T-cell response before transplant, but whether this response is maintained during the early time points following kidney transplant \[99\]. Similarly, in a prospective study of 148 kidney transplant recipients, our group did not find a significant difference in the frequency of CD4+ or CD8+ T-cells specific for VP1 or TAg between viremic and non-viremic patients, although the absolute number of CD8+ T-cells was lower at three months post-transplant in the viremic group.

Mechanisms of immune escape
In the SV40 model, viral miRNA appears to constitute a mechanism of escape from the antiviral cytotoxic T-cell response, by down-regulating the expression of TAg \[100\]. In addition, inhibition of the expression of host-cell ULBP3 by the JCPyV and BKPyV miRNA constitutes an escape mechanism from NK-cell lysis \[101\]. Therefore, the viral riposte to the host cellular immune response seems to be through the viral miRNA, rather than by the expression of viral proteins that interfere with epitope generation or presentation.

Diagnostic markers of BK polyomavirus infection

Serology
Currently, no commercial reagents exist for the detection of circulating BKPyV-specific antibodies. In practice, BKPyV serostatus is not determined before transplant, since greater than 80% of the adult population is seropositive for the virus.

Detection and quantification of the virus genome by molecular biology
BKPyV infection is diagnosed by the detection of virus DNA in urine and blood. BKPyV. The performance of real-time quantitative PCR for BKPyV DNA is generally excellent, both for commercial and “in-house” techniques, but it is difficult to directly compare results from different techniques. This implies that patient follow-up should always be assured by the same centre \[102\].

The quantification of viral RNA by real-time PCR is an alternative to the quantification of viral DNA. However, despite being technically more demanding, this technique has not been shown to improve the diagnosis or follow-up of BKPyV infection \[103\].

Detection of urinary PyV aggregates (“polyomavirus-haufen” test)
Aggregates of BKPyV particles form in the renal tubules, through lysis of infected cells and release of virions into the tubular lumen. Haufen is a German word meaning “aggregate”, and a positive “polyomavirus-haufen” test on a urine sample correlates with the presence of PVAN. The non-invasive nature of the test makes it a useful diagnostic tool for tissue involvement \[104, 105\].

Detection of urinary decoy cells
Decoy cells are dystrophic BKPyV-infected urothelial cells that can be observed after Papanicolaou staining. They are large basophilic cells containing large homogeneous nuclei that have a ground glass appearance with a central inclusion body that appears to occupy the whole nucleus. Their presence indicates viral reactivation and replication, and it is associated with high viral loads \[106\]. Decoy cells should not be confused with other pathological cell types which may have a similar appearance, such as carcinoma cells or cells infected by other viruses, like HCMV. Testing for decoy cells in urine can be used to screen for BKPyV reactivation, but is difficult to apply in large cohorts.

Immunohistochemistry on renal biopsies
PVAN is classified into three histological grades: A, B and C. Stage A is characterized by the presence of moderate cytopathic effects associated with minimal (< 10%) inflammatory lesions, tubular atrophy, and fibrosis. Renal
function is generally conserved at this early stage. Stage B is characterized by more extensive (10-50%) cytopathic effects and inflammatory lesions, associated with tubular atrophy and progressive fibrosis. Stage C is characterized by fibrotic lesions and severe, irreversible tubular atrophy (> 50%), associated with severe graft dysfunction [107]. Confirmatory diagnosis involves the detection of viral antigens by immunohistochemistry. The most frequently used antibodies are specific for SV40 TAg, but cross-react with BKPyV and JCPyV TAg. There are currently no commercial antibodies that are exclusively specific for BKPyV.

**Therapeutic strategies**

*Pre-emptive and curative therapy of polyomavirus-associated nephropathy by modulation of immunosuppression*

In the absence of a specific antiviral therapy with demonstrated efficacy and no harmful side-effects, and knowing that the degree of immunosuppression is the main risk factor for development of PVAN, the management of BKPyV infection is currently based on reduction of pharmacological immunosuppression (figure 4). This strategy can be

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**Figure 4.** Flow diagram for screening and management of BK polyomavirus (BKPyV) infections in kidney transplantation (from [55]).
applied in the case of histologically confirmed PVAN (curative treatment), as well as cases of probable nephropathy based on positive plasma viremia (pre-emptive treatment). The threshold for therapeutic intervention in the case of pre-emptive treatment is fixed at \(10^4\) DNA copies/mL plasma [55].

The reduction or disappearance of plasma viremia, in addition to the regression of histological PVAN lesions after modification of the immunosuppressive regimen has been reported in many patient cohorts [97, 108-110]. Somewhat surprisingly, the efficacy of this intervention has not been evaluated in prospective randomized trials, nor have the different modes of treatment reduction been rigorously compared.

Immunosuppression following kidney transplant typically involves induction treatment followed by an association of corticosteroids, a calcineurine inhibitor (cyclosporine A or tacrolimus), and an inhibitor of lymphocyte proliferation (mycophenolate mofetil). Different strategies of immunosuppressor modulation have been proposed [55]: in all cases corticotherapy is reduced or discontinued and one of the other two immunosuppressive agents is reduced by 50%. If this is not successful, the dose of the other immunosuppressor is reduced, or one of the two is discontinued. More radical reductions can be envisaged in advanced cases, and other strategies include the replacement of tacrolimus with low-dose cyclosporine or sirolimus, or replacement of mycophenolate mofetil by sirolimus or leflunomide.

Retransplantation

If patients lose the graft because of PVAN, retransplantation can be envisaged, and a three-year graft survival rate of 93% has been observed in a series of 118 retransplantations [111]. Although successful retransplantation has been reported despite the persistence of BKPyV viremia, it is generally admitted that intense immunosuppression and retransplantation should be avoided during active BKPyV replication [55].

Antiviral therapies

Antiviral activity has been demonstrated for several molecules in vitro. Their use as adjuvant therapies alongside reduction in immunosuppression has generally been investigated in case series or small single centre studies. However, their efficacy in vivo appears modest, and difficult to evaluate in the absence of prospective randomized trials.

Cidofovir and CMX001

Cidofovir (CDV) is an acyclic analog of deoxycytidine monophosphate. Its antiviral activity in vitro was first described for HCMV, and was then confirmed for other Herpesviridae (HSV1, HSV2, VZV, EBV, HHV6), and other DNA viruses (papilloma-, adeno-, polyoma-, hepadna-, and poxviruses). In vitro, it inhibits BKPyV replication, but has significant cytotoxicity [112]. In vivo, the use of CDV is limited by its nephrotoxicity and the data concerning its antiviral efficacy in the context of kidney transplant are somewhat contradictory [113, 114]. In the context of BKPyV-associated HC after HSC graft several investigators have reported reductions in BKPyV viral load and a positive clinical responses following CDV treatment in both adults and pediatric patients [115, 116].

CMX001 (hexadecyloxypropyl-cidofovir, Brincidofovir) is a lipid-conjugated form of CDV with increased oral bioavailability. Cells internalize CMX001 in a manner similar to lysophosphatidylcholine uptake, then CDV is liberated by a phospholipase and becomes active after intracellular phosphorylation. In vitro, CMX001 has greater antiviral activity than CDV in HRPTEC and urothelial cells [117, 118]. At the present time, these encouraging in vitro results have not yet been validated in clinical studies.

Leflunomide

Leflunomide is an immunosuppressor used in the treatment of rheumatoid arthritis. More recently, it has been used as an immunosuppressive agent after kidney transplant in order to reduce the administration of nephrotoxic drugs, to prevent chronic rejection, and to protect against viral infections, including HCMV, HSV and BKPyV. It appears to act firstly, by inhibiting the mitochondrial enzyme responsible for the synthesis of orotate, which is an intermediate in the de novo synthesis of pyrimidine nucleotides, and secondly, by inhibiting certain tyrosine kinases involved in T- and B-lymphocyte activation. In vitro, leflunomide inhibits BKPyV replication [119]. In vivo, the encouraging results reported by some investigators [120] have not been confirmed by others [121], and the only randomized study of leflunomide as treatment for PVAN found no beneficial effect on graft survival [122].

Fluoroquinolones

Fluoroquinolones are antibiotics that target bacterial topoisomerases and also inhibit the replication of BKPyV in HRPTECs [123]. The antiviral effect may be due to interference with the helicase activity of Tag, which interacts with cellular topoisomerase. The replication of SV40 in monkey kidney cells is also inhibited by several different fluoroquinolones [124]. Initial studies reported encouraging reductions in viral load in PVAN patients [120]. However, two recent double-blind randomized studies did not find levofloxacin to be effective as either curative [125] or pre-emptive [126] PVAN therapy.
Inhibiteurs de mTOR

mTOR inhibitors, such as sirolimus, reduce protein synthesis and limit progression through the cell cycle. In a retrospective study of 15 kidney transplant recipients use of mTOR inhibitors alone, or in combination with other antivirals was associated with favourable evolution of BKPyV infection [127]. These observations have not been replicated in subsequent patient series, and therefore require confirmation in larger patient cohorts.

**Intravenous immunoglobulins**

Intravenous immunoglobulins (IVIg) have been repeatedly tested as an adjuvant therapy for PVAN. IVIg contain neutralizing antibodies against all BKPyV genotypes, as shown by their ability to block BKPyV replication in HRPTEC [128, 129]. However, the elevated levels of BKPyV-specific antibodies already present in PVAN patients suggest that antibodies are not sufficient to control viral replication. *In vivo*, the efficacy of IVIg has not been formally demonstrated, particularly since they are generally used in association with reduction of immunosuppression. Some authors have reported favourable evolution of BKPyV infection following IVIg administration, while others have not found any beneficial effects, and serious adverse events, although rare, have also been reported. Once again, well-designed clinical trials will be required in order to determine real therapeutic efficacy of IVIg in the management of BKPyV.

**Conclusion**

More than 40 years after the discovery of BKPyV, several aspects of the host-virus interaction are still only partially understood. Outstanding questions include the following: what exactly is the mode of transmission? What is the mechanism of viral persistence? What events at the molecular and cellular level lead to virus reactivation? What elements of the innate and adaptive immune response participate in control of the infection? Are there some BKPyV-associated pathologies – particularly cancers – that remain to be identified? Answering these questions will allow us to improve the management of patients at risk for BKPyV infection and help us identify new therapeutic strategies.

**References**


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