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BK polyomavirus: emerging pathogen

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Abstract

BK polyomavirus (BKPyV) is a small double-stranded DNA virus that is an emerging pathogen in immunocompromised individuals. BKPyV is widespread in the general population, but primarily causes disease when immune suppression leads to reactivation of latent virus. Polyomavirus-associated nephropathy and hemorrhagic cystitis in renal and bone marrow transplant patients, respectively, are the most common diseases associated with BKPyV reactivation and lytic infection. In this review, we discuss the clinical relevance, effects on the host, virus life cycle, and current treatment protocols.

Keywords

BKPyV; Polyomavirus; Transplantation

1. Introduction

BK polyomavirus (BKPyV) was one of the first two human polyomaviruses identified. BKPyV and JC polyomavirus (JCPyV) were isolated from immunosuppressed patients and were described in back-to-back articles in *Lancet* in 1971[1,2]. BKPyV was named for the patient from whom it was originally isolated, a renal transplant patient with ureteric stenosis. BKPyV, JCPyV, and the closely related Simian virus 40 (SV40) were originally grouped with papillomaviruses in the genus *Papovavirus*, which was later separated into the genera *Papillomavirus* and *Polyomavirus*. Recently, the International Committee on Taxonomy of Viruses has recommended that the naming of all polyomaviruses be standardized [3]. Following taxonomical revisions, the family *Polyomaviridae* now contains two mammalian genera designated *Orthopolyomavirus* and *Wukipolyomavirus* [3]. In addition to BKPyV and JCPyV, the *Orthopolyomavirus* genus includes the more recently discovered human viruses Merkel cell polyomavirus and trichodysplasia spinulosa-associated polyomavirus [4,5]. KI polyomavirus, WU polyomavirus, and Human polyomavirus-6, -7, and -9 comprise the *Wukipolyomavirus* genus [6–9].

The BKPyV genome is divided into three major genetic regions: the early coding region, the late coding region, and the non-coding control region (NCCR) [10]. There are two different forms of the virus, termed archetype virus and rearranged variants, based on the DNA sequence structure of the NCCR. Archetype virus can be commonly isolated from the urine of both healthy people, as a result of transient reactivation events, and diseased patients [11]. In contrast, rearranged variants are most often found in the serum of patients with BKPyV-associated diseases.

While upwards of 90% of the population as a whole is persistently infected with BKPyV, the virus reactivates and is asymptomatically shed into the urine in 5–10% of immunocompetent adults at any given time [12]. Additionally, BKPyV viruria can be detected in up to 25% of pregnant women [13]. BKPyV is a significant human pathogen that causes disease in immunocompromised individuals. BKPyV is able to reactivate in renal transplant and bone marrow transplant (BMT) patients in whom a lytic infection results in polyomavirus-associated nephropathy (PVAN) and hemorrhagic cystitis (HC), respectively. Rarely, BKPyV reactivation and disease have been observed in other immunocompromised conditions such as systemic lupus erythematosus in other solid organ transplant recipients, and in patients with HIV/AIDS [12].

BKPyV is an emerging pathogen whose incidence continues to increase as a result of the growing number of transplant patients as well as the development of better immunosuppressive drugs. Unlike JCPyV, which was recognized early on as an opportunistic pathogen as a result of the HIV/AIDS epidemic, BKPyV was not acknowledged to be a pathogen until more recently. Historically, polyomaviruses, especially SV40, have been utilized to advance our understanding of basic eukaryotic cellular processes but have been less well studied for their significance as human pathogens. In this review we describe what is currently known of BKPyV infection both at the level of the affected patient and at the level of the infected cell.

2. Transmission and epidemiology

BKPyV transmission is thought to occur via a respiratory route, based on evidence of high seroconversion rates in the population by mid-childhood, with 65–90% of humans becoming seropositive by the age of ten, and the presence of viral DNA in tonsillar tissue [14,15]. There is also evidence for other possible routes of transmission such as fecal-oral, urino-oral, blood transfusion, and transplacental [12]. Archetype virus is thought to be the transmissible form of BKPyV because its NCCR structure can give rise to all known rearrangements and because it is isolated from both healthy people and diseased patients. The archetype NCCR is characterized by blocks of sequence that are deleted and/or duplicated in rearranged variants of BKPyV. Primary infection is thought to be asymptomatic or result in a mild respiratory illness [14]. BKPyV then disseminates to the urinary tract where it persists for the life of the host [16]. Immunostaining has shown the main sites of BKPyV replication to be in epithelial cells of the kidney, ureter, and bladder [17]. BKPyV DNA has been detected in peripheral blood mononuclear cells, which suggests that BKPyV may hijack the immune system to spread from the primary site of infection to its site of persistence [18]. It is unclear if the virus persists with minimal maintenance replication of its genome or if it enters a true state of viral latency. Periodically, BKPyV can reactivate and be shed into the urine of healthy hosts, which contributes to the evidence of a possible urino-oral infectious route [11].

In addition to variations in the NCCR, there are four BKPyV genotypes: I, II, III, and IV, which are differentiated based on sequence variation in VP1 [13]. Unlike JCPyV, which only has one serotype, the BKPyV genotypes correspond to four different serotypes, as

determined by hemagglutination inhibition assay: BK, SB, AS, and IV correspond to genotypes I, II, III, and IV, respectively [19]. The genotype system is the more commonly used nomenclature, and is often substituted for the original serotype nomenclature. There is evidence for geographical separation of genotypes and for subgroups within genotypes. For example, within genotype I, which is the predominant BKPyV genotype, subgroup I/b-2 was primarily observed in European and American populations, whereas subgroup I/c-2 was most often detected in Asian populations [20]. Possible genotypes V and VI were identified based on a phylogenetic analysis which reclassified distinct subgroups previously within genotype I, but these genotypes have not yet been widely recognized in the field and their significance is unknown [21]. It remains unclear if there is any correlation between genotype and clinical disease.

3. Reactivation and clinical diseases

3.1. Hemorrhagic cystitis

Hemorrhagic cystitis (HC) is a condition associated with high morbidity and is characterized by symptoms including dysuria, frequency, urgency, subpubic pain, and varying degrees of hematuria [22]. A grading system to assess the severity of HC was developed by Droller et al., where grade 1 is microscopic hematuria; grade 2, visible hematuria; grade 3, visible hematuria with small clots; and grade 4, gross hematuria with urinary tract obstruction caused by clots [23]. Mild cases of HC will usually resolve within two weeks with supportive care. In more serious cases, significant bleeding and clot formation can cause urinary tract obstruction, severe pain, uncontrollable bleeding, and acute renal failure. In rare instances, fatality may occur as a result of HC.

In BMT patients, HC can occur both pre- and post-engraftment but is attributed to different causes, chemotherapeutic agents or viral infection, respectively. Pre-engraftment HC occurs from the direct cytotoxic effects of antineoplastic treatments, generally a toxic build up of acrolein, which is a metabolite byproduct of the processing of cyclophosphamide or ifosfamide [24]. However, there is now an effective treatment of forced diuresis which has eliminated virtually all chemotherapeutic-induced HC. Cytomegalovirus (CMV), adenovirus, and BKPyV are able to cause HC post-engraftment, for which BKPyV makes up the majority.

BKPyV-associated HC occurs in 10–25% of BMT patients and typically presents two weeks post-transplantation [22]. However, BKPyV viruria can be detected in 50% of BMT patients. High levels of BKPyV viruria may be associated with increased risk of HC development. One study also found that plasma viral loads greater than 10^4 copies/ml were associated with increased risk of HC development [25].

Diagnosis of BKPyV-associated HC is often made by cytological examination of the urine or by PCR amplification of viral DNA in the urine or serum. Characteristic infected cells, called decoy cells, can be observed in the urine of affected patients. These cells have enlarged nuclei and one large basophilic intranuclear inclusion. However, these cells are not specific for BKPyV infection, as adenovirus can cause similar cytopathology. Therefore, PCR amplification of BKPyV viral DNA from the urine or the serum is more specific and is used commonly to diagnose BKPyV-associated disease in the presence of HC symptoms. BKPyV viral loads in the urine of HC patients can peak as high as 10^{14} copies/ml [26]. The current standard treatment protocol for BKPyV-associated HC is supportive care including pain management, hyperhydration, forced diuresis, and bladder irrigation [22].

3.2. Polyomavirus-associated nephropathy

Polyomavirus-associated nephropathy (PVAN) is characterized by an inflammatory interstitial nephropathy that presents on average 10–13 months post renal transplant [27]. PVAN most commonly occurs in kidney transplant patients and is rare in other solid organ transplant recipients. This is likely due to the level of immunosuppression and injury at the site of viral persistence, the kidney, rather than general immunosuppression alone. The development of better immunosuppressive drugs in the last two decades has lead to a decreased rate of acute rejection in renal transplant patients but an increase in the incidence of PVAN [28]. PVAN occurs in up to 10% of kidney transplant patients, up to 90% of whom will go on to lose their graft [28].

The immunosuppressive regimen for renal transplant patients varies somewhat but commonly consists of a calcineurin inhibitor, an antimetabolite, and a corticosteroid. Calcineurin inhibitors such as cyclosporine and tacrolimus inhibit immunophilins and reduce the transcription of IL-2, which has the overall effect of reducing T cell activation. Antimetabolites such as azathioprine and mycophenolate mofetil inhibit nucleic acid synthesis and cell proliferation, especially in lymphocytes. Corticosteroids are anti-inflammatory and have other immunoregulatory properties. Together these drugs reduce the host immune response to avoid graft rejection but unfortunately contribute to viral reactivation.

There is no single risk factor strongly associated with the development of PVAN in renal transplant recipients. Rather, a variety of weakly associated risk factors have been identified for BKPyV reactivation. Pre-transplantation risk factors can be divided into recipient and graft characteristics. Recipient characteristic risk factors include: older age, male gender, seronegative recipient, and previous graft loss caused by PVAN. Graft characteristic risk factors include: seropositive donor, degree of HLA mismatch, and ischemic or immunological injury. The immunosuppressive regimen is the major post-transplantation factor identified in virus reactivation [29]. It is thought that the degree of immunosuppression, and not the specific therapy, contributes to PVAN development. For instance, the incidence of PVAN increased with the advent of more potent calcineurin inhibitors such as tacrolimus and mycophenolic acid [28,30]. In addition, there are viral factors that may increase the relative risk of BKPyV reactivation. For example, mutations within the BKPyV VP1 capsid protein have been associated with increased pathogenesis [31]. None of these risk factors can explain a majority of BKPyV reactivation events; therefore there may be as yet unidentified risk factors or a combination of risk factors that contribute to the development of PVAN.

Viral reactivation in PVAN is characterized by intranuclear viral inclusions in epithelial cells of the renal tubules, collecting ducts, and glomeruli [32]. The first signs of PVAN disease are increased serum creatinine levels followed by BKPyV viruria, which can be detected in 35–57% of kidney transplant recipients [33]. BKPyV viruria can be diagnosed by PCR for viral DNA or identification of decoy cells in the urine. After viruria, 13–22% of PVAN patients develop a BKPyV viremia [33]. The final stage of PVAN is tubular atrophy, interstitial fibrosis, and inflammation leading to potential graft loss [34]. Lesions caused by BKPyV lytic reactivation can occur sporadically throughout the graft; therefore a negative biopsy result cannot rule out a PVAN diagnosis. The gold standard for PVAN diagnosis is a biopsy of the affected kidney.

3.3. Other diseases

Although rare, BKPyV is reported to cause other diseases in severely immunocompromised patients such as fatal pneumonia, native kidney nephritis and encephalitis [35–37]. BKPyV

is also rarely able to cause disease in the native kidney of transplant and non-transplant patients [38]. More recently, there have been increased reports of native kidney BKPyV nephropathy in pediatric heart and bone marrow transplant patients [39–41].

4. Cellular entry

In order to establish a persistent or lytic infection and cause disease, BKPyV must be internalized into a host cell type that is permissive to infection. As mentioned above, this may include a number of different cell types and different tissues and organs throughout the body. The main site of reactivation and replication, however, is within the kidneys and urinary tract, and thus the cell types commonly used for studies of cellular entry are cell lines or primary cells of renal epithelial origin.

BKPyV is a non-enveloped virus, and thus does not include a surrounding lipid bilayer but consists of only a protein capsid and DNA. The BKPyV particle consists mainly of three molecules that serve as the building blocks for the structure of the viral capsid: the major structural protein VP1 and less abundant minor capsid proteins VP2 and VP3. The capsid surrounds the double-stranded circular DNA genome that is complexed with histones in the form of a miniature chromosome. The outer shell of the virus capsid consists solely of VP1 proteins arranged in pentamers in a $T = 7$ icosahedral lattice, and these pentamers are stabilized by intra- and inter-pentameric disulfide bonds and calcium cations. Sixty of the 72 pentamers, or capsomeres, are coordinated with six adjacent pentamers, and the other twelve of the 72 pentamers are located on the icosahedral vertices of the capsid, five-coordinated with surrounding pentamers [42]. The importance of these different arrangements within the VP1 shell is likely seen during the capsid disassembly process, as differing inter-pentameric interactions require stepwise uncoating, and this gradual disassembly is used during intracellular trafficking towards the nucleus [43]. On the internal face of each VP1 pentamer, not surface-exposed on the intact viral particle, is one molecule of either VP2 or VP3. The minor capsid proteins are expressed from the same late mRNA transcript and share a C-terminal amino acid sequence, but VP2 has a unique set of N-terminal amino acids that include a putative myristoylation site. Both SV40 and mouse polyomavirus (MPyV) have been shown to contain myristoylated VP2 [44]. The stoichiometric ratio of VP2 to VP3 is not equal, and the functional significance of that ratio is unknown; however, it is believed that each of these minor capsid proteins plays a unique and vital role in cellular entry and assembly based what has been seen with SV40 [45,46]. Together, the features of this simple particle somehow play a role in both stabilizing the virus outside of the cell and enabling the virus to traverse the obstacle course of the cellular interior and eventually enter the nucleus.

4.1. Receptor binding

Successful infection of a cell requires attachment to the cell surface and functional interaction with the surface receptor that will lead to internalization of the virus and targeting to a productive pathway. The presence or absence of a functional receptor often plays a determining role in the tropism of viruses. Initial studies to identify the nature of the BKPyV receptor clearly pointed to gangliosides as an important factor. Hemagglutination was observed in the very first characterization assays for BKPyV, and this could be inhibited by preincubating with gangliosides [47]. Many of the first receptor studies in the context of virus infection were done with Vero cells, a monkey-derived kidney cell line, but were later repeated with the relevant cell culture model of human renal proximal tubule epithelial (RPTE) cells [48]. Pretreatment of RPTE cells with neuraminidase, but not proteinase K, abolished binding and infection. Studies with RPTE cells verified the presence of a sialic acid component, and went on to identify the specific gangliosides GD1b and GT1b as binding partners in a sucrose flotation assay. Infection of nonpermissive cells is made possible by the exogenous addition of each of these gangliosides [49]. Likewise, infection of

RPTE cells with SV40 is not possible unless its ganglioside receptor, GM1, is added to the cells exogenously [48]. A commonality between the GD1b and GT1b gangliosides is an α 2-8-linked disialic acid motif, but whether or not this presents a potential site of interaction between receptor and capsid remains to be determined, and this will require the 3-dimensional structure of the capsid with the receptor.

4.2. Internalization and intracellular trafficking

After binding its receptor, BKPyV must enter the cell and successfully traffic through the cytoplasm toward the nucleus, where the uncoated viral genome can utilize the cellular machinery for transcription and its genome replication. Early studies to determine the cellular entry pathway taken by BKPyV were based on transmission electron microscopy (TEM) of infected cells at various times post infection, and these images show virions taken into the cell by small, tight-fitting, non-coated vesicles, trafficking to smooth tubular structures contiguous with rough endoplasmic reticulum (ER), and entering the nucleus as relatively intact particles. Since single viral particles could be seen in the nucleus at a relatively early time before progeny would likely have been produced and because they were smaller than plasma-membrane localized virions, these are believed to be incoming virus [50,51]. Besides the information from TEM of BKPyV-infected cells, our understanding of the entry pathway of BKPyV was also initially based on inferences made from findings with SV40, and to some extent with the more distantly related MPyV, due to their earlier discovery and established cell culture models. However, it has recently become clear that interesting and significant differences exist between the biology of BKPyV and other polyomaviruses, including the role of different ganglioside receptors, as mentioned above. Thus, more detailed analyses of the specific interaction between virus and host cell will be necessary to gain a deeper understanding of how BKPyV establishes an infection.

Following receptor binding, internalization of BKPyV is dependent on a caveolae-mediated endocytic pathway [52]. This differs from what was found with JCPyV, which relies on clathrin-dependent receptor-mediated endocytosis [53]. Caveolae are a type of specialized microdomain invagination in the plasma membrane that are enriched in cholesterol, and formation is dependent on caveolin proteins such as caveolin-1 [54]. Caveolae-mediated entry by BKPyV was uncovered with data from both RPTE cells and Vero cells showing that expression of a dominant-negative caveolin-1 construct decreased infection, and depletion of membrane cholesterol with methyl- β -cyclodextrin had an inhibitory effect, since caveolae are disrupted with cholesterol depletion [55,56]. Additionally, expression of an Eps15 dominant-negative construct, which prevents clathrin-dependent endocytosis by targeting a necessary component in assembly of clathrin pits [57], did not prevent infection in Vero cells [56]. Verification of the pathway was also confirmed when co-localization of fluorescently labeled virions with cholera toxin B, which serves as a marker of caveolae endocytosis, was observed during synchronized entry [55,56]. Additionally, BKPyV allograft nephropathy analyzed by EM analysis shows virions close to the plasma membrane in 60–70 nm smooth vesicles whose description is consistent with that of caveolae, confirming this vesicular route in a natural infection setting [56].

Studies show internalization to be relatively slow and nonsynchronized [56,58]. The details of the endocytic pathway taken by BKPyV have not yet been defined. However, it is known that SV40 depends on the classical endocytic route through early and late endosomes towards endolysosomes and relies on acidification and maturation of endosomes for entry [59]. It is likely the route taken by BKPyV as well, and it has been shown that BKPyV requires an acidification step very early during entry in RPTE cells [56,58]. Although JCPyV enters via a clathrin-mediated route, it is taken into early endosomes like SV40, and the virus is then directed to an unidentified compartment by a caveolin-1 dependent step [60]. These studies together show that cross-talk between endocytic pathways allows for

differences in the initial entry steps of receptor-mediated binding and endocytosis between each virus, while later intracellular trafficking steps are likely more parallel as the virus reaches the ER [61].

Approaches using various inhibitor treatments of infected cells have established the need for microtubules during trafficking, but apparently not actin [58,62]. Treatment with the drug nocodazole, which destabilizes microtubules, was found to inhibit infection at an early time point, as well as tests with the microtubule destabilizing agent colcemid in RPTE cells. Paclitaxel, a microtubule stabilizer, inhibited infection in RPTE cells as well, which was surprisingly different from what was seen in Vero cells, and supports the relevancy of an *in vitro* primary host cell model [62,63]. Another study addressed the role of dynein, the microtubule motor protein, using the drug EHNA that inhibits the ATPase of the dynein complex, and showed independence of BKPyV infection from dynein in RPTE cells [55]. Interestingly, however, this study supports the idea that the Golgi apparatus is bypassed during the retrograde trafficking route of BKPyV, since EHNA disrupts the morphology of the Golgi apparatus but did not affect BKPyV infection.

4.3. Virus uncoating

Trafficking through the ER seems to be a necessary step for all polyomaviruses. This aspect of infection is unique among DNA viruses, which raises the question of what benefit the ER provides during entry. The main advantage within the compartment of the ER is that it contains chaperones, disulfide isomerases, and reductases of which the virus can make use to facilitate the capsid uncoating process. Evidence from SV40 and MPyV points toward involvement of the ER-localized protein disulfide isomerase family in providing the reduction and isomerization of the VP1 capsid proteins that provides an initial and vital step in the uncoating process [43,64]. The interactions and subsequent conformational changes that occur within the ER compartment likely also provide the means for the virion to continue on its pathway into the nucleus.

Many ER resident host factors that are involved in infection have been elucidated for SV40 and MPyV, providing a theoretical framework for the interactions of BKPyV during ER trafficking and uncoating. It was found for MPyV that interaction with the protein disulfide isomerase (PDI) family member ERp29 causes a conformational change in VP1, shown by exposure of a trypsin-sensitive site, and that this actually results in the viral particle being able to interact with a lipid bilayer [65]. Other PDI family members PDI, ERp57, and ERp72 were subsequently shown to work in concert for reduction and isomerization of specific disulfide bonds within the MPyV capsid, and these changes are necessary for uncoating [64]. Schelhaas et al. have shown that the same scenario and host factors are important for SV40, and also implicated a role for certain ER transmembrane proteins in facilitating trafficking of the virus out of the ER [43].

MPyV was also found to require the ER transmembrane protein Derlin-2 for infection [66]. Involvement of members of the Derlin-family proteins provides an interesting link between infection and the process of ER-associated degradation (ERAD), a cellular quality control mechanism for sending misfolded proteins out of the ER into the cytosol where they are degraded by the proteasome [67]. Derlins have been found to be important for infection with both MPyV and SV40, as well as BKPyV. Derlin-1 was found to be essential for BKPyV infection by transiently expressing a dominant-negative construct during infection, and was implicated in SV40 infection through siRNA-mediated silencing [43,58]. A role for the proteasome has also been shown during BKPyV infection, as well as for SV40, and this suggests the importance of the ubiquitin-proteasome system during entry, which along with the requirement for Derlin-1, implicates the ERAD pathway during polyomavirus infection [43,58,68]. A few recent studies with SV40 have identified components of the ERAD

retrotranslocation machinery in SV40 trafficking, and were able to demonstrate directly that viral trafficking from the ER to the cytosol was inhibited without the presence of ER chaperones BiP and its interacting partner DNAJB11, as well as the transmembrane shuttle BAP31 [69,70]. The minor capsid proteins VP2 and VP3 were also shown to be required for this retrotranslocation across the ER membrane by use of a VP1 pseudovirion, a particle containing VP1 proteins only, which was unable to enter the cytosol [69]. It has yet to be elucidated what mechanisms are used for BKPyV trafficking from the ER to the nucleus, but evidence suggests that use of the ERAD pathway to enter the cytosol is conserved among polyomaviruses.

4.4. Nuclear entry

The entry pathway of BKPyV into the nucleus is currently unknown. Evidence suggests for SV40 that the virus may use the canonical route through the nuclear pore complex. Because work by the Tsai lab on the exit of SV40 from the ER implies trafficking through the cytosol en route to the nucleus, it is reasonable to hypothesize that the virion would then use the established pore in the nuclear membrane to enter the nucleoplasm. The full sized virion at ~45 nm would be too big to fit through the nuclear pore, but partial disassembly could allow it to pass through if smaller than 39 nm [71,72]. A nuclear localization signal is located on each of the capsid proteins but none are exposed, so partial disassembly that occurs in the ER and possibly cytosol would be a likely prerequisite for the canonical use of this pathway, especially since evidence from SV40 suggests that the minor capsid proteins are required for nuclear entry [46].

Nakanishi et al. have provided a notable amount of evidence showing the use of the canonical nuclear import pathway by SV40 for nuclear entry through the nuclear pore. SV40 DNA can be co-immunoprecipitated with importin alpha and beta, and the importins can be coimmunoprecipitated with VP3 during time points early in infection. They also showed that the nuclear localization signal on the minor capsid protein VP3 is vital for infection and importantly nuclear entry, implying that the signal becomes exposed during infection and is used for nuclear import through the pore [73,74].

5. Transcription and replication

5.1. Regulatory proteins

Once the uncoated viral genome is inside the nucleus, BKPyV takes advantage of nuclear factors to allow the temporal expression of its viral genes. The three regulatory proteins, Large Tumor (T) Antigen (TAg), small T antigen (tAg), and truncated T antigen (truncTAg) are expressed first, followed by initiation of replication, and then late gene expression. The early and late promoters are located in the NCCR, in opposite orientations like their coding regions, on the 5.2 kb genome. The NCCR additionally contains the origin of replication, and multiple binding sites for regulatory factors of cellular origin have been identified within this regulatory region [75]. The early promoter drives expression of a primary transcript that is alternatively spliced to allow for expression of the three early proteins. Once the regulatory function of TAg is active, DNA replication begins followed by expression of the late proteins VP1, VP2, VP3, and agnoprotein [10].

TAg is an impressively multifunctional regulatory protein, responsible for directly interacting with the viral genome in its regulatory capacity to initiate DNA unwinding and transcription, as well as exhibiting functions that allow expression of the cellular DNA synthetic machinery that can support viral replication. These functions include binding to the retinoblastoma (Rb) protein and its family members (p107 and p130) to alleviate E2F-mediated transcriptional repression, as well as binding to p53 and inhibiting its downstream tumor suppressor targets to block apoptosis and allow maximal production of viral progeny

[76,77]. The tAg shares an N-terminus with TAg, and thus shares the interactions and functions mediated by the domains located in the N-terminus, such as the J-domain [78]. One unique function of tAg is its inactivation of protein phosphatase 2A via formation of a complex with the catalytic and regulatory subunits. This leads to increased cyclins D1 and A, and downregulated p27, which are factors in the control of cell cycle progression [79–81]. Any unique functions of the truncTAg protein have yet to be elucidated; however, it shares 133 N-terminal amino acids with TAg followed by three unique amino acids, and thus likely serves some redundant roles for TAg functions [82].

The cell cycle-altering functions of the regulatory proteins confer oncogenic properties to BKPyV; however, no convincing evidence of oncogenic transformation has been found in relation to BKPyV infection of human tissue. Injection of BKPyV into rodents does lead to formation of multiple tumors, prompting the question of what host factor interactions lead to specific outcomes during infection [83]. The viral genome remains episomal in human cells after infection, while in rodent cell lines it undergoes integration into the cellular DNA [75]. Identification of differences in the molecular mechanisms at work in the nuclei of each species may provide insights into the basis for maintenance, latency, and lytic replication.

5.2. Replication

After synthesis of the regulatory proteins and establishment of the DNA synthesis-active cellular environment, replication of the viral genome occurs. Replication begins at the origin of replication that sits within the NCCR. TAg binds to the viral origin and assembles into a double hexamer, which leads to its hydrolysis of ATP, initial unwinding of the viral chromatin through its helicase activity, and recruitment of the cellular DNA polymerase α /primase complex [83]. Synthesis occurs in both directions from the replication origin until the replication forks meet on the opposite side of the genome. Besides TAg, the minimal molecular requirements for the DNA replication of SV40 include, RPA, DNA polymerase α /primase, topoisomerase I, RFC, DNA polymerase delta, and PCNA; this provides a likely scenario for the minimal requirements of BKPyV as well [84].

Replication of archetype BKPyV has previously been a problem to study in cell culture models. Recently, however, Broekema and Imperiale showed propagation of archetype BKPyV in 293TT cells, a human embryonic kidney cell line that overexpresses SV40 TAg [85]. The abundance of this exogenous regulatory factor was able to compensate for the usually undetectable level of BKPyV TAg expression from the archetype virus. Rearranged NCCR BKPyV variants, however, grow in cell culture, and thus far these variants have been used as the experimental laboratory system. The archetype BKPyV NCCR is characterized by a linear O-P-Q-R-S block structure, where O is named for the origin of replication and the blocks that follow are named arbitrarily. The early and late region promoters are located in the P-Q-R-S region. Rearranged variants are characterized by deletions and duplications of these blocks of sequence within the NCCR. It has yet to be determined which differences between the archetype and rearranged NCCR variants affect replication ability, since archetype is usually the shed form and thus is able to replicate somewhere within the host. It is possible that the permissive cell type for archetype BKPyV has not yet been discovered. Some investigations have addressed the levels of replication and progeny production in different NCCR variants, but little information nor a pattern has emerged as to which of the arbitrary blocks, duplications, or deletions affect the ability of BKPyV to replicate in cell culture [75,86,87].

Robust late mRNA expression for production of the structural proteins occurs after the regulatory proteins have been produced and begun initiation of DNA replication [75]. The capsid proteins VP1, VP2, and VP3 are produced in the cytoplasm and contain nuclear localization signals in order to be imported into the nucleus, where virion assembly occurs.

Progeny virus particles form when capsomeres assemble around newly synthesized genomes. These particles can also incorporate plasmids of non-viral origin or small cellular DNAs [88]. Assembly can also result in empty particles that do not contain any encapsidated nucleic acid [42]. New progeny virions accumulate within the nucleus and can be seen clearly by TEM, often in a crystalline lattice arrangement, as a sizeable nuclear inclusion. The means of viral egress is not clearly defined; however it is believed that profuse accumulation of viral progeny particles within the nucleus causes engorgement and strain on the nuclear envelope and eventual lysis of the cell [50].

6. Host immune responses to BKPyV

6.1. Intrinsic immunity

Intrinsic immunity refers to cell-based responses, usually expressed at a constitutive level, which have antiviral properties. One example of intrinsic immunity is promyelocytic leukemia nuclear bodies (PML-NBs), which are nuclear substructures possessing antiviral effects against both DNA and RNA viruses. BKPyV is hypothesized to interfere with these antiviral effects by reorganizing PML-NBs and causing the dispersion of two of the major protein components constitutively present in PML-NBs, Daxx and SP100 [89].

6.2. Innate immunity

The innate immune system is important for host antiviral defense. Currently, however, there is dearth of data about the interaction between BKPyV and the innate immune system. One study identified a group of pre-transplant patients with reduced dendritic cell numbers who were at increased risk of developing BKPyV viremia following transplantation [90]. Dendritic cells are important for antigen presentation and activation of T cells. Reduced numbers of dendritic cells could therefore impair the immune response to BKPyV. These data suggest that PVAN development may be linked to a host characteristic which predisposes an individual to BKPyV disease and highlights the importance of the innate immune system in controlling viral reactivation.

Other innate immune mechanisms, such as human antimicrobial defensins and antiviral cytokines, have been shown to be important for controlling BKPyV. Defensins are small excreted peptides that form intramolecular disulfide bonds and have been shown to inhibit both enveloped and non-enveloped virus infection. Human alpha defensin 5 was shown to inhibit infection of BKPyV by interacting directly with the virus particle, causing aggregation and reduced cellular attachment [91]. In addition to defensins, cytokines have been shown to regulate BKPyV infection. Interferon- γ inhibits BKPyV replication by inhibiting viral transcription through an as yet unknown mechanism [92]. However, microarray analyses of infected RPTE cells have shown that BKPyV does not induce the expression of genes of the host innate immune response such as cytokines [93]. This suggests that the virus may have a means to evade or interfere with the innate response. Nevertheless, these host immune mechanisms may be compromised in a transplant patient receiving high levels of immunosuppression.

Recently, a possible active mechanism of BKPyV immune evasion was described. BKPyV and JCPyV encode a miRNA on the late strand that, by analogy to SV40, targets early mRNAs for degradation [94]. These miRNAs have an identical 3-prime arm that also targets the stress-induced ligand ULBP3. ULBP3 is recognized by NKG2D, a receptor for natural killer (NK) cells. Therefore, the viral miRNA aids in the evasion of immune recognition and killing of infected cells by NK cells [95].

6.3. Adaptive immunity

BKPyV-specific T cell responses have been shown to control BKPyV replication. Studies have shown that weak T cells responses are linked to recurrent BKPyV viremia, and when BKPyV-specific T cells are reconstituted, viral replication is repressed [90]. However, there are also data to suggest that high levels of BKPyV-specific T cells can contribute to immunopathogenic disease in PVAN patients [90]. In this study, two patients who lost their grafts due to PVAN were found to have an accumulation of BKPyV-specific CD8 T cells in the graft. Babel et al. suggest that T cell responses may serve a beneficial role early in BKPyV infection but an immunopathogenic role late in infection [90].

In contrast to cellular immunity, humoral immunity is not thought to play a major protective role in patients with BKPyV disease. One study found that in PVAN patients a strong cytotoxic T lymphocyte (CTL) response and low BKPyV antibody titers were associated with reduced viral loads in the blood and urine, while a weak CTL response and high BKPyV antibody titers correlated with viral load persistence [96]. This suggests that the T cell response, and not the antibody response, is more important for controlling BKPyV reactivation. The role for neutralizing antibody as a possible treatment for BKPyV diseases will be discussed in the following section.

7. Drug treatment of BKPyV-associated diseases

The current treatment for PVAN is reduction of immunosuppression, which risks graft rejection. The FDA has not approved drugs specifically for the treatment of BKPyV infection. However, several drugs that are approved to treat infections with other DNA viruses such as herpes simplex virus (HSV), CMV, and hepatitis B virus have been tested for their efficacy against BKPyV [97]. All of these drugs act by inhibiting DNA polymerases, effectively halting viral replication. Many of these drugs have been used in combination with immunosuppression reduction in patients with BKPyV reactivation. This review will address cidofovir, leflunomide, fluoroquinolones, mammalian target of rapamycin (mTOR) inhibitors, and intravenous immunoglobulin (IVIG).

7.1. Cidofovir

Cidofovir is a nucleoside analog that reduces CMV replication by inhibiting the viral DNA polymerase. The mechanism of action against BKPyV remains unclear. Cidofovir is currently approved for the treatment of CMV-induced retinitis in HIV-infected patients [98] and has *in vitro* activity against BKPyV; however, there are conflicting reports of *in vivo* activity. Cidofovir has been used to treat BKPyV-associated HC with simultaneous CMV infection and was found to reduce both CMV replication and the level of BKPyV viruria, and resulted in clinical improvement [99]. Low-dose cidofovir treatment has been used in BMT with positive clinical outcomes and decreased viruria and viremia in 84% and 47% of patients, respectively [99]. In contrast, there are also reports claiming the deleterious effects of reduced renal function and increased viral load in cidofovir-treated PVAN patients: cidofovir has limited treatment potential in renal transplant patients due to its nephrotoxicity and its limited oral bioavailability [100]. The variable and conflicting results of cidofovir treatment in BKPyV disease highlight the need for randomized clinical drug trials. The first randomized clinical trial for the use of cidofovir in the treatment of PVAN in renal transplant patients is in progress (ClinicalTrials.gov).

7.2. CMX001

Recently, a trial was completed with the compound CMX001 (phosphonic acid, [[(S)-2-(4-aminio-2-oxo-1(2H)-pyrimidinyl)-1-(hydroxymethyl)ethoxy)methyl]mono[3-(hexadecyloxy)propyl] ester; hexadecyloxypropyl cidofovir), which is a derivative of

cidofovir (ClinicalTrials.gov). CMX001 is superior to cidofovir in that it is orally delivered, rather than intravenously, is a bioavailable lipid conjugate with reduced nephrotoxicity, and is able to inhibit BKPyV replication in RPTE cells more rapidly and with a longer-lasting effect [101]. This compound was tested in renal transplant and BMT recipients but the results of this clinical study were not yet available at the time this review was written.

7.3. Leflunomide

Leflunomide is an immunosuppressive agent and is known to block dihydroorotate dehydrogenase, tyrosine kinase, and pyrimidine synthesis, and has modest activity *in vitro* in blocking BKPyV viral replication [102]. Leflunomide is licensed for the treatment of rheumatoid arthritis and has been used in combination with immunosuppression reduction in the treatment of BKPyV-associated diseases. It also has *in vivo* activity against other DNA viruses such as CMV and HSV [102]. Like cidofovir, there are conflicting reports of *in vitro* activity against BKPyV replication in RPTE cells [102,103]. A retrospective study of 26 kidney transplant recipients found that leflunomide treatment decreased viral loads in the plasma and urine compared to those not treated with leflunomide [104]. Recently, a randomized phase 2 trial was completed with FK778, a derivative of leflunomide, and was found to significantly decrease viremia in patients, although graft function was not improved and a higher incidence of acute rejection occurred compared to untreated controls [105].

7.4. Fluoroquinolones

Fluoroquinolones such as ciprofloxacin and gatafloxacin have also been used in BKPyV treatment in combination with immunosuppression reduction and have been shown to have *in vitro* activity against BKPyV [106]. These antibiotics inhibit bacterial DNA replication by inhibiting type II topoisomerases and are thought to have activity against the viral helicase, TAg [107]. In a retrospective study to assess the use of fluoroquinolones as a prophylactic therapy for PVAN in kidney transplant patients, a one month course of ciprofloxacin or levofloxacin resulted in a significantly reduced rate of BKPyV viremia [108]. Ciprofloxacin has also been used to treat BMT patients prophylactically and was shown to decrease HC incidence compared to immunosuppression reduction alone [109]. Seven of ten renal transplant patients treated with gatifloxacin had an 80% or greater reduction in viremia or a disappearance of decoy cells in the urine; however this drug has subsequently been pulled from the market due to severe adverse side effects such as liver damage.

7.5. mTOR inhibitors

Recently, mTOR inhibitors have gained popularity as a possible drug treatment for PVAN. mTOR inhibitors such as sirolimus reduce translation and cell cycle progression, and act as immunosuppressants themselves. In a retrospective study of fifteen renal transplant recipients, those patients receiving an mTOR inhibitor compared to immunosuppression reduction alone or in combination with other drug treatments cleared BKPyV viruria and viremia faster and had greater improvement of allograft function compared to those patients not receiving mTOR inhibitors [110]. Recently, mTOR inhibitors were reported to improve CD8 T cell memory, which could account for improved BKPyV clearance [111]. However, there is one account of BKPyV viral clearance only after termination of sirolimus in a PVAN patient and one study in pediatric renal patients listed BKPyV viremia as a complication of sirolimus treatment [112,113]. Therefore, like most drugs used in the treatment of BKPyV reactivation, a randomized controlled study will be necessary to determine if mTOR inhibitors are beneficial for the treatment of BKPyV-associated diseases.

7.6. Intravenous immunoglobulins

BKPyV neutralizing antibodies present in intravenous immunoglobulins (IVIG) have been used in patient trials with immunosuppression reduction in treating BKPyV-associated diseases. Although, the humoral response is not sufficient to limit BKPyV replication, IVIG treatment is thought to have anti-inflammatory properties as well as contain BKPyV neutralizing activities. There are studies which appear to have positive outcomes in patients treated with IVIG therapy. However, recently there was a report of an increase in the plasma BKPyV viral load in a PVAN patient after IVIG therapy [114]. High antibody titers in the recipient do not correlate with protection from PVAN [115]. However, recipients who receive kidneys from donors with high titers are more likely to develop viruria [116].

8. Future areas of research

BKPyV-associated diseases are a significant problem in a growing number of immunosuppressed transplant patients. There are many unanswered questions in the field of BKPyV infection. One major impediment in the field is the lack of animal models for this human-specific pathogen. There is an MPyV model of PVAN but this virus has a somewhat different genomic structure and it is unclear how well this model mimics human PVAN as mice do not undergo acute rejection. Future research is needed to determine the mechanism of viral persistence and reactivation in the host. While reduction in immunosuppression remains the standard of care, currently there is no specific efficacious antiviral treatment for BKPyV-associated diseases. Therefore, an *in vitro* drug screen against BKPyV replication in RPTE cells would be useful to identify new viral targets for drug treatment. Alternatively, screens could be conducted to identify host components necessary for BKPyV infection. Additionally, it will be important to conduct randomized clinical trials to determine which, if any, antivirals should be used in treating BKPyV-associated diseases.

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