MOLECULAR VIROLOGY AND CLINICAL ASPECTS OF THE HUMAN POLYOMAVIRUS BK INFECTION

S. Slavov, Z. Kalvatchev
National Center of Infectious and Parasitic Diseases, Laboratory of Molecular Virology, Sofia, Bulgaria

ABSTRACT
Polyomavirus BK (Polyomavirus hominis 1) is a nonenveloped human polyomavirus, belonging to Polyomaviridae family. It infects up to 90% of the general population. Infection is acquired during early childhood and is mainly asymptomatic in individuals with normal immune status but in those with impaired immune system and renal allografts the situation is quite different. They develop hemorrhagic cystitis, interstitial nephritis, vasculopathy, even interstitial pneumonia, retinitis, autoimmune diseases and graft failure. There are data regarding its role in different human neoplasia of the central nervous system, renal pelvis and bones. Proper laboratory diagnosis is of critical importance in monitoring transplantation patients thus lowering the risk of allograft rejection. This review summarizes actual information about molecular characteristics, clinical manifestations and virology diagnosis of BKV infection.

Introduction
Polyomavirus hominis 1, better known as BK virus (BKV), is a member of the Polyomaviridae family. It is ubiquitous agent throughout the world population causing asymptomatic infection during the early childhood in up to 90% of the people. It may be occasionally associated with clinical illness, especially in individuals with impaired immunity. BKV induced clinical signs are hemorrhagic cystitis, ureteral obstruction, vasculopathy, pneumonia, retinitis and even multi-organ failure. Now there are data of its implication in autoimmune diseases and possibly cancer. BKV disease is a complicated process requiring complementing determinants in the host, the target organ, and possibly the virus. These aspects are connected in the so called polyomavirus associated nephropathy, a disease causing allograft dysfunction and graft loss. There are no specific antivirals and improving immune control plays a vital role in graft recipients. The quick laboratory diagnosis including urine cytology and BKV-load measurements in plasma increases the success of transplantation. Thus reduction of immunosuppressive therapy together with some antivirals provides better outcome for the transplant patients.

In this review we summarize information connected with its molecular characteristics, clinical manifestations and laboratory diagnosis of BKV infection.

BKV molecular biology
BK virus is a nonenveloped virus measuring approximately 50 nm in diameter (Fig. 1). The capsid has icosahedral symmetry and is built of 72 capsomers that are all pentamers of the protein VP1 arranged in a T=7 icosahedral lattice.

All known polyomaviruses have three structural proteins (VP1, VP2, and VP3), of which VP1 is the major capsid protein. Overall amino acid sequence homology between BKV and the other human polyomavirus, JCV, is 75%, and that with the simian polyomavirus (SV40) is 69%.
Due to the high similarity, the solved atomic structure of VP1 of SV40 creates a model of the BKV VP1 protein folding. The VP1 pentamer of SV40 is built as a ring of five β-barrel-shaped VP1 monomers, tightly linked by interacting loops between the frameworks of β-strands. The C-terminal subdomain of each VP1 monomer “invades” a neighboring pentamer, thereby tying the pentamers together in the virion shell. There are six unique monomers building up the capsid (monomer α, α’, and α” at the local threefold; β, β’ around the icosahedral threefold, and γ at the twofold). The major structural differences between the six unique monomers are found in their C termini and are essential for the formation of the icosahedral capsid. The most flexible region is the outermost C terminus of the peptide chain (amino acids 355 to 364). The structure of this region has not been defined in three of the six unique monomers (α, α”, and β). Under certain conditions the capsid disassembles into pentamers, which can be reassembled. The reassembled particle may have different configurations and sizes depending on the conditions. This was shown for recombinant SV40 virus like particles (VPLs) and recombinant murine polyoma VLPs. Through computational modeling is concluded that there are two types of symmetry of the reassembled particles icosahedral symmetry - composed of 12 and 72 pentamers, in a $T=1$ and $T=7$, respectively, surface lattice and octahedral symmetry of 24 pentamers. Mutations in VP-1 region of the genome are associated with increased pathogenicity of polyomavirus in experimental systems. VP-1 sequences undergo continual modification. This genetic instability could conceivably have implications for evasion of host immunity and development of resistance to antiviral drugs (19, 25).

BKV encodes three non-capsid regulatory proteins: large T-antigen, small t-antigen, and agnoprotein. These proteins interact with a number of cellular target proteins to exert effects that dysregulate pathways involved in the control of various host cell functions including the cell cycle, DNA repair, and others (33).

Large T antigen (LT-Ag) is responsible for the transforming functions of the virus. It can both inhibit the cellular response to DNA damage and induce proliferation, allowing for potential accumulation of mutations in cellular growth control genes. This suggests a possible role for BKV LT-Ag in cellular transformation and tumor formation in the human host (13). LT-Ag contains multiple domains some of which inactivate proteins of the retinoblastoma family and the p53 to overcome cell cycle control and subvert apoptosis. One of the many functions of LT-Ag DNA is unwinding/helicase activity thus LT-Ag
regulates DNA replication as well as late gene expression in concert with activated and basic host-cell transcription factors (15).

The small t antigen is cysteine-rich protein whose 82 terminal acids are shared with LT-Ag. Small t antigen is located both in the nucleus and the cytoplasm. It is important for the lytic virus cycle and promotes G1 phase progression. Also it increases the efficacy of virus transformation through activation of growth factor stimulated signaling pathways (19).

The late region of the BKV encodes a small, highly basic protein known as agnoprotein. It has become increasingly evident that agnoprotein has a critical role in the regulation of viral gene expression and replication, and in the modulation of certain important host cell functions including cell cycle progression and DNA repair (13, 19).

The viral genome (Fig. 2) is a circular double-stranded DNA of 5153 base pairs (bp), coated by host-cell histones (H3, H4, H2A, H2B). The BKV genome shares an overall homology of 75% with JCV and of 70% with SV40, and can be divided into regulatory, early, and late region. After virion uptake by endocytosis of non-coated caveolae, BKV genomes are released into the host-cell nucleus. Early gene expression and replication of BKV is needed before late gene expression and replication of the BKV minichromosome is needed before late gene expression ensues and the virions are assembled in the nucleus. Electron microscopy at this stage shows dense, even crystal-like arrays of particles in the nucleus, which are in fact nuclear inclusion structures. "Decoy cells" exfoliated into the urine are prototypes of this stage. Infectious viral progeny is released by host cell lysis rendering BKV replication per se cytopathic (15).

The noncoding control region (NCCR) is believed to contain enhancer elements that are important activators of viral transcription (26). It is located between the origin of replication and the start site of the late leader protein (agnoprotein). In most BKV strains, this regulatory region contains tandem repeats varying in size. Recently, however, new BKV strains are lacking such repeat sequences (29). The BKV NCCR readily undergoes DNA sequence rearrangement during passage of the virus in cell culture. Therefore, the NCCRs of BKV isolates obtained by viral culture could contain alterations introduced in vitro. In contrast, those obtained by molecular cloning or PCR represent naturally occurring BKV transcriptional control regions. Analysis of BKV NCCRs isolated using the latter method thus revealed that naturally occurring BKV NCCRs have a common structure named the archetype (31). Various strains of human polyomavirus BKV show marked heterogeneity in the NCCR (5, 10). The results show that rearranged BKV NCCR is associated with BKV nephropathy and high virulence and different biological characteristics of the BKV strains (30). The rearrangements of NCCR change the number of transcription factor binding sites NF1, SP1, CREB, c-myc, c-myb, oestrogen, and also glucocorticoid receptor. The rearrangements are probably adaptive changes bestowing in-
creased “viral acclimatization”, in the respective host cells.

Early genes encode the LT-Ag, as main regulatory protein and the small T-Ag with supporting function. Late gene expression is initiated after viral DNA replication has started. After translation in the cytoplasm, the viral capsid proteins VP1, VP2, and VP3 are transported to the nucleus for viral assembling and this is supported by the agnoprotein. The antigenic determinants of the BKV subtypes are located between aminoacids 61-83 of the VP1, which is also responsible for adsorption to the host cell. Serological and genetic studies indicate that there are at least four distinct subtypes I (prototype PT, Dunlop Dun, Gardner GS, and MM), II (SB), III (AS), and IV (IV and MG) circulating with the type I being the most prevalent (15). Little is known of the membrane receptor and entry pathway for BKV. BKV was found to interact with gangliosides GT1b and GD 1b. The terminal alpha 2-8 linked disialic acid motif, present in both of these gangliosides, is likely to be important for this interaction. It was determined that the addition of GD1b and GT1b to LNCaP cells, normally resistant to BKV infection, makes them susceptible to the virus. In addition BKV interacts with membranes extracted from the endoplasmic reticulum and infection is blocked by addition of blefeldin A, which interferes with the transport from the endoplasmic reticulum (ER) to the Golgy apparatus. These data demonstrate that BKV uses the gangliosides GT1b and GD1b as receptors and passes through ER on the way to the nucleus (18).

Clinical manifestations

BKV latency. BKV can exist in latent phase in different organs. It can remain in the cell in nonreplicating or minimally replicating form. The genome may be left over in episomal or integrated condition. In either state, few genes are transcribed, thus keeping the virus out of reach of the immune system. The kidney is the most common site of latent infection. Other places reported for latent infection are brain and peripheral blood leukocytes. There are data concerning as places of latency pituitary gland, tonsils, nasopharynx, genital tissues, sperm, liver, bones. It is important to stress that reactivation is observed in patients with relative or absolute immunodeficiency, generally cellular immunodeficiency rather than humoral (27).

BKV viremia. Once activated the virus (viral DNA gains access to the blood stream) does not only affect the urothelium, but also spreads to collecting ducts and tubules in the highly vascularized renal medulla. It is proposed that BKV infects new cells via cell-to-cell spread because viral particles are seen on apical tubular cell surfaces by electron microscopy. Thus, BKV could follow ascending route of infection from the superficial transitional cell containing α-(2, 3) linked sialic acid is a critical component of the cellular receptor for BKV (7).
layer to collecting ducts and tubules. Lysis of inclusion bearing tubular cells releases viral particles into the tubular lumen leaving behind denuded basement membranes. The virus may enter the blood stream when tubular fluid containing viral particles leaks into the interstitium that is rich in capillaries (22).

**BKV induced interstitial nephritis and inflammation.** Interstitial nephritis (ICN) is a syndrome consisting of severe refractory bladder symptoms of unknown etiology. The disease tends to affect caucasian women in the age interval of 40 years with 25% of patients under the age of 30 (9). Interstitial inflammation in BK nephritis (BKN) is poorly understood and the interpretation of the finding is controversial. The major problem is to distinguish between virally induced interstitial nephritis and cellular rejection. This distinction is very important, because it seems to be crucial since the only current therapeutic option in BKN is reduction of immunosuppression. BKN is accompanied by a heterogeneous inflammatory reaction. The inflammatory cell infiltrate is composed of lymphocytes, macrophages, and occasional plasma cells. Non-specific inflammation contains polymorphonuclear leucocytes and is seen in response to markedly damaged tubules with urinary leakage. Tubulitis is rare and inconspicuous (22).

**BKV and hemorrhagic cystitis.** Haemorrhagic cystitis (HC) is a distinct clinical disorder of multiple etiologies. It is characterized by painful haematuria due to haemorrhagic inflammation of the urinary bladder mucosa. In allogeneic haematopoietic stem cell transplantation, HC occurring before engraftment is mostly transient and self-limiting, whereas that after engraftment is severe and sometimes life-threatening. Pre- and post-engraftment HC represent distinct disorders with different etiologies and treatment implications. Reactivation of BKV plays a cardinal role in post-engraftment HC. Urotoxicity of the conditioning regimen and allogeneic reaction accompanying graft-versus-host disease upon engraftment are also important pathogenetic factors. Based on data from BKV studies, HC may be divided into three phases. In the first phase, the conditioning regimen damages uroepithelial cells, providing a milieu for BKV replication. In the second phase, uroepithelial BKV replication leads to BK viruria. In the last phase after engraftment, allosimmunity against BKV-infected uroepithelial cells leads to HC (16). Several observations suggest an association between HC in bone marrow transplantation recipients and human polyomavirus BKV reactivation. BKV reactivation can cause, together with other factors, the majority of late HC in transplantation recipients as well as in patients treated for acute refractory lymphoblastic leukemia (1).

**BKV and transplantation.** BKV in donor kidney is a determinant of BKV infection in the recipient. Previous studies of serum specimens have shown that about a quarter of BKV infections in renal transplant recipients occur in individuals who have no detectable antibodies in their first serum, i.e. they are primary infections. This is in contrast to BKV infections in bone marrow transplant recipients and in pregnant women, which are almost always due to reactivation of latent BKV. BKV infection in renal transplant recipients occurs most frequently when kidney from a BKV-seropositive donor is transplanted into a BKV seronegative recipient. Such a donor/recipient antibody combination may be a risk factor for BKV nephropathy (28). As primary infection occurs in childhood, BKV may be particularly important in the pediatric transplant population (23). In BKV ureteritis, the graft prognosis is poor; graft loss could be due to the progress of BKV nephropathy (12). A correct differential diagnosis of the etiologic agent responsible for the ureteritis is mandatory, because treatment and outcome of the infec-
BKV and co-infections. It is reported that co-infection with BKV and SV40 occurs in renal transplant patients with BKN, suggesting that SV40 may contribute to BKN after renal transplant (17). Infections caused by BKV and JC virus occur independently, but concomitant infections and the simultaneous persistence of both viruses have been observed in renal transplant recipients. Several studies have showed a correlation between BKV and JCV induced interstitial nephritis in renal transplant recipients (21).

BKV and central nervous system infections. Although reports of BKV infections in the nervous system are rare, there is now evidence for its occurrence in immunocompromised patients and the diagnosis should be considered in such patients with neurological symptoms and signs of renal disease. The diagnosis is simple to verify and is important to establish. There are reports that BKV can cause subacute meningoencephalitis in HIV positive and HIV negative patients. In all cases the meningoenephelitis was developed in combination with severe tubulointerstitial nephropathy (27). BKV and JCV DNA in the cerebrospinal fluid of the patients suspected to have either meningitis or encephalitis suggests that these viruses may be etiologic agents for these conditions thus, diagnostic tests for BK and JC viruses should have been included in the investigation for meningitis or encephalitis (2).

BKV and immunosuppression. Inherited immune dysfunction increases the susceptibility to various infectious complications. In such cases, the major determinants of BKV disease seemed to reside in high level BKV replication in permissive organs - i.e., the autologous kidneys, most likely after primary infection of children who were unable to mount complete cellular and humoral immune responses. HIV-1 infection represents the most frequent cause of acquired immunodeficiency today. Clinical manifestations of BKV in AIDS patients are rare despite frequent reactivation. The prevalence of BKV shedding increases from 4 - 8%, to 27 - 51% when, CD4 counts fall below 200 μl. Also decay cell shedding became significantly more prevalent in HIV-patients with CD4 cell counts of less 200μl/ml. Systemic BKV disease, with multi-organ failure, has been described in HIV patients (15).

In severe long-lasting immunosuppression, highly effective virus multiplication can be accompanied by extended cytolytic damage of viral target cells leading to fatal disease (6). Patients with AIDS, develop BKV infection of the lung and kidney that progress to diffuse alveolar damage and death. The infected type II pneumocytes in the lung and the tubular epithelial cells in the kidney show large, homogenous purple intranuclear inclusions. The absence of necrosis and destruction make it possible to distinguish BKV infection from herpes simplex. The size of the infected cells and the lack of a halo around the nuclear inclusion rejected cytomegalovirus as the cause of infection (4).

Laboratory (virology) diagnosis

Primary infection is diagnosed by the detection of viral genes or gene products in a seronegative individual and secondary infection is defined as BKV replication in a previously seropositive individual. Reactivation is considered if replication of the latent or a new subtype is detected, respectively. Seroconversion or rising in antibody titers without direct proof of BKV replication is difficult for interpretation (15).

The gold standard for BK nephropathy diagnosis is still immunohistochemical staining for large T antigen in graft biopsy specimens (20). Diagnostic strategies using urine cytology and BKV load measurements in plasma have led to earlier diagnosis of BK nephropathy, which increased the success rate of intervention (15). “Decoy” cells are caused by infection of the urinary
epithelial cells with BKV and can be identified by light microscopy. The nuclei are enlarged and nuclear chromatin is completely homogenized by viral cytopathic effect. "Decoy" cells are often present before BKV disease occurs. The combined presence of inflammatory sediment and decoy cells (>10 cells/high power field) with allograft dysfunction is described as a more specific marker for BKV nephropathy. The presence of intranuclear viral inclusions, primarily within the tubular and parietal epithelium of Bowman’s capsule, helps to distinguish BKV disease from rejection (23). Cytology analysis of urine is an important diagnostic tool for screening renal transplants patients at risk of BK nephropathy (3). Compared with PCR, urine cytology is a less sensitive technique, which requires morphologically intact cells, and cannot distinguish BKV from JC virus (24).

BKV diagnosis on a renal biopsy is complicated and molecular detection by polymerase chain reaction (PCR) on serum, urine and renal tissue is recognized as a sensitive and specific method for detecting BKV in clinical samples (32). The BKV load is better measured in urine than in tissue, because a urine sample represents material from the entire kidney. There are data describing the development of a semiquantitative - nested PCR assay to simultaneously detect BKV viral load in urine and serum. The assay offers several advantages including: (a) rapid submission of clinical samples to screening; (b) verification of the absence of Taq polymerase inhibitors with the use of an internal control; (c) sensitivity threshold of 10 copies/reaction; and (d) assay running is less labor intensive, cheap, and easy to perform. The assay may be easily used to monitor viral loads in urine and serum samples from renal transplant recipients thus to detect those at risk of BKV-related nephropathy, and to monitor their response to immunosuppression reduction therapy if it occurs (21).

Monitoring the polyomaviral load in the urine and the blood of the patients using a quantitative PCR technique is a useful tool in the diagnosis and subsequent management of this infection. Even before viremia is present, an important rise in the urinary viral load should draw the attention of the transplant clinician and raise the issue of adapting the immunosuppression (14).

Multiplex nested PCR (nPCR) method was developed for detecting and differentiating simultaneously the DNA of polyomaviruses JC, BK and SV40 in a single tube. The assay is a good tool for supporting the diagnosis of polyomavirus infection and could be used for epidemiological purposes and in other studies in order to define better the role of polyomaviruses in human disease (9). Molecular biology methods are the most sensitive tool for detecting polyomavirus urinary infection in HIV-positive patients and the only reliable method of differentiating JCV and BKV viral genotypes (3).

Other methods for the diagnosis of BKV infection include ELISA and Hemagglutination Inhibition Assay. Using an antigen solid-phase immunosorbent assay (SP-ELISA), is a good method for detecting serum antibodies- IgG, IgM, IgA. The same sensitivity shows Hemagglutination inhibition assay (11).

REFERENCES
5. Degenerer A.M., Pietropaolo V., Di Taranto C., Jin L., Ameglio F., Cordiali-Fei P., Trento E.,