

# Reactivation of human polyomaviruses in immunocompromised states

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**Abstract** Infection with various human polyomaviruses (HPyVs) is prevalent, with rates as high as 80 % within the general population. Primary infection occurs during childhood through respiratory or urino-oral transmission. While the majority of individuals exhibit asymptomatic latent infection, those immunocompromised persons are at risk for viral reactivation and disease progression resulting in conditions such as progressive multifocal leukoencephalopathy (PML), trichodysplasia spinulosa, Merkel cell carcinoma, and polyomavirus associated nephropathy. Individuals with altered immune systems due to HIV, organ transplantation, lymphoproliferative diseases, and monoclonal antibody therapy are particularly susceptible to reactivation of various HPyVs. While the specific factors that induce lytic infection have yet to be defined, it is evident that dysfunctional host cellular immune responses allow active infection to occur. Immunosuppressant conditions, such as in chronic alcohol abuse, may serve as added risk factors for reactivation of HPyVs. Since the human HPyV family is rapidly expanding, continuing studies are needed to characterize the role that known and newly discovered HPyVs play in human disease.

**Keywords** Immunosuppression · Polyomavirus · Reactivation · Alcohol abuse

## Introduction

Human polyomaviruses (HPyVs) are small non-enveloped double stranded DNA viruses believed to be acquired through the respiratory system during childhood. Evidence of early infection is seen in age related seroprevalence rates of Washington University polyomavirus (WUV), which infects 69 % of the population with 44.6 % of children age 5 years and younger carrying the virus (Kean et al. 2009). Overall, HPyVs are prevalent in the general population, with seroprevalence rates in adults as high as 90 %; however, disease only manifests in immune compromised individuals (Carter et al. 2009; Nguyen et al. 2009). HPyVs share common characteristics, such as genome size and organization, but are unique in their tissue tropism and associated pathology. The first two members of the viral family, JC polyomavirus (JCV) and BK polyomavirus (BKV), were discovered in 1971 from brain and kidney tissues of immunosuppressed individuals (Gardner et al. 1971; Padgett et al. 1971). Significant time elapsed before additional viruses were discovered with the help of newly developed techniques, such as rolling circle amplification and transcriptome subtraction in conjunction with high throughput sequencing (White et al. 2013). Currently 12 distinct human-specific HPyVs have been discovered (Korup et al. 2013).

## Polyomavirus and diseases

The most frequently observed members of the viral family are the BKV, JCV, Karolinska Institute polyomavirus (KI), WU, and Merkel cell polyomavirus (MCV), but not all have been linked to disease (Kean et al. 2009). A correlation exists between immunosuppression and reactivation of JCV, BKV, MCV, and trichodysplasia spinulosa-associated polyomavirus (TSV), leading to disease pathogenesis. BKV has been associated with acute

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interstitial nephritis and hemorrhagic cystitis in stem cell transplant recipients (Kalvatchev et al. 2012). JCV DNA is significantly increased in the kidneys and central nervous system (CNS) in immunosuppressed individuals, with 42 % of immunosuppressed patients having detectable JCV as compared to 21 % of immunocompetent patients (Delbue et al. 2010). JCV is primarily associated with progressive multifocal leukoencephalopathy (PML), a debilitating opportunistic infection that affects AIDS patients, and in rare cases occurs in individuals undergoing immunosuppressive therapy. PML manifests as dementia, confusion, and vision impairment resulting from demyelination in the CNS (Bellizzi et al. 2012; Berger and Houff 2006; Van Assche et al. 2005). BKV has been associated with acute interstitial nephritis and hemorrhagic cystitis in stem cell transplant recipients (Dalianis and Hirsch 2013). MCV, one of the most recently discovered polyomaviruses, has been associated with cellular transformation and development of an aggressive form of skin cancer—Merkel cell carcinoma (Spurgeon and Lambert 2013). An additional dermatrophic pathology results from TSV infection of hair follicles in immunocompromised individuals and is characterized by facial spines and papules often in conjunction with facial hair alopecia (van der Meijden et al. 2010b).

### Human polyomavirus replication and latency

Viral attachment requires specific cell surface receptors that mediate entry into permissive cells and direct tissue tropism (Schowalter et al. 2012). Permissive cells are capable of supporting early and late stage viral protein production resulting in active infection (White et al. 2013). Early and late coding regions are initiated from a bidirectional regulatory region (Ault 1997). The early coding region is responsible for the production of transforming proteins, large T-antigen, and small t-antigen, which are produced in all HPyV members (DeCaprio and Garcea 2013). The late coding segment is responsible for structural protein production and, in the case of JCV and BKV, a small multifunctional protein known as agnoprotein (Khalili et al. 2013). The large T-antigen contains a DNA J domain and an LXCXE motif which interact to promote efficient viral replication and promote cell cycle progression (DeCaprio et al. 1988). Viral replication is dependent on host cell enzymes that are abundant in the S-phase of the cell cycle (Traylen et al. 2011). In order to induce the expression of the necessary enzyme, HPyV large T-antigen binds to the tumor suppressor protein, p53, to induce proliferation and prevent apoptosis associated aberrant cell cycle check points (Orba et al. 2010). While this process can theoretically result in cellular transformation, HPyVs generally induce host-cell lysis to release viral progeny. The mechanism of HPyV cell lysis has yet to be characterized (White et al. 2009).

Not all cells that are infected with HPyVs undergo cell lysis; in some of the virally infected cells, the virus remains in a latency state with very low or no replication. Transcription of the late coding region results in the production of viral capsid proteins, VP1, VP2, and VP3. The transition from latency to active replication can be detected by the presence of late transcription genes, such as the viral capsid protein, VP1, and identification of virions by electron microscopy (White et al. 2013).

The tissue tropism and mechanism of viral latency and persistence remain poorly defined but likely vary within the viral family, partially accounting for the differences in disease observed upon reactivation. In cells unable to support late coding products required for capsid formation, cellular transformation can occur, as in the case of Merkel cell carcinoma (MCC) cells, where knock-down of large T-antigen results in diminished growth and cell death of transformed cells infected with the MCV polyomavirus (Houben et al. 2010).

### Human polyomavirus reactivation and immunosuppression

While the primary infection with HPyVs is usually asymptomatic, viral reactivation can occur in immunosuppressed individuals resulting in active replication and disease development. Reactivation of various HPyVs has been noted following immunosuppressive therapy (Chen et al. 2009). Tacrolimus and mycophenolate mofetil, immunosuppressive drugs administered in coordination with organ transplants, have been associated with increased polyoma reactivation following transplantation (Hirsch and Steiger 2003). In addition, there was an increase in JCV from 19 % to 63 % in the urine of patients after 12 months of natalizumab immune suppressant therapy to combat symptoms of multiple sclerosis and Crohn's disease (Mengel et al. 2003).

Similar to other polyomaviruses, TSV reactivation occurs under immune suppressed conditions resulting from lymphocytic leukemia and organ transplantation (Sadler et al. 2007; van der Meijden et al. 2010a). Whereas reactivation is associated with disease progression, increases in viral production is not always associated with pathogenesis. For example, asymptomatic viruria can occur in the late stages of pregnancy and in the elderly who experience a decline in immune competence (Vanchiere et al. 2009).

The exact facet of the immunosuppressive state responsible for reactivation is yet to be characterized but probably relies on a combination of replication-driven promoter rearrangements and peripheral-stimulatory signals in concert with decreases in immune surveillance that manifest in the immunocompromised individual. Viral transcription is mediated by a bidirectional promoter, known as the non-coding regulatory region (NCCR), that contains binding sites for a multitude of transcription factors (Ferenczy et al. 2012). Recombination

events associated with the NCCR in JCV allow for adaptation to different cell types and progression to virulence in the context of immunosuppression (Johnson et al. 2013). Rearrangement of NCCR sequences in JCV may occur within lymphocytes, which are suspected to be viral reservoirs (Chapagain and Nerurkar 2010). NCCR regulation of viral replication is altered in MCV, which integrates into Merkel cell carcinomas expressing a mutated large T-antigen gene which disables viral replication due to the deletion of DBD and Tag helicase domain. The resulting integrated viral genome is limited from replication but maintains expression of oncogenic viral proteins such as the truncated large T-antigen (DeCaprio and Garcea 2013; Feng et al., 2008).

While sequence alterations in the viral genome are characteristic of JCV prior to the onset of PML, they have also been detected in individuals who do not suffer from that debilitating disease (Bag et al. 2010). This suggests that an additional trigger is required for reactivation of JCV and, most likely, all HpyVs. Immune mediators secreted by peripheral immune cells may also modulate viral expression. SF2/ASF is an alternative splicing factor that suppresses viral expression in glial cells through binding of specific tandem repeats located within the viral promoter. Secretory products from induced peripheral immune cells increase expression of SF2/ASF, limiting viral gene expression and replication (Khalili et al. 2013; Uleri et al. 2013). In addition, the NCCR also contains binding sites for pro-inflammatory cytokines, suggesting a possible mechanism for cytokine release in reactivation of various HpyVs (Romagnoli et al. 2009).

An important aspect of immunosuppression that allows for unmitigated viral reactivation is the decrease in the cellular immune response. Studies investigating T-cell response to JCV in PML have shown a correlation between impaired CD8<sup>+</sup> cytotoxic T-cell responses and a fatal progression of PML (Du Pasquier et al. 2004; Gheuens et al. 2011). Another study indicated that the number of JCV specific CD4<sup>+</sup> T-cells as well as immunoglobulin G (IgG) antibody responses were significantly increased in PML survivors suggesting that T-cell responsiveness and antibody production are essential to combating polyomavirus infection (Khanna et al. 2009). A correlation between T-cell response and IgG against TSV in seropositive individuals also suggests the importance of both facets of the immune response in control of TSV infection (Kumar et al. 2012). Similarly, MCV specific CD8<sup>+</sup> cells appear to inhibit MCV reactivation thus, limiting progression of oncogenesis through immune surveillance and IFN- $\gamma$  release (Iyer et al. 2011).

### Human polyomaviruses and HIV

Human polyomaviruses have been associated with individuals infected with the human immunodeficiency virus-1 (HIV-1)

(Degener et al. 1997). HpyVs are more prevalent in the peripheral blood of immunocompromised HIV-positive patients compared to healthy individuals (Behzad-Behbahani et al. 2004). HIV infection remains the primary risk factor for JCV disease. JCV can be reactivated in patients with HIV-1, leading to PML which predominantly affects individuals afflicted with HIV, and characterizes some of the neurocognitive impairments associated with AIDS. Additionally, BKV viral load has been shown to increase as the levels of CD4<sup>+</sup> decrease in HIV infected individuals (Jiang et al. 2009; Knowles et al. 1999). The correlation between the increase in PML and the AIDS pandemic suggests that the reduction in CD4<sup>+</sup> T-cells characteristic of HIV infection could lead to a parallel unregulated JCV infection (Holman et al. 1991).

The prolonged and severe nature of immune suppression in the context of HIV is a primary factor in the increased occurrence of polyomavirus reactivation (Dörries 2002). Both the CD8<sup>+</sup> and CD4<sup>+</sup> T-cell responses are suboptimal in the HIV afflicted individual, allowing reactivation of latent infection (De Gascun and Carr 2013). Reduced numbers of CD4<sup>+</sup> TH1 cells are detrimental to anti-viral defense. Activated TH1 cells produce a number of cytokines, including IL-2, IFN- $\gamma$ , and TNF- $\alpha$ , which defend against viruses either directly, by inducing anti-viral state in nearby cells, or indirectly, by activating cytotoxic T lymphocytes (CTL) or natural killer (NK) cells.

Another component of HIV that promotes opportunistic infection is the transactivating viral protein, Tat, which increases JCV transcription (Mischitelli et al. 2005). Additionally, activation of JCV infection in the brain may involve transcriptional activation caused by factors such as NF- $\kappa$ B and C/EBP $\beta$ , which are persistently active in immune pathologies such as HIV (Romagnoli et al. 2009; Wollebo et al. 2011). The observed increase in cytokines, cell adhesion molecules, and blood–brain barrier (BBB) defects induced by HIV progression in the CNS may also facilitate the movement of JCV-infected lymphocytes across the BBB (Chapagain and Nerurkar 2010).

While no specific treatment exists against JCV reactivation in HIV-positive patients, the use of highly active antiretroviral therapy (HAART) is associated with immune reconstitution and improved survival rates in individuals suffering from PML (Kraemer et al. 2008). The introduction of HAART improves immune response through increases in CD4 T lymphocytes and decreases in HIV viral load. The rise in CD4 T cells is relevant to overall immune functioning and improved outcome in PML patients but does not appear to be the only contributor to positive PML outcome since many patients still show limited improvement in neurological symptoms of PML following HAART therapy (Du Pasquier et al. 2001). Additionally some cases of PML develop after starting HAART treatment, a phenomena known as unmasking (Sidhu and

McCutchan 2010). It is likely that the initiation of a JCV specific cellular immune response mediated by CTL's, in addition to the rise in CD4 T cells, is essential in prevention of disease progression. Long-term clinical improvement in parallel with JCV DNA clearance has been observed following virus specific CTL infusion, illustrating the importance of the CTL response in controlling JCV (Balduzzi et al. 2010).

### Human polyomaviruses and alcohol abuse

Alcohol mediated immunosuppression could serve as a potential risk factor for HPyV reactivation. Alcohol abuse has been shown to suppress multiple immune system responses, including cell-mediated viral detection and clearance, which could enable viral reactivation in individuals immune compromised due to alcohol abuse (Brown et al. 2006). Monocytes, which differentiate into macrophage at the site of infection, play an important role in clearance of invading pathogens. Prolonged alcohol exposure has been shown to modulate the anti-viral activity of monocytes by reducing production of Type 1 interferon (IFN) which acts by targeting viral protein synthesis (Pang et al. 2011). In addition, these IFNs can induce an antiviral response or resistance to viral replication by binding to the IFN- $\alpha$ - $\beta$  receptor, thereby activating the JAK/STAT pathway and the production of new transcripts, one of which encodes an enzyme that leads to viral RNA degradation. Viral inhibition of BKV by IFN has been exhibited in vitro cultures of infected renal proximal tubule epithelia cells (Abend et al. 2007). Similarly, renal transplant recipients lacking IFN producing BKV-specific T cells developed BKV-associated nephritis and increased BKV viral load associated with the decreased IFN response (Comoli et al. 2004).

The CD8<sup>+</sup> cytotoxic T cell responses critical to viral detection are also inhibited by alcohol due to alcohol-mediated dendritic cell dysfunction. Dendritic cells are critical to antigen specific T-cell activation. Ethanol exposure inhibits antigen

presentation by dendritic cells which limits the virus specific adaptive response gained through CD8<sup>+</sup> T cell activation and contributes to an immunosuppressed state (Szabo et al. 2004). Furthermore, the ability of cytotoxic CD8<sup>+</sup> T cells to secrete perforins and granzymes upon recognition of virally infected cells is compromised through chronic alcohol exposure. The importance of the cytotoxic T cell response is demonstrated by viral progression that results from T cell exhaustion in individuals with residual JCV infection. T cell exhaustion is prevalent in chronic viral infections and limits the ability of T cells, specifically CD8<sup>+</sup> cytotoxic T cells, to proliferate in response to antigen and to produce antiviral cytokines (Wherry 2011). The ability of JCV to cause T cell dysfunction, in conjunction with alcohol's impairment of immunity against viral infections, could facilitate viral reactivation. The regulatory receptor, PD-1 (programmed cell death), modulates immune T cell exhaustion. The binding of the PD-L1 and PD-L2 ligands to PD-1 prevents CD8<sup>+</sup> expansion and the production of IL-2, allowing for unregulated viral replication (Goldberg et al. 2007). The PD-1 receptor is increased in both CD4<sup>+</sup> and CD8<sup>+</sup> T cells in patients with PML. Additionally, JCV-specific CD8<sup>+</sup> T cells express the PD-1 receptor more frequently than nonspecific CD8<sup>+</sup> cells (Blackburn et al. 2010). When the PD-1 receptor is blocked in a subset of individuals with PML, the JCV-specific immune response appears to be enhanced by increasing the number of CD8<sup>+</sup> and CD4<sup>+</sup> T cells, suggesting that a limited CD8<sup>+</sup> immune response, augmented by T cell exhaustion, could play a role in the progression of polyomavirus infection (Blackburn et al. 2010; Tan et al. 2012).

In addition to its detrimental effects on cytotoxic T cell populations, alcohol decreases NK cell activity, which can interfere with an individual's ability to fight viral infections (Andoniou et al. 2006). As a result, alcohol abuse increases the risk for and the progression of chronic viral infections, such as HIV-1. Chronic alcohol consumption during HIV-1 infection increases the serum viral load and promotes the progression of the disease (Poonia et al. 2006).

**Table 1** Immunosuppressant drugs associated with HPyV reactivation and mechanisms of action (Bayliss et al. 2011; Carson et al. 2009; Van Den Brande et al. 2002)

Therapy	Disease	Mechanism of action
Rituximab	B cell dysfunction Lymphoma Leukemia	Binding the B cell receptor CD20 <sup>+</sup> resulting in apoptosis of CD20 <sup>+</sup> B cells in the periphery
Infliximab	Rheumatoid arthritis Crohn's disease Rheumatoid arthritis	Antibody specific for TNF- $\alpha$ resulting in reduction of the cytokine and the TH1 cells that produce the chemical messenger
Natalizumab	Ulcerative colitis Multiple sclerosis Crohn's disease	$\alpha$ 4b1 and $\alpha$ 4 $\beta$ 7 integrin inhibitor resulting in limiting of cell migration and infiltration
Efalizumab	Psoriasis (withdrawn from market in May 2009)	Targets T cell receptor CD11a resulting in decreased T lymphocyte trafficking, downregulation of adhesion molecule VLA-4 and T cell hyporesponsiveness

The mechanisms of action induced by immunomodulatory agents associated with polyomavirus activation provide insight into facets of immune regulation that are paramount in active HPyV infection (Table 1). Many of these identifying immunosuppressant characteristics have also been documented in response to alcohol abuse.

For instance, alcohol has been shown to suppress TNF- $\alpha$  messenger in humans and rhesus macaques (Friedman et al. 2003; Stoltz et al. 2000). In addition, chronic alcohol consumption has been shown to reduce the TH1 response similar to infliximab therapy. Impaired TH1 responses resulting from excessive alcohol consumption have been shown to exacerbate hepatitis C in humans and the retrovirus LP-BM5 which causes an acquired immunodeficiency in mice (Jerrells 2002; Meyerholz et al. 2008).

Organs especially sensitive to alcohol insult have been documented as sites of HPyV replication. The liver is a primary site of alcohol induced damage and is also a host tissue for JCV, MCV, and the newly discovered, HpyV12, whose role in human health has yet to be defined (Korup et al. 2013). Development of PML has been described in two HIV negative patients with alcoholic cirrhosis, demonstrating that alcohol based immune suppression and associated tissue damage could serve as an unconventional risk factor for reactivation (Gheuens et al. 2011).

Alcohol can also damage the integrity of the BBB, allowing the movement of JCV-laden lymphocytes into the CNS, which may be one mechanism of JCV dissemination prior to the onset of PML (Atwood et al. 1992). In addition to JCV, polyomaviruses KI and WU have been detected in the brain of HIV-positive individuals who also commonly exhibit increased BBB permeability, a result of HIV-induced neurodegeneration (Barzon et al. 2009; Persidsky et al. 2011). Damage to the BBB shows similar characteristics in both HIV-positive individuals and chronic alcohol abusers (Shiu et al. 2007). Membrane permeability in the CNS, resulting from continual alcohol exposure, could allow for movement of polyomaviruses, and their reservoirs into the brain as has been suggested for HIV-induced BBB dysfunction. Taken together, alterations in host immune functioning in concert with alcohol induced tissue damage provide evidence that the burden of disease incurred through alcohol abuse should be further investigated to determine the potential for reactivation of the HpyV family resulting from alcohol-induced immune suppression. HIV infection, a significant risk factor for polyomavirus reactivation, and alcohol abuse often occur together, with 50 % of HIV-infected individuals surveyed having heavy alcohol intake (Samet et al. 2004). Alcohol exposure in HIV patients has been associated with accelerated AIDS wasting, diminished circulating CD4<sup>+</sup> T cells, and accelerated disease progression (Marcondes et al. 2008; Persidsky et al. 2011). The combined effects of these immunosuppressive states may put individuals at increased risk for polyomavirus associated disease and reactivation of various HpyVs.

## Conclusions and future directions

Immunosuppression appears to be paramount in HPyV reactivation. Due to the increasing use of immunosuppressant therapy in autoimmune diseases and organ transplantation, the frequency of disease resulting from infection of various HPyVs is expected to increase. In addition, immunosuppression, one of the consequences of chronic alcohol abuse, may facilitate persistent infection of various HPyVs (Molina et al. 2010). However, further studies are needed to elucidate the precise mechanisms that are paramount to viral progression. For instance, the ability of polyoma viruses to evade immune responses and cause direct immunosuppression via the down-regulation of MHC class I molecules, secretion of complement neutralizing factors, or production of anti-inflammatory cytokine analogues, such as IL-10, remains to be addressed. The recent discovery of a multitude of HPyV members suggests that many more HPyVs are likely to be discovered. The polyomavirus family may play a more significant role in public health than previously expected as additional studies shed light on the role of HPyVs in cancer and disease pathogenesis.

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