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Review

The human polyomaviruses

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Abstract. The Polyomavirus family includes two members, BK virus (BKV) and JC virus (JCV), that naturally infect humans. These viruses are widely distributed among the population worldwide. Primary infection occurs in early childhood and remains for life clinically unapparent in immunocompetent individuals. In the context of severe immunosuppression and other predisposing factors BKV and JCV may reactivate and cause serious illnesses known as Polyomavirus-induced nephropathy and progressive multifocal leukoencephalopathy, respectively. Here we briefly examine the biological and physical characteristics and the lifecycle, namely receptor(s) interaction, mode of entry, intracellular trafficking, viral transcription and replication, and progeny assembly of these two human Polyomaviruses. We also provide an overview of the clinical manifestation of Polyomavirus-induced disorders in affected individuals and discuss the potential involvement of BKV and JCV in human cancer.

Keywords. Polyomavirus, BK virus (BKV), JC virus (JCV), Polyomavirus-induced nephropathy (PVN), progressive multifocal leukoencephalopathy (PML), viral lifecycle.

Introduction

Humans are the natural host species for two members of the Polyomavirus family, JC virus (JCV) and BK virus (BKV). Both viruses establish subclinical persistent infection in the kidney and peripheral blood in 35–85% of the population worldwide [1–3]. The urotheliotropic nature of BKV virus is characterized by infection of the epithelial lining of the collective ducts, the transitional epithelial cells of the renal calyces, the parietal epithelium of the Bowman's capsule and the transitional epithelium of the renal pelvis and the urinary tract [4, 5]. JCV also targets the epithelial cells that line the collective tubules in the kidney as a site for persistent infection. JC viral DNA has been isolated from B-lymphocytes residing in the bone marrow and the peripheral blood of the human host, implicating these cells as carriers and propagators of the virus [6–8]. Upon reactivation in the context of profound immunosuppression, JCV spreads to the central nervous system (CNS), where the virus cytolytically infects oligodendrocytes and establishes a non-productive infection in astrocytes [9, 10].

The lifelong Polyomavirus persistent infection does not present a clinical threat to the immunocompetent host despite the residual level of viral gene expression [11]. Sporadic reactivation of these human parasites resulting in limited viral replication is seen in 0.5–20% of healthy seropositive individuals. However, no histopathological changes are observed in the kidney parenchyma, and renal function is left unaffected [12]. As evidenced by serological data, rise in the titer of serum antibodies against BKV and JCV is observed in pregnant women towards the end of the second trimester and persists until term [13, 14]. The altered immune state during pregnancy, spe-

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cifically higher monocyte and lower neutrophil counts, are associated with Polyomavirus reactivation and subsequent excretion [14]. The Polyomavirus activity observed in pregnant women is more often due to JCV than BKV, and is shown to result from reactivation of persistent virus rather than from a primary infection [15, 16]. Dramatic increase in viral activity yielding to disease progression occurs predominantly in the setting of relative or absolute cell-mediated immunosuppression. Rise in the incidence of Polyomavirus viruria is observed in human immunodeficiency virus (HIV)-infected individuals and recipients of bone marrow, kidney and heart transplants [7, 17–25]. The urothelic tropism of BKV confines viral-induced pathogenesis, commonly referred to as Polyomavirus-induced nephropathy (PVN), to the urogenital tract and the kidneys of affected individuals [26, 27]. In the case of JCV, virus-associated disease, known as progressive multifocal leukoencephalopathy (PML), is restricted to the CNS [28–31].

To date there are no adequate anti-viral treatments to resolve the detrimental, and in some cases fatal Polyomavirus-induced illness in affected individuals. A more thorough understanding of the biology of these human pathogens would ensure the development of effective therapies for PVN and PML in the future.

Discovery and biologic characteristics

BK virus was first identified and described in 1970 by Sylvia Gardner. Viral particles were isolated from a urine sample of a Sudanese kidney transplant recipient (with initials B. K.), who presented with ureteral stenosis 3 and a half months after transplantation [32]. The newly discovered infectious agent was shown to be a member of the Polyomavirus family.

In 1965, 7 years after the first cases of PML were recorded, the presence of virus particles in the nuclei of glial cells in PML tissue confirmed previous speculations about the viral etiology of the disease [30, 31]. The defining experiments identifying JC virus as the infectious agent in PML were performed by Padget et al. in 1971. The group was successful in demonstrating biological activity by the viral particles seen in preparation of a PML brain taken from a patient with initials J. C. Their further observations suggested that the virions are similar in size and shape to the Polyoma-SV40 subgroup of Papovaviruses [33, 34]. The Polyomaviridae comprises 13 distinct viruses that have all descended from a common ancestor. The natural hosts for these viruses include humans, other primates, rodents, rabbits and birds. The Polyomaviruses exhibit a restricted host range and do not productively infect other species [35]. Simian virus 40 (SV40) and mouse Polyomavirus (PyV) are the prototypical and most well characterized members of the Polyomaviridae. BK virus and JC virus are the only two Polyomaviruses that naturally infect humans. The BKV genome displays 75% overall homology with the JCV genome and 70% overall homology with the SV40 genome [2]. In spite of their high resemblance, these closely related Polyomaviruses exhibit distinct biological behavior and disease pathogenesis [36].

Each Polyomavirus particle measures 40.5–44.0 nm in diameter and has a sedimentation coefficient $S_{20,w}$ of 240S, and a buoyant density in CsCl of 1.34 g/cm^3. The virions comprise 88% protein and 12% DNA. A non-enveloped icosahedral capsid composed of three virus-encoded proteins, VP1, VP2 and VP3, encloses a single molecule of double-stranded viral DNA genome of 5130 base pairs (bp) in the case of the JCV Mad-1 strain [37] or of 5153 bp as seen in the BKV Dunlop strain [38]. The circular viral minichromosome is in complex with cellular histone proteins H2A, H2B, H3 and H4. The viral genome is organized in three functional regions, namely the genetically conserved early and late coding regions, which are separated by the hypervariable non-coding regulatory region (Fig. 1) [35, 36, 39–41].

As the notation implies, the early region is the first part of the genome to be transcribed and translated in the viral lifecycle. The early region spans 2.4 kb and encodes two viral regulatory proteins also known as the tumor or simply T antigens. These proteins are produced by alternative splicing of a common pre-messenger RNA (mRNA) precursor, and are distinguished as large T and small t antigens (T-Ag and t-Ag) based on their size [36, 40]. T-Ag is

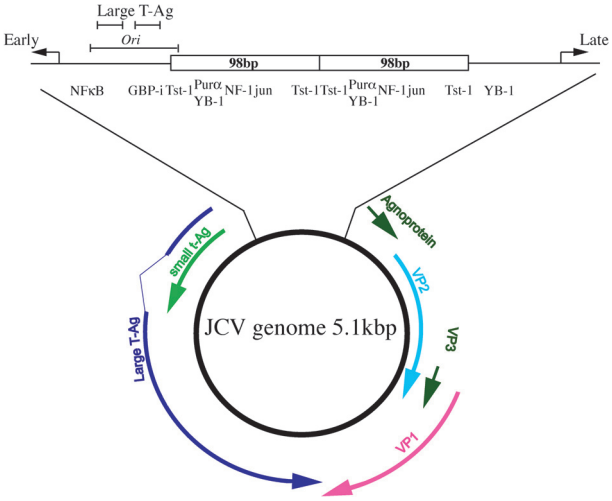

Figure 1. Schematic of the JCV Mad-1 genome and its regulatory region. The Polyomavirus genome is a closed circular, double-stranded DNA molecule approximately 5 kb in size. The coding regions for the early genes, T-Ag and t-Ag, are transcribed in a counterclockwise direction, and the late genes, agnoprotein and VP1, VP2 and VP3, are transcribed in a clockwise direction. The genome is in black with the arrows representing the coding regions of the viral proteins. The non-coding regulatory region is emphasized, and depicts the tandem 98-bp repeats along with some of the known transcription factor sites. The origin of DNA replication and binding sites for the viral T-Ag are also represented.

considered the master regulator of the infectious process. Through its multiple enzymatic activities and ability to bind DNA and a number of cellular proteins, T-Ag orchestrates production of early mRNA, initiation of viral DNA replication and activation of late gene transcription [42]. Binding of T antigen to the hypophosphorylated form of the retinoblastoma susceptibility protein (pRb) allows for premature release of the transcription factor E2F, which in turn stimulates resting cells to enter the S-phase of the cell cycle [43]. Having established a suitable environment, T-Ag then directly recruits the host cell DNA polymerase complex to the origin of viral replication in order to initiate bi-directional viral DNA synthesis [44, 45]. Activation of the late viral promoter by T-Ag and associated cellular transcription factors leads to viral late gene expression [46].

The role of small t antigen in the lifecycle of human Polyomaviruses is not yet fully defined. Viral replication proceeds successfully in the complete absence of the protein [36]. It is believed that t-Ag serves an ancillary role for T-Ag activity and cell transformation [42].

The late coding region spans 2.3 kb and contains the genetic information for the major structural protein VP1 and the two minor structural proteins VP2 and VP3. All three capsid proteins are derived from a common precursor mRNA by alternative splicing. Translation of VP2 and VP3 occurs from alternative start codons on the same mRNA transcript [36, 47]. Based on extrapolations from the solved crystal structures of SV40 and PyV, the BKV and JCV capsids are predicted to contain 360 molecules of VP1 arranged in 72 pentameric subunits [48–50]. Each VP1 pentamer associates with a single VP2 or VP3 molecule to form the individual capsomeres. The C-terminals of the VP1 molecules extend to anchor together neighboring capsomeres and form the complete capsid [51, 52]. The late region also encodes the agnoprotein. The role of this protein in the lifecycle of the Polyomaviruses, which encode and express this protein remains under investigation. SV40 agnoprotein has been suggested to facilitate the efficient packaging of capsid proteins, thereby increasing virus yield [53]. Through direct interaction with the JC T-Ag agnoprotein appears to modulate viral transcription and replication during JCV infection [54]. Functional association between the agnoprotein and the cellular transcription factor, YB-1, downregulates the YB-1-mediated activation of the JCV early and late promoters [55].

The viral non-coding regulatory region (NCRR) spans 300–500 bp and is located between the early and late coding regions (Fig. 1). The NCRR contains the origin of DNA replication (*ori*), the TATA box, T-Ag binding sites, cellular transcription factor-binding sites, and bi-directional promoter and enhancer for transcription of early and late genes [35, 36, 56]. The enhancer region of the BKV Gardener strain consists of three 68-bp repeats, which are subject to deletions, duplications and rearrangements in different BKV variants [2, 57]. For example, only one 68-bp sequence is found in the NCRR of the archetypal WW strain of BKV [58]. The regulatory region of JC virus contains two 98-bp tandem repeats that serve as enhancer elements and are positioned on the late side of the *ori* [59]. In general, there is a great deal of sequence variability seen in the NCRR within BKV and JCV variants, which is believed to confer selective replicational and transcriptional advantage to these viruses in their hosts [36, 60, 61].

The infectious lifecycle of Polyomaviruses

As previously mentioned, the infectious lifecycle of Polyomaviruses is arbitrarily divided into early and late stages. The early stage begins with the initial interaction of the virus with the surface of the host cell and continues until the onset of viral DNA replication. The late stage includes all subsequent events that lead to the assembly of new virions and concludes with the release of viral progeny, which in turn marks the completion of the entire viral replication cycle.

Receptors

The lifecycle of the two human Polyomaviruses is initiated by adsorption of the virions to the cell surface. Both BKV and JCV share the requirement for interaction with cell surface sialic acids, albeit different linkages, for efficient entry [62, 63]. Evidence from earlier work demonstrates the involvement of the gangliosides GD1a and GT in the initial interaction between BKV and the susceptible cell [64–66]. More recent experiments argue that an N-linked glycoprotein with α(2,3)-linked sialic acid serves as the receptor for BK virus on host cells. Enzymatic removal of this sialic acid linkage with specific neuraminidases and subsequent rescue re-sialylation with a corresponding sialyltransferase eloquently demonstrated that the N-linked α(2,3)-carbohydrate residue mediates productive BKV attachment and entry into host cells [62]. The identity of the proteinaceous component of the cellular receptor(s) for BKV remains to be determined.

In vitro studies have demonstrated that JCV requires N-linked glycoprotein containing terminal α(2,6)-linked sialic acid to successfully infect human glial cells. Treatment of susceptible cells with crude neuraminidase from *Vibrio cholerae,* but not with recombinant α(2,3)-specific neuraminidase, blocks infection by JCV. Also, JCV competitively inhibits binding of α(2,6)-specific lectin to glial cells [63]. Recent work examining the nature of the proteinaceous portion of the JCV receptor revealed the identity of the serotonin receptor $5HT_{2A}$ [67]. Experimental evidence compellingly showed that $5HT_{2A}$ receptor antagonists and antibodies inhibit JCV infection with

a high degree of specificity. Additionally, supplementing receptor-negative and JCV-resistant cells with the serotonin receptor renders them susceptible to infection [67].

Mechanisms of entry and intracellular trafficking

In a manner similar to other DNA viruses, BKV and JCV penetrate into the cytoplasm by endocytosis [68]. Viruses take advantage of the endocytic machinery to gain access to the cytosol and/or the nucleus of the target cell, where their replication and multiplication take place. BKV and JCV employ different internalization mechanisms to enter the host cell [69–72]. Dissection of the early events in the BKV lifecycle revealed that the virus enters cells by caveolae-mediated endocytosis. The entry process of BKV is relatively slow, dependent on a functional caveolin-1 scaffolding domain, independent of clathrin-coated pit assembly, sensitive to tyrosine kinase inhibition and requires intact membrane cholesterol levels. Inside the cell, BKV co-localized with the caveolae marker Cholera toxin subunit B. Taken together, these observations argue in favor of a caveolae-mediated endocytosis as the major route for BKV infectious entry [71]. In-support of the experimental evidence, ultrastructural analysis of BKV-infected renal tissue revealed that BK virions were localized to smooth, flask-shaped invaginations of the plasma membrane, which correspond to caveolae [72, 73].

Conversely, JCV enters the target cell rapidly and requires proper assembly of clathrin-coated pits. JCV uptake is sensitive to agents that disrupt clathrin endocytosis, but not to those that interfere with caveolae-mediated internalization. Following entry, the virus co-localizes with the clathrin marker, transferrin in endosomes [69, 70]. These data led to the conclusion that JCV utilizes clathrin-dependent endocytosis as means of entry into cells. This notion was further substantiated by the established fact that the $5HT_{2A}$ receptor is also internalized by clathrin-dependent endocytosis [74].

Having penetrated into the interior of the cell, Polyomaviruses are next transported through the cytosol to the nucleus (Fig. 2). For such directional intracellular migration, viruses depend on the active cytoskeletal transport machinery [75]. Close examination of the involvement of the different cytoskeletal components demonstrated a critical role for intact microtubules (MTs) during early infection by both BKV and JCV [76, 77]. In the case of BKV, a timecourse study showed that by 8 h post-infection, the virus had advanced beyond the critical MT-dependent step. Arrest in MT dynamics did not interfere with BKV infectivity, indicating that the virus does not rely upon active rearrangement of these structures for its transport, but rather uses them as tracks to advance to its intracellular destination [76]. BKV infection proceeds successfully under the circumstances of induced disas-

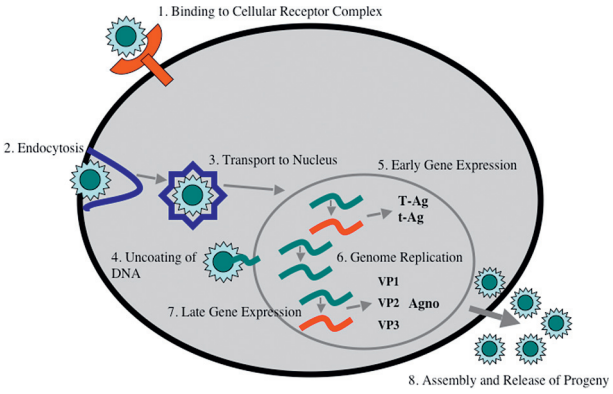

Figure 2. The replication cycle of Polyomaviruses in a permissive cell. The lifecycle begins when the virus attaches to a cellular receptor complex (1). Following this initial interaction the virus is internalized into the cytosol by endocytosis (2). The virus is then transported to the nucleus (3) where uncoating (4) takes place to expose the genome for early gene expression (5). Viral DNA synthesis (6) precedes the production of the late proteins (7). This in turn allows the assembly of new virions, which are released in the exterior (8), thereby marking the successful completion of productive infection.

sembly of actin filaments [76]. In contrast, JCV infection of SVG cells, an immortalized human fetal astroglial cell line, is severely inhibited by disruption of the actin cytoskeleton [77]. The authors of the latter study speculate that this inhibitory effect during early JCV infection is most likely attributed to actin participation in assembly of the clathrin machinery rather than direct involvement of actin filaments in viral trafficking. Intact intermediate filament network is also necessary for productive infection by JCV [77].

In the nucleus

Aided by the endocytic and cytoskeletal transport machinery, BKV and JCV arrive at nucleus – the site of viral replication and virion assembly. It is thought that uncoating of Polyomaviruses occurs inside the nucleus [78, 79]. As with the other members of the family, the two human Polyomaviruses have lifecycles that exhibit temporal regulation. Initially upon entering the nucleus there is transcription of the early viral genes, large T and small t antigens. Following early viral transcription there is a switch to DNA replication. Subsequent to complete DNA replication there is a second switch to the expression of late viral genes, VP1, VP2 and VP3, as well as the regulatory protein, agnoprotein.

JCV transcription has been extensively described, although many of these studies have been limited to the Mad-1 strain. The Mad-1 promoter region is composed of tandem 98-bp repeats that function in either direction to control both early and late viral transcription (Fig. 1). The narrow host cell range of JCV is believed to be in part determined by the significantly higher rate of early

transcription seen in glial cells as compared with non-glial cells [59, 80–83]. Experiments swapping JCV and SV40 promoters indicate that neurotropism is largely determined by the JCV promoter [84]. Other experiments have demonstrated that DNA replication and late gene expression can occur in non-glial cells that express JCV T-Ag [80, 85]. As a result, efforts have focused on transcription factors that may be responsible for the restriction of viral tropism during early transcription. Numerous viral and cellular proteins are known to be involved in controlling transcription throughout the viral lifecycle (Fig. 1). Although a number of these, including NF-1, Sp-1 and Tst-1 (Fig. 1), have been demonstrated to contribute to tropism [86–88], it is likely that no single glial-specific transcription factor is responsible for the restriction of JCV transcription. Instead, a combination of factors that occurs specifically in glial cells is probably responsible for determining neurotropism.

In contrast, the BKV promoter region has been less well characterized. As is the case with the other Polyomaviruses, the transmissible archetype strain undergoes several rearrangements, which leads to a high degree of variability in transcriptional activity between the different strains. Previous studies analyzing the promoter region have highlighted binding sites for NF-1, Sp-1, AP1, C/EBPβ and NFκB [57, 61, 89–91]. The significance of these transcription factors and their potential role in the development of PVN is yet to be determined. The NCRR of BKV contains a non-consensus glucocorticoid-response element (GRE)/progesterone-response element (PRE)-like motif and a fully consensus estrogen-response element (ERE), which successfully mediate an increase in viral promoter activity upon hormone stimulation [92]. Addition of steroid hormones to BKV-infected cells enhances the production of late viral capsid proteins that in turn potentiates the assembly of a greater number of viral particles compared with untreated BKV-infected cells. Further studies revealed that activation of the BKV promoter by the estrogen receptor (ER) can be mediated by either the ERE or by the two AP-1 binding sites present in the viral NCRR. Co-expression of the early viral proteins, T-Ag and t-Ag, synergistically amplifies the activity of the ER-stimulated BKV promoter [93]. The authors of these studies speculate that elevated estrogen levels, such as those seen in pregnant women, might contribute to viral reactivation, hence the increase in BK viral shedding observed in pregnant women [93].

Subsequent to early transcription is the switch to DNA replication. This switch is believed to be primarily initiated by T-Ag, which binds to the origin of replication and instigates bi-directional DNA replication. T-Ag is also a strong activator of the late viral promoter [94], which aids the switch to late viral transcription after DNA replication has been completed. If early transcription has been highlighted as a determinant of viral neurotropism, then DNA replication has been identified as a determinant of species specificity. T-Ag determination of species specificity is a feature of the Polyomavirus family. Host cell DNA polymerase α-primase interacts with large T-Ag in a species specific manner. Cell-free extract experiments demonstrate that replication of PyV in HeLa cell extracts can only be rescued by the addition of murine DNA polymerase α-primase, while the converse is true for SV40 [95–97].

The role of T-Ag in the viral lifecycle is vital to productive infection. Control of T-Ag expression is therefore crucial to productive infection. In addition to transcriptional regulation, T-Ag expression is controlled post-transcriptionally. Recent studies have found that SV40 expresses a microRNA that downregulates the expression of viral T-Ag [98]. Downregulation of T-Ag in this manner appears to render the cells less sensitive to cytotoxic T cells. Similar sequences also exist in both JCV and BKV, although their function is undetermined.

Virion assembly and release

Once the viral genome has been replicated, T-Ag mediates repression of early gene transcription, and stimulates transcription of the late genes. Expression and subsequent nuclear localization of the viral structural proteins VP1, VP2 and VP3, leads to the assembly of the virion capsid. The capsids of BKV and JCV consist of 360 molecules of the major coat protein VP1, which are arranged in 72 pentamers creating an icosahedral shape. There is 77% sequence similarity between JC and BK VP1. The C′ terminal residue of a VP1 molecule invades the next VP1 in order to tie the pentamer and the capsid together [50]. One of the minor coat proteins, VP2 or VP3, lies in the center of each pentamer. VP3 is identical to the C′ terminal two-thirds of VP2, where this shared domain comprises the DNA binding domain and the VP1 interacting domain [99]. The DNA is packaged with histones H2A, H2B, H3 and H4, and creates a mini-chromosome structure that is almost indistinguishable from the host's chromatin.

Virus-like particles (VLPs) composed of VP1 alone are capable of self-assembly for both JCV and BKV when expressed in eukaryotic expression systems [100, 101]. These studies have elucidated the importance of disulfide bonds and calcium ions for maintaining a stable structure [102]. These VLP will not be able to package the viral genome bound by the minor proteins.

Unlike the bipartite BK nuclear localization signal (NLS), JC VP1 has a weak monopartite NLS. JC VP1 needs VP2/3 in order to achieve proper import, suggesting that assembly of the pentamers occurs in the cytoplasm [103]. Further evidence for the cytoplasmic association of capsids is presented by NLS mutagenesis experiments. In these experiments, the removal of the NLS of one struc-

Table 1. Comparison of biological properties of BKV and JCV.

	BKV	JCV	Refs.
Receptor(s)			
Carbohydrate moieties	α(2,3)-linked SA, GD1a, GT	α(2,6)-linked SA	[62–66]
Protenaceous component(s)	undetermined	serotonin receptor $5HT_{2A}$	[67]
Mode of entry	caveolae-mediated endocytosis	clathrin-dependent endocytosis	[69–73, 78]
Intracellular trafficking:			
Vesicles	vesicular tubules associated with Golgi and ER;	endosomal compartments	[69, 72] [76–78]
Cytoskeletal components	intact MT and dynamic actin filaments	intact MT, actin filaments and IF	
pH	dependent	dependent	[71, 77]
Structure of NCRR:			
Enhancer sequence(s)	three 68-bp repeats (Gardner); one 68-bp sequence (WW); non-consensus GRE/PRE; consensus ERE	two 98-bp repeats (MAD-1)	[2, 57–59, 92, 93]
Cellular transcription factors	NF-1, Sp-1, AP1, C/EBPβ, NFκB	NFκB, GBP-I, Tst-1, NF-1, jun, Purα, YB-1	[80–82, 86, 88–91]
Clinical manifestation	Polyomavirus-associated nephropathy; hemorrhagic cystitis; ureteral stenosis; tubulointerstitial nephritis	progressive multifocal leukoencephalopathy	[26, 28–34, 114, 115, 122–124, 146]
Reported association with human cancers	glial tumors; insulinoma, osteosarcoma, colorectal tumor	glial tumors, colon cancer	[150, 151, 153, 154, 156, 158–160]

SA, sialic acid; ER, endoplasmic reticulum, MT, microtubules; IF intermediate filaments.

tural protein determined that this capsid component associates with others to allow for its nuclear import [104]. In the case of BKV, VP1 is imported on its own. The nuclear fraction from infected cells shows that the ratio of available BKV structural proteins is similar to that seen in the capsids of assembled virions. This would suggest that regulation of nuclear import is connected with capsid assembly [105]. Additionally, the importance of minor proteins has been shown for post-translational modifications for other Polyomaviruses [106, 107]. Assembly seems to be predominantly in the nucleus. It has been suggested this is due to the higher calcium concentrations. The loss of the minor proteins of JCV is detrimental to the completion of its viral lifecycle and creates loose packaging, as demonstrated by DNase assays [M. Gasparovic and W. I. Atwood, unpublished observations].

It has been shown recently that JC VP1 requires VP2/3 to localize to the specific nuclear domain ND10. VP1 alone is unable to localize to this domain. VP1 was shown to exhibit both diffuse staining around and to accumulate into the ND-10 domains when many cells expressing wild-type virus were evaluated [108]. The nuclear localization of BK has not yet been evaluated. Other viruses are known to utilize the ND10 domain during their replication cycle. The genomes of both HPV and SV40 have been detected in this nuclear compartment. The localization for SV40 is dependent upon large T-Ag binding to the viral DNA, suggesting that only actively replicating DNA is targeted to the ND10 domain [109]. The special concentration of genome replication and capsid formation in the ND10 domain would greatly facilitate the production of complete virions.

The newly packaged virion progeny is thought to be released by lytic rupture of the host cell, although electron microscopy observations report secretion of virions from the plasma membrane of intact cells [110, 111]. It remains to be determined whether cell lysis or at times intracellular vesicular transport is the preferred pathway for the release of JCV and BKV progeny virions.

Disease manifestation of Polyomavirus infection

Although BKV and JCV pathology targets distinct anatomical regions and is influenced by different risk factors, the unifying theme in Polyomavirus disease presentation is the incapacitation of the cell-mediated immunity in the human host. The profound immunological impairment experienced by HIV-1/AIDS (acquired innunodeficiency syndrome) patients and transplant organ recipients pro-

vides the necessary background for the opportunistic reactivation and uncontrolled infection by JCV and BKV that in turn may result in overt disease development (reviewed in [112, 113]).

Polyomavirus-induced nephropathy

PVN has recently emerged as a significant cause of severe renal allograft dysfunction and ultimate graft loss [26, 114, 115]. It is well established that BK virus (BKV) is the etiological agent of PVN. Active BKV infection in the renal allograft induces morphological cytopathology, which directly translates into functional impairment [26, 116]. The underlying cause of renal graft dysfunction in PVN is virus-induced necrosis of tubular cells with a secondary denudation of the underlying basement membrane. Sustained high levels of viral replication lead to repeated rounds of lytic destruction of the epithelial cells lining the tubules. Such severe and extensive tubular damage ultimately permits consistent leakage of tubular fluid into the interstitial compartment, which in turn causes irreversible interstitial fibrosis and tubular atrophy [26, 27, 116].

PVN is almost exclusively restricted to the renal allograft; the native kidneys or any other organs remain unaffected [116, 117]. Although intensive immunosuppressive therapy is a key factor in conferring predisposition to PVN development, there are other promoters of the disease process [112, 118]. Several risk factors have been implicated to exacerbate viral replication and nephropathy progression. Most common among them include proinflammatory state of the graft brought upon by surgical injury, warm ischemia and reperfusion during implantation of the organ, a higher number of human leukocyte antigen mismatches between donor and recipient, BKV seronegativity prior to transplantation and tubular injury due to drug toxicity [26, 27, 112, 116, 118]. Because the exact mechanism of BKV reactivation and development of PVN is poorly understood, the exact role of the above risk factors in the disease process also remains unclear.

Although steady progress has been made in recognizing and diagnosing PVN, clinical management and treatment of the disease remains a challenge. To date, there is no specific anti-viral therapy to control BKV infection in the renal allograft of a PVN patient. The use of the acyclic nucleoside analog cidofovir has shown limited success in reducing viral burden in PVN patients [119]. Unfortunately, this anti-viral agent is generally contraindicated in patients with renal dysfunction due to its nephrotoxicity [27, 119]. Currently, the primary clinical approach to counteract PVN is achieved through a reduction in immunosuppression to permit recovery of host anti-BKV immune response in order to resolve the infection [120]. A decrease in the immunosuppressive regimen, albeit beneficial to the allograft function, heightens the inherent risk of graft rejection [118, 121].

PML

Profound immunosuppression provides suitable conditions for increase in JCV replication and spread to the CNS, where the virus selectively targets and destroys the myelin-producing glial cells, the oligodenrocytes [30, 33]. The loss of these cells results in a patchy and subsequently confluent demyelination of the CNS referred to as progressive multifocal leukoencephalopathy [28, 122]. Histopathologically the PML lesions are poorly outlined and are characterized by a discoloration of the white matter, presence of giant, multinucleated astrocytes, and loss of oligodendrocytes [9, 28, 122–124]. The nuclei of the remaining oligodendrocytes are enlarged and laden with viral particles [30, 31]. The clinical manifestation of this neurological disorder typically includes motor function abnormalities, dementia, visual and cognitive impairment, and cranial nerve palsies [28, 125]. The AIDS pandemic has coincided with a surge in the incidence of PML, as HIV-1-associated immunosuppression is the underlying disease in 85% of the PML cases [113, 126]. In addition, involvement of HIV-1 secreted products has been proposed to stimulate JCV replication in immunocompromised patients and ultimately aid JCV spread to the CNS [127–130].

The mode of viral dissemination from the periphery where primary infection occurs to the CNS where the disease develops remains undefined. Microscopic examination of early-stage PML brain reveals multiple perivascular areas of white matter deterioration suggestive of viral spread to the CNS facilitated by a hematogenous route [122, 123, 131]. Subsequent investigations of JCV association with circulating lymphocytes revealed that JCV DNA is indeed present in the blood of healthy individuals and at higher rate in AIDS patients [18–20, 132]. The B lymphocyte population appears to be the main target of JCV in peripheral blood [133–135]. The presence of viral DNA and proteins has been detected by polymerase chain reaction (PCR) and immunohistochemistry in B-cell-rich lymphoid organs such as tonsil, spleen and lymph nodes [136, 137]. The putative lymphotropic nature of JCV outside the CNS strongly suggests that in addition to the kidney, the lymphoid organs could be another site of viral persistence and may facilitate viral spread to the CNS by infected B-cells [138, 139].

PML is a rapidly progressive disease and proves fatal in 90% of patients within 1 year after onset of the symptoms [140, 141]. To date, there is no effective treatment for PML. The inability of the nucleoside analogs cidofovir and cytosine arabinoside to cross the blood-brain barrier renders these agents inept to combat this disease [142]. The use of highly active antiretroviral therapy (HAART) has shown limited success with variable duration in some PML patients [143].

More recently, development of PML has been reported in two multiple sclerosis (MS) patients who were under-

going treatment with the selective adhesion molecule inhibitor natalizumab. This new therapeutic agent, a humanized monoclonal antibody against α_4 integrins, blocks the interaction between $\alpha_4\beta_1$ integrin and the adhesion molecule VCAM-1, thereby preventing the emigration of lymphocytes and monocytes from the bloodstream to sites of inflammation [144]. A speculation for the rise in JC viral loads and progression to PML proposes that the disruption of lymphocyte trafficking and the subsequent suppression of the cellular immune response grant the virus unrestricted multiplication activity [145]. Other factors are certain to have contributed to the JCV pathogenesis in these MS patients; however, these PML cases have alerted us to the risk that this Polyomavirus presents upon the use of immunomodulatory drugs and the requirement to monitor the clinical behavior of the virus during the course of treatment with natalizumab and similar therapeutics.

The prevalence rate of PVN among kidney recipients is currently reported to be approximately 5% [112, 114, 118]. PVN significantly reduces the survival rate of the grafted kidney. Once established, the disease leads to rapid graft failure and subsequent loss in 45–60% of PVN patients [26, 27, 146]. Similarly, statistics show that 5% of all AIDS patients succumb to PML [113]. These numbers demonstrate that Polyomavirus-induced pathogenesis affects and offers a grim outcome for a significant portion of the population worldwide. The need for identifying effective therapeutics to combat BKV and JCV infection is acute. The key to novel clinical therapies lies in furthering our insight into the biological behavior and intricate lifecycle of these human pathogens.

Role of JCV and BKV in human cancer

The clinical significance of the Polyomaviruses in human cancer remains under heated debate marked by largely opposing views. The putative tumorogenic potential ascribed to JCV and BKV lies in the transformation capabilities of the viral-encoded oncoproteins – T-Ag and t-Ag. As described earlier, T-Ag ensures a propitious cellular environment for viral DNA replication by driving resting cells into S-phase through interactions with cell cycle regulators such as pRb and p53 (reviewed in [147–149]). Experimental evidence shows that both JCV and BKV are able to induce tumors in a variety of laboratory animals. However, the oncogenicity of the two viruses is manifested in distinct anatomical locations in the same animal models, which reflects the tissue and cell restricted tropism of JC and BK (summarized in [150, 151]). In general, the degree of transforming capacity displayed by JCV in experimental animals and cultured cells is greater than that seen with BKV. The latter virus transforms cell lines with limited success and often requires the auxiliary function of other more potent oncogenes [152]. Similarly, in animal models the transformation efficiency of BKV is contingent upon the route of inoculation and the strain of the virus [151]. Despite the demonstrated *in vitro* and experimental oncogenic properties of human Polyomaviruses, their involvement, either causative or compounding, in human cancers awaits irrefutable proof. The ubiquitous presence of these viruses in the population worldwide has presented a major hindrance in obtaining conclusive data to support or confound the role of JCV and BKV in tumor formation in humans. There are numerous reports that demonstrate the presence of human Polyomaviruse DNA sequence, viral RNA and proteins in tumor tissues obtained from patients with different malignancies: oligoastrocytoma, medulloblastoma, colon cancer, pancreatic islet tumors (extensively reviewed in [150, 151, 153]). Various techniques such as PCR, Southern blotting, immunohistochemistry, and *in situ* hybridization have been employed to probe for the presence of JC and BK viral products in tumors in an effort to establish the oncogenic power of these viruses. The findings published in studies designed to address this issue vary significantly; the reported prevalence of Polyomavirus sequences in brain and other tumor tissues ranges from <5 to 100% [154–159]. The claim for association between JCV and BKV and human cancer, in studies that detect viral products in human malignancies, is merely observational and is rarely substantiated by a clearly characterized molecular mechanism and chronology for the alleged viral oncogenesis [160]. The general notion in the field, shared by both critics and supporters of the role for JCV and BKV in malignant transformation of human tissues, is that more thorough and consistent interlaboratory studies are needed to establish an unequivocal causal or contributory function for Polyomaviruses in carcinogenesis in humans.

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