CHAPTER 10

The Pathobiology of Polyomavirus Infection in Man

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Abstract

This article traces the discovery of polyomaviruses and outlines investigations, which shed light on potential modes of transmission of this increasingly important group of human pathogens. The pathobiology of the virus is summarized with particular reference to interactions with host cell receptors, cell entry, cytoplasmic trafficking, and targeting of the viral genome to the nucleus. This is followed by a discussion of sites of viral latency and factors leading to viral reactivation. Finally, we present biochemical mechanisms that could potentially explain several key elements of tissue pathology characteristic of BKV mediated damage to human kidney.

Biology of Polyomaviruses

Polyomaviruses (PV) are 45nm sized particles with a 5 kb genome.[1,2] The viral genome is comprised of double-stranded, circular, supercoiled DNA. The viral genome is typically arranged in three general regions: non-coding control region (NCCR), the early coding region coding for the small and large T antigens, and the late coding region coding for the viral capsid proteins (VP-1, VP-2, VP-3) and agnoprotein. The direction of early and late transcription is divergent, with opposite DNA strands participating in these processes.[3] The NCCR contains (a) the origin of replication (ori), and (b) regulatory regions containing enhancer elements that are important activators of viral transcription.[4] There is clinical and laboratory evidence that NCCR variants determine host cell permissivity and rate of viral replication.[5,6] The T antigens bind to tumor suppressor proteins Rb and p53 and stimulate host cell entry into the cell cycle.[7,8] This observation provides a theoretical basis for multiple lines of accumulating evidence that PV may be carcinogenic in man, as discussed elsewhere in this book. The viral capsid proteins VP-1, VP-2, and VP-3 are structural proteins required for the assembly of complete virions. The viral capsid coding regions display considerable genetic heterogeneity, and this feature has been used to divide polyomavirus BK (BKV) into distinct genotypes I, II, III, and IV,[9-13] and polyomavirus JC (JCV) into Types 1, 2A, 2B, and 3-8.[14-20] In mice, specific mutations in the viral capsid protein VP-1 region have been associated with increased viral pathogenicity.[21,22] The existence of potential relationships between viral genotype and clinical virulence is illustrated by the observation that progressive multifocal leucoencephalopathy is associated primarily with JCV Type 2B infection. Agnoprotein protein localizes primarily to the cytoplasmic and perinuclear regions of the host cell. This distribution has led to the suggestion that agnoprotein may promote virion release from cell.[23] Other proposed roles for this protein include participation in host cell lysis, enhanced nuclear localization of viral capsid protein VP-1, and help in viral capsid assembly.[24] Cultured cells infected with agnogene mutants show a 17-100 fold reduction in

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virion burst size.[25] A detailed discussion of the molecular biology of the polyomaviruses is presented elsewhere in this book.

Historical Aspects

The polyomavirus species most relevant to human disease are BKV, JCV, and simian virus 40 (SV40).[3] SV40 was first discovered in 1960 as a contaminant of poliovirus vaccines prepared in monkey kidney cell lines.[26] Millions of human subjects developed iatrogenic infection as a result of mass vaccinations programs carried out in the late 1950s and early 1960s. These individuals did not develop any acute sequelae, nor any definitely proven long term effects, although the potential role of SV40 in some human neoplasms is currently an area of active investigation. BKV was discovered in 1970 by Dr. Sylvia Gardner while examining a urine specimen from a Sudanese kidney transplantation recipient with a ureteric stricture. This specimen was found to contain numerous cells bearing viral inclusions.[27] Electron microscopy demonstrated viral particles that resembled papillomavirus. However, inoculation of the urine into secondary rhesus monkey kidney cells and human embryonic kidney cells produced a viral cytopathic effect indicating that the virus was different from papillomavirus. Hence, this microbe was identified as a new virus and named BKV after the initials of the patient from whom it was isolated. Subsequently, BKV was shown to be distinct from polyomavirus JC (JCV), a virus cultured from a patient with progressive multifocal encephalopathy. Electron microscopic evidence suggesting that this disease is viral in etiology was published by two independent laboratories in 1964.[28,29] However, the medical community remained unconvinced until isolation of JCV from a patient with progressive multifocal encephalopathy in 1971. Interestingly, this finding was reported in the same issue of The Lancet, which reported the discovery of BKV.[30]

Following these discoveries extensive epidemiological studies showed that up to 90% of some human populations become exposed to polyomaviruses BKV or JCV by adulthood.[1] After transplantation, 10-60% of renal allograft recipients were noted to excrete virus in the urine. However, infection was typically asymptomatic or associated with only transient graft dysfunction. There were only rare reports of viral inclusions being present in specimens examined following nephrectomy or at autopsy. Sporadic cases of virus-induced kidney damage were also observed in the setting of congenital immunodeficiency[31] and human immunodeficiency virus infection.[32] A new era in the study of polyomavirus infections after renal transplantation was ushered in by a patient with full blown BKV nephropathy diagnosed by a needle biopsy of the allograft kidney, This case, which was diagnosed in 1993 at the University of Pittsburgh, but published in 1996,[33] led to a flurry of additional cases reported from virtually all major kidney transplant centers around the world.[34-36] The emergence of BKV nephropathy in the 1990s is generally attributed to the widespread use of potent immunosuppressive drugs such as tacrolimus, mycophenolate mofetil, and sirolimus.

Modes of Natural Transmission

Primary polyomavirus infection occurs typically in childhood. Adult levels of seroprevalence, on the order of 65-90% in most studies, are reached between 5 and 10 years of age. This high incidence of polyomavirus infection raises obvious questions about the mode of transmission from one individual to another. Given the known latency of the virus in the kidney, urine would appear to be a natural vehicle for spread within and between families. A variety of laboratory techniques have accordingly been used to assess the prevalence of viruria in the pediatric age group. Urine cytology investigations show viral inclusions in 0-1.2% of children. Viral cultures give a similarly low yield varying from 0-1%. Higher rates of viruria can be detected using PCR, but the results vary in different studies ranging from 4% to 26.7%, with all but one study in children reporting values <5%.[12] In adults, viral DNA has been amplified from 0-40% of urine samples, with a tendency to higher values in older subjects. Viral DNA concentrations reported have varied from <3 fg/ml to 5 pg/ml. Other body fluids may also be involved in viral transmission. Thus, BKV DNA has been amplified in 1% of nasopharyngeal aspirates obtained from hospitalized infants with serious

respiratory infections.[37] The possibility of feco-oral transmission has been recently raised by the demonstration of viral DNA in urban sewage.[38] Blood, semen, genital tissues, and normal skin biopsies have also been shown to contain BKV.[39,40] Hence, it is possible that the virus may be transmitted by intimate contact with infected individuals.

Transplacental transmission of polyomaviruses from mother to fetus is controversial. BKV specific IgM antibodies were demonstrated in three of six infants whose mothers seroconverted during pregnancy.[41,42] On the other hand, Shah et al[43] could not detect anti-BKV IgM antibodies in the cord blood of 387 infants. Admittedly, only three of the mothers evaluated in the latter study had anti-BKV IgM antibodies in the serum. Coleman et al[44] studied 309 mothers, 39 of whom excreted viral inclusion bearing cells in the urine during pregnancy. Neonatal and cord blood samples drawn from the offspring consistently tested negative for BKV-specific IgM. Transplacental transmission of polyomavirus has been demonstrated in mice,[45] and it is conceivable that the same could occur in man.

Transmission of Polyomavirus via Organ Transplantation

Given that polyomavirus is latent in the kidney, it is reasonable to believe that the donor kidney will be the source of infection in a proportion of transplant recipients. Attempts to determine the frequency with which this occurs have relied on serologic analysis of pre-transplant donor and post-transplant recipient sera. Gardner et al[46] found 6 of 48 (12%) kidney transplant patients to be seronegative for BKV at the time of transplantation. Two of these six (33%) patients subsequently developed seroconversion indicative of primary BKV infection, and one patient developed viruria. The incidence of primary and secondary JCV infection in this study was 23% and 46% respectively.[46]

Noss detected anti-BKV antibodies in103/168 (61.3%) of renal transplant recipients using indirect immunofluorescence or virus neutralization assays. In an analysis of 62 paired donor and recipient sera, it was determined that primary infection occurred in 18 (29%) patients, typically within 3 months of transplantation. The remaining 44 patients developed presumed reactivation infection, usually after 3 months following transplantation.[47]

Andrews et al[48] conducted a serological study of 496 renal transplant recipients and donors for BKV and JCV infections. They found that a seropositive donor increased the risk of primary and reactivated infections with BKV and of primary infection with JCV. Specifically, a donor seropositive and recipient seronegative combination was associated with a 43% incidence of primary infection defined by serologic methods. In comparison, a 10% incidence of reactivation infection was observed in seropositive recipients of seropositive organs.[2]

In a more recent study, BKV specific hemagglutination inhibition antibodies were found in 59/78 (77%) serum samples collected before transplantation.[49] Of 23 patients with post-transplant decoy cell shedding, 18 (78%) were seropositive and 5 (22%) seronegative prior to receiving the donor organ. It was observed that 3 of 5 seronegative patients developed BK viremia, and one went on to develop nephropathy

Viral Interactions with Host Cell Receptors

Defining the cellular mechanism of BK infection is important, because this may lead to the identification of biochemical molecules that could be targeted in drug discovery studies. Viral receptor interactions are believed to be important determinants of host range and tissue tropism. The primary receptor binding determinant on all polyomaviruses is the VP-1 molecule, which is arranged in the form of icosahedrally symmetric pentamers. Despite the fact that BKV, JCV, and SV40 are related viruses, there is growing evidence that these microorganisms use distinct mechanisms to target their host cells.

The mouse polyomavirus has small and large plaque strains, which recognize a cell surface associated N-linked glycoprotein containing terminal α (2-3)-linked sialic acid.[50] The small plaque strain also recognizes a branched disialyl structure containing α (2-3)- and α (2-6)-linked sialic acids. The ability of the small and large plaque strains to distinguish between these two sialic acid

configurations has been attributed to a single amino acid polymorphism at position 92 in the VP-1 protein.[51] Specifically, the replacement of a negatively charged glutamic acid by glycine at this position correlates with the increased in-vitro pathogenicity of the large plaque strain. Recently α4β1 integrins have been found to act as cell receptor for murine polyomavirus.[52] Treatment with blocking antibodies before and after virus adsorption indicate that the effect on cell permissivity is at the post-attachment level. Binding of virus to α4β1 is mediated by integrin binding motifs located in the DE and EF loops of the VP-1 protein. These motifs were not found in a BKV AS strain and a JCV ML-6 strain analyzed by the authors. The mouse lymphotropic papovavirus interacts with a O-linked glycoprotein containing terminal α (2-6)-linked sialic acid. This receptor is restricted to B-cells, and accounts for the limited tissue tropism of this virus.

The simian virus SV40 is unlike other polyomaviruses in that cell entry is independent of surface sialic acids. Instead, SV40 VP-1 interacts with major histocompatibility class I proteins and O-linked glycan molecules. Accordingly, anti-MHC class I antibodies inhibit infection of glial cells by SV40 but not JCV.[50] SV40 does not compete with sialic acid dependent polyomaviruses for binding to host cells.

Considerable work has been done on characterization of receptors for the human virus JCV. Receptors on the surface of glial cells and B-cells have been shown to bear a N-linked glycoprotein containing terminal α (2-3)- and α (2-6)-linked sialic acids.[53] Treatment of cultured glial cells with proteases, phospholipases, and neuraminidases inhibits viral binding, indicating that virus can bind to a wide variety of cell surface ligands. However, only neuraminidase inhibits infectivity, and this has been attributed to the ability of this enzyme to cleave both α (2-3) and α (2-6)-linked sialic acids from the cell surface. A recombinant neuraminidase that specifically cleaves the α (2-3) linkage of sialic acid has no effect on either virus binding or infection. Competitive binding assays with sialic acid specific lectins also support the notion that viral interaction is primarily with α (2-6)-linked sialic acids.[50] Indirect overlay assays have demonstrated that virus like particles (VLP) comprised of recombinant VP-1 can bind to a number of sialoglycoproteins, including α1-acid glycoprotein, ferritin and transferrin receptor. Binding was also demonstrated to glycolipids, such as lactosylceramide, and gangliosides, including GM3, GD2, GD3, GD1b, GT1b, and GQ1b. VLP bound weakly to GD1a, but did not bind to GM1a, GM2, or galactocerebroside. Furthermore, a chemically synthesized neoglycoprotein containing the terminal α 2-6-linked sialic acid and the ganglioside GT1b inhibited JCV infection in the susceptible cell line IMR-32. These results suggest that the oligosaccharides of glycoproteins and glycolipids work as JCV receptors and may be appropriate targets in the quest to develop effective anti-JCV drugs.[54]

There is very limited information available about the early steps of BKV binding to the host cell. Digestion of human red blood cells by Vibiro cholerae neuraminidase inhibits virus induced hemagglutination activity suggesting a role for α (2-3)-linked sialic acid residues.[55] However, the chemical nature of the associated receptors has not been described. The presence of glycolipids has been impicated based on phospholipases digestion experiments.[56] Unfortunately, such studies cannot distinguish between specific and non-specific interactions with cell surface ligands, unless parallel investigations are performed to determine if actual entry of the virus into the cell is also affected.

Entry of Virus into Host Cells

The mechanism of polyomavirus intra-cellular entry is species dependent. Thus, JCV enters the cell by clathrin dependent endocytosis, which is believed to be a constitutive process, and unlike SV40 entry, does not depend on a virus dependent extracellular signal. Clathrin facilitated endocytosis can be blocked by chlorpromazine and clozapine.[57] Clathrin dependent endosomes have an acidic milieu, which induces conformational changes in viral glycoproteins, thereby promoting uncoating of viruses.[58] Viruses that enter by endocytosis generally disassemble in the endosomes. Neutralization of endosomal pH by ammonium chloride or bafilomycin A2 inhibits viral infection of host cells.

In contrast to JCV, SV40 viral entry is a caveolae dependent endocytosis susceptible to nystatin, rather than chlorpromazine.[59] The sequence of events begins with the virus binding to MHC class 1 molecules in the plasma membrane. At this point, the virus transmits an extracellular signal that promotes virus enclosure in caveolae with in 30 minutes of surface binding.[60] This signal activates the tyrosine kinase and calcium independent protein kinase pathways, as evidenced by upregulation of c-myc, c-jun, and c-sis.[61] There is no activation of the Raf or mitogen activated protein kinase (MAP/ERK1). Caveosomes are 'pH neutral' organelles (i.e., their function is not affected by intra-cellular pH), and do not contain markers for endosomes, lysosomes, golgi complex or endoplasmic reticulum. In passing, it should be noted that caveolae are also involved in the intracellular trafficking of mouse polyomavirus virions,[62] human immunodeficiency virus, *Toxoplasma gondii*, *Plasmdium falciparum* and *Campylobacter jejuni*.[63]

Little published information is available about the mechanisms used by BKV for intracytoplasmic transport, but electron microscopic observations on human biopsy material show that the virus is associated with non-clathrin coated vesicles, which may represent caveolae.[64]

Cytoplasmic Trafficking

It is now well recognized that, following receptor mediated cell entry, the transport of viral particles in the cytoplasm is not a matter of simple diffusion. Rather, it is an active process mediated by interactions between virus containing vesicles and the cellular cytoskeleton. Detailed studies of the interactions between virus particle containing vesicles and the host cytoskeleton have been carried out for the mouse polyomavirus. It appears that this virus moves predominantly along microfilaments.[62] Actin filaments are involved in the early stage of infection, when the viral VP-1 protein colocalizes with disorganized actin microfilaments. In later stages of infection, VP-1 is associated with microtubules. Colocalization of VP-1 and tubulin can be detected around the nuclei, in mitotic spindles, and in centromeres. Lack of free viral particles in this location, however, led one group of investigators to speculate that the microtubular compartment might be involved in viral disassembly. In apparent conflict with this interpretation, it has been observed that the microfilament destroying agent cytochalasin D has no effect on viral infectivity, while the microtubule disrupting agent nocodazole inhibits viral transport efficiently.[65] These discrepant results could mean that there are two different trafficking mechanisms, the relative importance of which depends on the experimental conditions.

Cytoplasmic trafficking of SV40 virions resembles the mouse polyomavirus in being susceptible to nocodazole, but not cytochalasin.[66] Video enhanced live fluorescence microscopy has demonstrated a two-step vesicular transport pathway to the endoplasmic reticulum. The first step consists of SV40 entry into caveolin rich vesicles, which subsequently direct virions to a caveolin-free microtubules. Microtubules in turn target the virus to a syntaxin 17-positive smooth endoplasmic reticulum compartment.[67] During its transport to the endoplasmic reticulum, SV40 passes thru an intermediate compartment containing b-COP, a protein best known for its association with Golgi cisternae and their derivative budding vesicles. b-COP is believed to be involved in the Golgi to endoplasmic reticulum recycling pathway. An unusual feature of caveola-mediated SV40 entry is that the virus bypasses the endosomal compartment, and is transported to the endoplasmic reticulum.[63] More commonly, endocytic cargo is channelized to the endosomal-lysosomal pathway. Microfilaments are not required for early entry steps of SV40, but do facilitate viral trafficking following entry of the virus into caveolae.[58]

JCV has a complex intra-cellular transport mechanism involving sequential involvement of several classes of cytoskeletal elements. Unlike SV40 transport, which is inhibited only by nocodazole, JCV transport is inhibited by nocodazole, cytochalasin B, as well as acrylamide, indicating that microtubules, microfilaments, and intermediate filaments all play a role in intra-cellular trafficking. Actin polymerization facilitates clathrin-mediated endocytosis. The plasma membrane is intimately linked to the underlying cytoskeleton, and surface events such as vesicle budding require remodeling of the actin framework. The microtubular system, with

its microtubule organizing center located close to the nucleus, is anatomically well suited for the intra-cellular transport of viruses that replicate within the intranuclear compartment. The role of intermediate filaments in viral trafficking is not yet well understood. The mechanisms of endocytosis and intra-cellular trafficking utilized by BKV have not been investigated to date.

Nuclear Targeting

Viral entry into the cytoplasm does not in itself ensure subsequent transport to the nucleus. Thus, synthetic virus like particles composed of VP-1 protein (with no enclosed viral DNA) can enter the cytoplasm, but fail to enter the intranuclear compartment, where viral replication normally occurs.[62] Instead, empty viral particles are internalized in large vesicles prior to undergoing degradation.

The mechanism by which polyomavirus traverses the nuclear envelope to enter the nucleus is controversial. Monoclonal antibodies to nucleoporin (a protein associated with the nuclear pore complex) have been shown to block the entry of SV40 into the nucleus of 3T3 cells. However, this observation needs to be reconciled with the fact that the maximal diameter of a particle that can pass through the nuclear pore is 23 nm,[68] whereas the diameter of the encapsidated polyomavirus particle is approximately 50nm. Observations on human biopsy material suggest that fully assembled viral particles tend to accumulate in the nucleus as large crystalline arrays until complete cell lysis. This may be due to fibrils attached to the nuclear pore, which prevent egress of SV40 particles from the infected nucleus.[69]

The uncoating process of polyomaviruses has been stated to occur after the virions have entered the cell nuclei. However, Richterova et al[62] found no convincing VP-1 signal in the nuclei of fibroblasts infected with the mouse polyomavirus. Norkin et al[63] showed viral capsid proteins VP-2 and VP-3 overlapping the endoplasmic reticulum within 5 hours of infection. Since these antigens are normally not exposed on the surface of the viral capsid, this finding implies that SV40 disassembly can occur in the endoplasmic reticulum. VP-2 and VP-3 normally insert into the axial cavity of the VP-1 pentamer by hairpin-like loops anchored by strong hydrophobic interactions. Dissociation of these high affinity interactions is likely facilitated by molecular chaperones, one of which may be the viral T-antigen. Interestingly, VP-2 and VP-3 contain a nuclear transport signal and also have a non-specific DNA binding domain[70] These latter properties provide the functions required for the nuclear delivery of the viral minichromosome. Consistent with this concept, injection of antibodies to VP-2 and VP-3 in the cytoplasm blocks the transport of viral DNA to the intranuclear compartment.[71]

Clinical Sequelae of Primary Infection in Man

No systematic clinical observations have been made in individuals undergoing polyomavirus seroconversion. When recorded the most frequent symptom is an upper respiratory infection. Unfortunately, some of the reported cases also had concurrent infections with other known respiratory viruses, making it difficult to attribute the observed symptoms entirely to primary polyomavirus infection.[37,72] Sporadic case reports of children presenting with cystitis, with or without hematuria, are also on record.[73-75] Unusual clinical manifestations associated with BKV seroconversion include Guillane-Barre syndrome and encephalitis.[76,77]

Sites of Viral Latency

After primary infection has resolved the virus enters a latent phase. It appears that viral latency can be maintained in a number of different sites:

1. Viral DNA is detected most often in the urogenital tract including the kidneys, urinary bladder, prostate, cervix, vulva, and semen.[78]
2. Peripheral blood mononuclear cells are the second most important site of polyomavirus latency. In healthy individuals rates of detection vary from 0-94%.[79] JCV has a particular propensity for B-lymphocytes. Limited BKV infection has been shown in T-cells and B-cells maintained in culture. In one study, monocytes showed BKV attachment and penetration,

but no viral replication, unless the cells were treated with anti-macrophage antiserum.[80] BKV mRNA has been detected in circulating mononuclear cells of healthy donors by RT-PCR and in-situ hybridization.[81,82]

3. Mucosa-associated lymphoid tissue is a potential site of latency, since BKV DNA has been demonstrated in throat washings and tonsil tissue obtained from children.[37,83]
4. Other proposed sites of viral latency include the brain,[84] normal bone, and bone tumors.[81]

Reactivation of Latent Virus

Activation of latent virus has been reported in a variety of clinical settings summarized below:

1. Asymptomatic viruria can occur in old age, pregnancy, and diabetes mellitus, presumably as a result of hormonal effects on anti-viral immunity.[78]
2. Co-infection with other viruses has been proposed to be one of the mechanisms of polyomavirus reactivation. Thus, there is evidence that polyomavirus JCV reactivation can be triggered by infection with herpesvirus 6 (HHV6).[85] Human immunodeficiency virus encoded HIV-1 Tat protein can transactivate polyomavirus JCV by induction of the JCV promoter.[86, 87]
3. Immunosuppression is a well-known risk factor for viral reactivation. This likely reflects interference with the normal cell mediated immune mechanisms that keep viral replication in check. Accordingly, BK viruria has been reported in 20-44% of HIV infected individuals, 22-100% of bone marrow transplant recipients, 10-60% of kidney transplant recipients, and 50% of heart transplant recipients.[79] A small proportion of viruric patients go on to develop BKV nephropathy, ureteric stricture, or hemorrhagic cystitis. Progressive multifocal encephalopathy is well known complication of JCV infection in HIV infected patients. In recent years, SV40 DNA has been documented in allograft kidneys, native kidneys with glomerular disease, and in a variety of neoplasms, particularly mesotheliomas, brain tumors, and non-Hodgkin lymphomas. A detailed discussion of polyomavirus associated clinical syndromes is provided elsewhere in the book.

Pathogenesis of Tissue Damage in Polyomavirus Infected Tissues

Our understanding of the actual metabolic pathways utilized by polyomavirus to initiate and sustain tissue damage is rudimentary. Using Affymetrix HG-U133 DNA microarray analysis of BKV infected WI-38 cells, our laboratory has obtained data indicating that viral infection causes up-regulation of several major groups of intra-cellular mRNA's.[88] These virus-induced changes in host gene expression offer potential insights into the pathogenesis of BK virus allograft nephropathy, as summarized below:

1. Cell cycle proteins were by far the largest group of proteins that were upregulated following BKV infection. Initiation of the host cell cycle is an important event because polyomavirus is dependent upon host cellular factors for replication, and the required cellular factors are not present in quiescent host cells
2. Several pro-inflammatory cytokines were up regulated, including molecules participating in both the early (IL-1 and TNF induced proteins) and late (IL-6, IL-11) phases of the inflammatory response. It is pertinent to note that IL-1 is known to be induced by nearly all microbes. It initiates a febrile response and stimulates IL-6 and IL-11. IL-6 and IL-11 share the gp130 signaling pathway and are capable of further intensifying the acute phase response associated with viral infection.[89]
3. There was increased expression of cytokine receptors IL-4R, IL-13R, and TNF soluble factor 15. IL-4 has a biological role in antibody production. It is significant that increased B-cells and plasma cells have been noted in human biopsy material with BKV nephropathy.[90] IL-13 is a cytokine that is similar to IL-4 in its anti-inflammatory properties and ability to enhance antibody production by B-cells.[91]

4. The chemokines RANTES (regulated on activation T-cell secreted chemokine) and IL-8 were found to be stimulated by viral infection. RANTES is known to be expressed by tubular epithelium in inflammatory disease states such as acute cellular rejection. IL-8 facilitates influx of polymorphonuclear cells and offers a plausible explanation for the presence of polymorphonuclear cells in biopsies with viral interstitial nephritis.[92]
5. BKV infection led to increased mRNA for the intercellular adhesion molecule ICAM-1 (CD54). ICAM-1 is expressed by tubular epithelial cells and is believed to play a role in the pathogenesis of interstitial inflammation in the kidney.[92]
6. There was enhanced transcription for three groups of major histocompatibility complex class I molecules (HLA-B, HLA-C, and HLA-F) in BKV infected cultured cells. Occurrence of the same phenomenon in the transplanted kidney would put the graft at increased risk for acute cellular rejection. This would explain why the pathology of BKV nephropathy overlaps with acute cellular rejection.
7. Increased mRNA was demonstrated for Collagen VII and extracellular matrix protein 1. Collagen type VII is the major component of the anchoring fibrils at the dermal-epidermal junction. It is usually not present in normal glomeruli, but has recently been shown to be actively synthesized in areas of glomerular and/or tubular scarring in many kidney diseases.[93] Viral induced synthesis of collagen VII may contribute to the progressive scarring that can accompany BKV nephropathy.
8. Expression of vascular endothelial growth factor (VEGF) mRNA was increased. There is evidence that VEGF participates in the pathogenesis of chronic renal allograft rejection.[94] This raises the possibility that BKV may accelerate this process in the transplanted kidney by a similar mechanism.

The participation of interleukins, cytokines and cellular adhesion molecules in the pathogenesis of BKV nephropathy raises the possibility of using pharmacological means of ameliorating virus mediated allograft injury. Such intervention may be particularly indicated in patients with progressive interstitial nephritis, who do not respond to reduction of immunosuppression and cidofovir therapy. Several molecules shown to be up regulated by BKV infection are targets for currently ongoing efforts to devise anti-inflammatory therapy using cytokine antagonists. For example: (a) anti-IL-4 antibody has potential utility in the treatment of rheumatoid arthritis,[95] (b) IL-1 receptor inhibitors are being developed and investigated in the setting of rheumatoid arthritis, septic shock and steroid resistant graft versus host disease, (c) Patients with Crohn's disease and refractory rheumatoid arthritis are being treated with anti-TNF antibody (Infliximab, Remicade), anti-IL-8 (Abgenix), and anti-IL-6 antibodies, and (d) anti-ICAM-1 monoclonal antibody (enlimomab) has been used for the prevention of acute rejection in cadaveric renal transplantation.[96-98] Proof of concept studies showing the applicability of these treatment modalities to viral disease are yet to be performed. We do not know whether abolishing the inflammatory response will have a deleterious or beneficial effect on the natural history of BKV nephropathy. It is also possible that cytokine redundancy may make the use of single pharmacologic agents ineffective.

Concluding Remarks

There is increasing recognition of the importance of polyomavirus infections in clinical medicine. The spectrum of human disease caused by polyomaviruses BK, JC, and SV40 has expanded considerably as a result of investigations in the past two decades. A frustrating issue is our currently limited ability to treat these diseases by effective drug therapy. Further progress in this field would require more intensive investigations into the mechanisms of virus mediated tissue injury. There is also an urgent need for high throughput assays capable of screening currently available libraries of chemical compounds for anti-polyomavirus activity.

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