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Immune surveillance and response to JC virus infection and PML

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Abstract

The ubiquitous human polyomavirus JC virus (JCV) is the established etiological agent of the debilitating and often fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML). Most healthy individuals have been infected with JCV and generate an immune response to the virus, yet remain persistently infected at subclinical levels. The onset of PML is rare in the general population, but has become an increasing concern in immunocompromised patients, where reactivation of JCV leads to uncontrolled replication in the CNS. Understanding viral persistence and the normal immune response to JCV provides insight into the circumstances which could lead to viral resurgence. Further, clues on the potential mechanisms of reactivation may be gleaned from the crosstalk among JCV and HIV-1, as well as the impact of monoclonal antibody therapies used for the treatment of autoimmune disorders, including multiple sclerosis, on the development of PML. In this review, we will discuss what is known about viral persistence and the immune response to JCV replication in immunocompromised individuals to elucidate the deficiencies in viral containment that permit viral reactivation and spread.

Keywords

JCV; progressive multifocal leukoencephalopathy; HIV-1; immune surveillance; CNS; multiple sclerosis; natalizumab

Introduction

It has been estimated that the majority of individuals worldwide are latently infected with the human polyomavirus, JC virus (JCV) (Knowles et al. 2003). Initial infection with JCV is asymptomatic, typically occurs in the first two decades of life, and results in a lifelong persistent subclinical viral infection. However, in severely immunocompromised patients, most notably those infected with HIV-1 or receiving immunosuppressive monoclonal antibody therapies, JCV can reactivate and infect oligodendrocytes, the myelin producing cells of the CNS, where a productive infection leads to their lytic destruction. Accordingly, axons become stripped of their protective myelin sheath, rendering them dysfunctional. As the viral infection progresses, focal demyelinated lesions appear rapidly, leading to progressive multifocal leukoencephalopathy (PML). Patients with PML present with a variety of severe visual, cognitive, and motor impairments, mirroring the brain regions most

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affected. Currently, other than restoring the underlying immunodeficiency, there are no effective treatments for PML, making the prognosis of this disease very poor.

While it has been known for decades that JCV is the etiological agent responsible for PML, our understanding about immune control of JCV infection both in healthy individuals as well as in immunocompromised individuals remains ambiguous. For instance, neutralizing antibodies against JCV, typically found in most individuals, neither protect against the development of PML nor effectively clear the virus from the body. However, given the rarity of PML, cell mediated immunity to JCV is clearly capable of controlling viral infection and preventing JCV from reactivating in healthy adults. Many questions also remain regarding viral reservoirs, as well as how and when JCV traffics to the brain. In this review, we will summarize what is known about the delicate interplay between JCV and the host immune system, and discuss potential mechanisms of reactivation and immune-mediated control of CNS infection in immunocompromised patients.

The JC virus life cycle

Similar to other members of the growing polyomavirus family, including SV40, BKV, and Merkel cell polyomaviruses, JCV has a double-stranded, DNA genome enclosed within an icosahedral capsid. The noncoding, control region acts as a bidirectional viral promoter separating the viral genome into early and late genes. The early JCV transcript encodes the major regulatory proteins large and small tumor antigen proteins (T-antigen and t-antigen, respectively) and a series of T prime proteins are also generated by alternative splicing of the early transcript. T-antigen protein is the key viral regulatory protein, which acts as a transcription factor to direct DNA replication and drive expression of the late viral transcript, which encodes the accessory protein, Agnoprotein, and capsid proteins VP1, VP2, and VP3 (Lashgari et al. 1989).

To gain entry into cells, JCV first attaches to the cell surface via an interaction between JCV VP1 and the pentasaccharide LSTc (Neu et al. 2010). Viral particles then bind either $\alpha 2,3$ - and $\alpha 2,6$ - linked sialic acid receptors and utilizes a clathrin-dependent endocytosis mechanism. Previous studies have also identified the 5HT_{2A} serotonin receptor as a receptor for the virus, but most evidence suggests that the initial steps of viral infection can occur in a broad range of cell types. Viral particles are thought to travel via endosomal vesicles to the endoplasmic reticulum and then the nucleus. The JCV genome is readily transcribed in permissive cells due to its strong promoter, containing many binding sites for common transcription factors including NF- κ B, AP-1, and HIF-1 α . Once capsid proteins are encoded, the newly transcribed viral DNA is encapsulated and virions are released in a lytic process, resulting in host cell death (for review Ferenczy et al. 2012).

In directing viral replication, T-antigen proteins hijack cell cycle machinery, via sequestration of key cell cycle regulators, including p53 and Rb, thus forcing cells into an S-phase like state to obtain the necessary machinery for further viral replication (Caracciolo et al. 2006). JCV T-antigen selectively binds to wild type p53, and disrupts this potent inducer of G1 phase cell cycle arrest, apoptosis, and DNA repair (Krynska et al. 1997). This protein complex also enhances the expression of Insulin-like Growth Factor-1 (IGF-1), whose receptor is another T-antigen binding partner, to further stimulate cell growth (Del Valle et al. 2002). Functioning as “pocket-proteins”, members of the Rb family of proteins sequester E2F transcription factors to prevent the expression of several proteins involved in the G1 to S transition of the cell cycle (Helt and Galloway 2003). T-antigen binds preferentially to hypophosphorylated forms of all three known Rb family members and promotes the release of E2F proteins (for review Caracciolo et al. 2006). Mechanisms of inactivating Rb and p53 are also utilized by human papillomavirus (HPV) E6 and E7 proteins, and adenovirus E1A

and E1B proteins, indicating that their inactivation is an essential step in the propagation of many DNA viruses (Helt and Galloway 2003; Levine 2009). The induction of early gene transcription by JCV T-antigen is thus the first step in viral replication and a key potential target for blocking reactivation of JCV.

JCV exploits several pathways to maintain cells in an immature, virus producing state. In addition to its direct impacts on the cell cycle, JCV proteins can also promote a stem cell-like phenotype. T-antigen stabilizes key members of the Wnt signaling pathway, β -catenin and c-myc, allowing for them to more readily enter the nucleus (Bhattacharyya et al. 2007; Enam et al. 2002; Gan and Khalili 2004). Once there, β -catenin and c-myc act as transcription factors for several genes, such as Oct4, Nanog and Sox2, which promote self renewal, a characteristic of stem cells. Expression of T-antigen, as well as the JCV late protein, Agnoprotein, can also prohibit the differentiation of glial cells (Merabova et al. 2008; Tretiakova et al. 1999). Further, T-antigen can allow infected cells to evade apoptosis. Through its interactions with the IGF-1 receptor, T-antigen enhances mTOR signaling and expression of the anti-apoptotic protein, survivin (Gualco et al. 2010). Alternatively, BAG3, another inhibitor of apoptosis, can suppress T-antigen expression and halt JCV replication (Saryier et al. 2012). Though the JCV genome is relatively simplistic compared to most viruses, encoded proteins produce a multifaceted attack on infected cells to ensure further propagation of JC viral particles.

Cell type specificity of JCV infection

JCV, like all known polyomaviruses, displays strict species specificity. *In vivo*, JCV has been shown to productively infect a broad range of human cells including kidney epithelial cells, tonsillar stromal cells, bone marrow derived cells, granule cell neurons, oligodendrocytes, and astrocytes (Wei et al. 2000). Attempts to study JCV in human cell culture systems have been restricted to human primary fetal astrocytes, oligodendrocyte progenitor cells and progenitor derived astrocytes, embryonic cells, brain microvascular endothelial cells, and fetal Schwann cells (Khalili et al. 2003). Due to the inability of JCV to replicate in non-human cells, animal models for studying PML by viral inoculation, have been largely unsuccessful at recapitulating the pathology seen in humans, instead resulting in the induction of a broad range of neural-origin tumors in non-human primates and several rodent species (Khalili et al. 2003). However, deficiencies in myelination induced by T-antigen in the absence of viral infection have been observed in transgenic mice, and humanized mice have recently been used to study the immune response to JCV infection (Khalili et al. 2003; Tan et al. 2013).

Initial JCV infection occurs in the first two decades of life via an oral or respiratory route, as indicated by infection of tonsil cells and the presence of JCV in cells of the gastrointestinal tract (Tavazzi et al. 2012). Typically, JCV establishes a persistent, low-level infection in kidney cells, where viral shedding can be detected in the urine of 10–30% of healthy individuals (Dörries et al. 1998). JCV has also been repeatedly detected in human PBMCs, more specifically B cells, and it is possible that the virus establishes a subclinical infection in other immune cells, such as bone marrow progenitor cells (Ferenczy et al. 2013; Tan et al. 2009). T-antigen expression alone can transform bone marrow derived mesenchymal stem cells, indicating that T-antigen may also drive viral replication in permissive cells residing in the bone marrow (Del Valle et al. 2010). Persistent infection of stromal cells of the bone marrow is one mechanism that could account for the association of JCV DNA with a broad range of circulating immune cells in healthy, as well as immunocompromised, individuals.

More recently, JCV DNA and proteins have been found in the brains of patients with and without PML or other underlying immunosuppressive condition, leading some to speculate

that JCV may traffic to and perhaps reactivate within the brain prior to the development of PML (Bayliss et al. 2012). Indeed, studies using laser capture microdissection, to avoid possible contamination of brain tissues with B cells from the circulation, have found JCV DNA sequences in several regions of gray and white matter (Bayliss et al. 2012; Piña-Oviedo et al. 2006). JCV has similarly been detected in cortical neurons in PML patients, suggesting that these cells can also be infected (Wüthrich and Korálnik 2012). In either scenario, the route and timing of JCV entry into the CNS are unclear. Initially, reactivation and dissemination of JCV into the brain would necessitate a loss of immunosurveillance mechanisms and require a vehicle for CNS penetration.

Pathological hallmarks of PML and immune cell infiltrates

Most of what is known about the immune response in PML has been determined based on detailed histopathological analysis of PML lesions. During active replication of JCV in the brain, oligodendrocytes serve as the permissive host cells, allowing productive infection. The nuclei of these infected oligodendrocytes become greatly enlarged during viral capsid formation and develop intranuclear eosinophilic inclusions. These oligodendrocyte inclusion bodies occur prior to their lytic destruction and are necessary pathological hallmarks for the diagnosis of PML (Enam et al. 2004; Wüthrich et al. 2006). Following oligodendrocyte loss, denuded spared axons can be seen within white matter lesions. The other hallmark of a PML lesion is the presence of bizarre, reactive astrocytes, which have a transformed appearance similar to glioblastoma cells (Astrom et al. 1958). The bizarre astrocytes of PML do not appear to be permissive for virus replication, though viral capsid proteins can be detected by immunohistochemistry and some intranuclear icosahedral capsids have been observed by electron microscopy, suggesting an abortive or semi-permissive infection of these cells (Boldorini et al. 1993). Initially, PML lesions develop as individual foci within the white matter, with the center of lesion typically exhibiting denuded axons. Active infection occurs at the periphery of the lesion, where oligodendrocyte inclusion bodies and bizarre astrocytes can be detected. Small lesions then coalesce to form larger lesions as the infection spreads to neighboring oligodendrocytes, usually along white matter tracts. In addition to the typical white matter lesions, lesions associated with cerebellar granule cell neurons have been recognized since the initial description of PML, but have typically received little attention (Astrom et al. 1958; Gheuens et al. 2013). More recently, infection of cortical pyramidal neurons within the gray matter has also been described (Gheuens et al. 2013).

While PML lesions do not exhibit appreciable inflammation, as might be anticipated due to the patient's underlying immunosuppressive state, some immune cells can be found at the periphery of active lesions, in proximity with oligodendrocyte inclusion bodies and bizarre astrocytes (Wüthrich et al. 2006). Foamy, lipid consuming macrophages can be found within PML lesions, but are likely a secondary consequence in response to the myelin sheath damage, rather than a specific anti-viral response (Cinque et al. 2009). As discussed further below, the type and function of the immune cells may depend, in part, upon the underlying immunosuppressive condition. For example, in PML patients with the underlying B cell lymphoproliferative disorder, chronic lymphocytic lymphoma (CLL), CD20+ B cells can frequently be seen accumulating in perivascular cuffs adjacent to PML lesions. CD4+ T cells are infrequently detected within PML lesions, which would be expected in HIV-1+ patients, especially those with reduced CD4+ T cell counts. The predominant immune cells in proximity to the PML lesion are CD8+ T cells, with their expression tapering off with distance from the lesion. These cells are thought to be the primary suppressors of viral resurgence, and their loss in the CNS is a predisposing factor to PML development. Interestingly, the profile of immune cells associated with PML lesions reflects the underlying mechanism of immune dysfunction seen across the range of evolving populations most vulnerable to the development of PML (Table 1).

The evolution of PML

Initially, PML was first described by Astrom, Mancall and Richardson as an extremely rare disorder seen in older patients with underlying lymphoproliferative disorders, including those caused by uncontrolled proliferation of B lymphocytes, such as chronic lymphocytic leukemia (CLL) and Hodgkin's lymphoma (Berger and Major 1999). It was named for the pathological features, a progressive multifocal disease of the white matter, which can now be thought of as the classical form of PML. With the beginning of the AIDS pandemic in the 1980's, the incidence of PML and the context of immunosuppression have made the disease more frequent. Though still a rare disorder, the lack of treatment and the poor outcomes associated with PML have continued to increase our awareness of the disease.

PML in the AIDS era

PML is the 3rd most common infectious neurological disease seen in HIV-1+ patients, and was an extremely rare disorder prior to the AIDS pandemic (Delbue et al. 2012). PML associated with HIV-1 infection can be separated into two distinct phases; before and after the introduction of highly active anti-retroviral therapy (HAART). In the pre-HAART era, PML was considered an AIDS defining illness, effecting 7–10% of HIV-1 infected individuals (Berger and Concha 1995). In the era of HAART, an estimated 3–5% of AIDS patients developed PML (Major et al, 2010), with more current combined anti-retroviral therapy (cART), it is estimated that the number of AIDS patients who now develop PML is predicted at 1.3 – 3.3 per 1000 person-years (Ensig et al. 2009). While cART can currently retain HIV-1 plasma viremia to a minimally detectable level, viral reservoirs remain in the periphery and CNS. HIV-1 replication predominantly occurs in CD4+ T cells, which are either lytically destroyed to release more virus or become latently infected reservoirs (Chun and Fauci 2012). Currently, it is presumed that the lack of CD4+ T cells and their roles in immune surveillance and activation of CD8+ T cell CTL responses are responsible for the high instances of PML in HIV-1+ patients. Though the incidence of PML in HIV-1+ individuals has declined somewhat due to cART, interest in JCV and PML spiked recently with the advent of a class of potent immunomodulatory therapies for auto-immune disorders.

Monoclonal antibodies and PML vulnerability

The monoclonal antibody era of PML began with the unexpected complication of PML in two multiple sclerosis (MS) patients being treated with the monoclonal antibody therapy, natalizumab (trade named Tysabri). MS is the most common autoimmune demyelinating disease in the CNS; however the underlying antigen triggering the autoimmune response remains unknown. In MS, inflammatory lymphocytes, particularly CD8+ T cells directed against endogenous myelin proteins, attack the protective myelin sheath of neurons leading to vast areas of demyelination, axonal damage, and inflammation (Compston and Coles 2008). As a result, visual defects, sensory abnormalities, pain, and loss of motor coordination are characteristic signs and symptoms of MS. Similar to a PML lesion, the most predominant immune cells present in an MS lesion are the CD8+ T cells (Gay et al. 1997). An effective strategy to control the disease is to reduce the infiltration of T cells into the CNS, thereby preventing their destruction of myelin. Based on this principle, natalizumab was developed to target $\alpha 4$ integrins present on the surface of T cells, to block their ability to bind to the adhesion molecule VCAM1 and thus block intravasation into the CNS (Steinman 2005). Natalizumab was shown to be successful at reducing T cell infiltration into the CNS, resulting in a reduction in brain lesions and fewer debilitating relapses in MS patients (Miller et al. 2003; Niino et al. 2006; Stüve et al. 1996). Similarly, natalizumab has been a useful agent in preventing the T cell infiltration which leads to the destruction of normal gut tissue seen in Crohn's disease patients (Van Assche et al. 2005).

While successful at reducing the devastating effects of an overactive immune system, natalizumab, by its very design, leads to a reduction in immune surveillance mechanisms in the CNS. To date, natalizumab treatment has been linked to nearly 400 cases of PML in MS patients and two cases of PML in Crohn's patients worldwide, with approximately 23% of the MS cases being fatal (FDA Drug Safety Communication 2012). Consequently, natalizumab was voluntarily removed from the market, and shortly thereafter reintroduced, with its associated warning of the risk of PML development. Several factors including prior immunosuppressive therapy, prolonged treatment with natalizumab, and the presence of high levels of anti-JCV antibodies have been identified as placing natalizumab patients at higher risk for the development of PML.

PML has been reported as an adverse event in the context of treatment of other auto-immune disorders with a variety of monoclonal antibody therapies, suggesting that immunosuppression can predispose these patients to the development of PML as well. Efalizumab (trade named Raptiva), targeting CD11a on T cells to their prevent binding to ICAM and infiltration into layers of the skin, was introduced for use in plaque psoriasis but was voluntarily withdrawn from the market in 2009 following the development of PML (Vugmeyster et al. 2004). Three monoclonal antibodies targeting TNF, adalimumab (Humira), etanercept (Enbrel), and infliximab (Remicade) for the treatment of psoriasis, rheumatoid arthritis, and Crohn's disease have also been associated with cases of PML. Interestingly, rituximab (trade named Rituxan), which targets CD20 on circulating B cells and is thought to lead to their depletion, has been associated with considerable cases of PML in B cell lymphoma and rheumatoid arthritis patients (for review Atzeni et al. 2013). The association between rituximab and PML may be partly due to the fact that lymphoproliferative disorders are clearly an underlying disorder predisposing patients to PML in itself. However, given the many lines of evidence suggesting a role for B cells in JCV trafficking and the virus life cycle, the association may extend beyond this observation.

The development of PML in patients treated with drugs targeting trafficking, as well as function of immune cells, reinforces the concept that a loss of immune surveillance mechanisms of T and B cells in the periphery can allow for JCV reactivation in the CNS and the initiation of PML (Carson et al. 2009; Schwab et al. 2012). The complexity of this issue is highlighted by the observation that the monoclonal antibody, alemtuzumab (trade name Campath), an anti-CD52 antibody that depletes both T and B cells, recapitulating many of the immune abnormalities of HIV-1 infection, has not yet been demonstrated to increase the risk of PML (Berger 2010).

B cells: CNS viral trafficking and anti-JCV antibody production

As classical PML was initially seen in patients with lymphoproliferative and myeloproliferative disorders, this prompted early speculation that the virus could infect PBMCs (Astrom et al. 1958; Houff and Berger 2008). B cells, the antibody producing cells of the immune system, are identified by the cell surface marker CD20 antigen. JCV can nonproductively infect naïve B cells, immortalized cell lines, and B cells previously infected by Epstein Barr Virus (Atwood et al. 1992; Major et al. 1990). JCV infected B cells can be found in the spleen and bone marrow, as well as the brain (Houff et al. 1988). Near the PML lesion, CD20+ B cells are found within the perivascular cuffs, indicative of their travel to the CNS from the periphery (Wüthrich et al. 2006). Virions released from infected B cells can be taken up by primary human fetal glial cells, suggesting that B cells can carry associated JCV into the brain (Chapagain and Nerurkar 2010).

The percentage of the individuals within a population producing anti-JCV antibodies varies greatly from 35–90%, though similar trends can be observed (Table 2, Weber et al. 2001).

For instance, earlier studies performed by hemagglutination inhibition assay (HAI), to detect antibodies to the virus capsid, reported the presence of anti-JCV antibodies in 10% of individuals within the first 4 years of life, which steadily increases to 50 and 75% by the age of 70 years old (Knowles et al. 2003; Padgett and Walker 1983). More recently, studies performed by ELISA with recombinant VP1 or virus-like particles (VLPs), reported similar increases with age across a range of populations (Egli et al. 2009; Kean et al. 2009). Taken together, these remarkably similar results clearly demonstrate increasing seroconversion during the first two decades of life, followed by a persistent increasing antibody response, throughout adulthood in the majority of the population. Though, these studies also suggest that a proportion of adults may indeed be naïve to the virus.

While it is generally accepted that the majority of the human population produces circulating antibodies against JCV VP1, these antibodies are not protective against the development or progression of PML. In fact, substantial antibody titers can be obtained from PML patients during disease, suggesting that the humoral response alone is inefficient at clearing JCV infected cells (Lindå et al. 2009). Anti-JCV antibody titers have been posed as a risk factor for the development of PML in MS patients receiving natalizumab, and are currently being used to stratify natalizumab patient populations using an FDA approved ELISA assay. However, the cutoff for the assay has created a high false negative rate, such that many patients who test negative for serum antibodies counts also have JC viruria, suggesting that this ELISA assay more accurately reflects the presence of high versus low antibody titers (Berger et al. 2013b). It is unclear why natalizumab patients with higher JCV antibody titers would be a greater risk of developing PML.

Immune surveillance of JCV by T cells

Though healthy individuals worldwide have with measurable amount of JCV antibodies, they do not have a concomitant viremia (Koralnik et al. 1999). This indicates that the immune system is capable of detecting and arming itself against JCV, as well as containing viral spread. Further, MHC class I and MHC class II molecules are highly expressed within PML lesions, eliminating the possibility that the lack of response is due to a lack of antigen presentation (Achim and Wiley 1992). Antigens presented on these molecules are recognized by CD8⁺ and CD4⁺ T lymphocytes, respectively, in a fashion typical of a normal cell mediated immune response to viral infection.

Several lines of circumstantial evidence exist suggesting that CD4⁺ helper T cells are crucial to the prevention of JCV spread. Initially it was recognized that JCV reactivation is most common in patients with deficiencies in CD4⁺ T cells, such as HIV-1⁺ patients who have progressed to AIDS and patients with CD4⁺ T lymphocytopenia (Haider et al. 2000; Puri et al. 2010). Accordingly, a low CD4⁺ T cell count would correlate with a greater viral load in the CSF, and a worse clinical outcome in PML patients (Ferenczy et al. 2012). More recently, it was discovered that individuals harboring the allele for MHC-II molecule HLA0DRB1*04:01 display a dampened proliferation of CD4⁺ T cells when stimulated with JCV specific antigens, perhaps making this population more susceptible to viral resurgence (Jelcic et al. 2013). These JCV specific T cells can then infiltrate the CNS and be detected within the PML lesion, albeit at a low frequency compared to other immune cells (Yousef et al. 2012). However, CD4⁺ T cell count alone cannot predict disease progression, suggesting that other cell types are also crucial for viral containment (Du Pasquier et al. 2001).

In PML patients, CD4⁺ T cell counts correlate with the cytotoxic action of CD8⁺ T cells, the most common immune cell infiltrate seen within the PML lesion and CSF of these patients (Du Pasquier et al. 2005; Gasnault et al. 2003; Wüthrich et al. 2006). With the help of cytokines secreted by CD4⁺ T cells, CD8⁺ T cells can differentiate into effector or

memory T cells. Effector T cells, when coming in contact with a cell presenting their primed target, attempt to eliminate these cells via the cytotoxic T lymphocyte (CTL) response (Kaeck and Cui 2012). This CD8⁺ CTL response plays an important role in controlling replication of many viruses, most notably the herpesvirus Epstein–Barr virus (Steven et al. 1996). CD8⁺ T cells from healthy, HIV-1⁺, and HIV-1⁺ PML individuals are all capable of mounting a robust CTL response against the JCV VP1 capsid protein (Du Pasquier et al. 2004). *Ex vivo* experiments in individuals with the HLA-A*201 haplotype have identified two highly immunogenic regions of the JCV VP1 capsid protein, VP1 p36 and VP1 p100. Though a number of other antigens spanning the VP1 sequence are predicted to elicit a similar response, VP1 p36 and VP1 p100 are capable of inducing a CTL response (Du Pasquier et al. 2003). Since JCV is a slow growing virus, it is conceivable that even a low amount of these armed CD8⁺ T cells can effectively prevent viral resurgence, indicating these cells to be the most important in immune surveillance of JCV (Lima et al. 2007).

Insights from immune reconstitution

Currently, there are no effective treatments for PML, and patients who do not survive will succumb to disease within 6–12 months of onset, while those that do survive are often left with significant physiological and cognitive impairments (Brew et al. 2010). Restoration of underlying immune dysfunction has become the mainstay of treatment. This is based on early observations that the availability of HAART treatment improved patient survival in HIV-1⁺ PML patients (Clifford et al. 1999; Gasnault et al. 1999; Miralles et al. 1998; Tassie et al. 1999). Presumably, the deficiency of CD4⁺ T cells seen in treatment naïve HIV/AIDS patients results in a lack of JCV specific CD8⁺ effector T cells, thus predisposing HIV-1⁺ patients to the risk for PML development (Wuthrich et al. 2006).

Heterogeneity in clinical outcomes has been noted in several contexts. For example, there are rare reports of spontaneous remission of PML, as well as reports of PML in immunocompetent patients, suggesting that these patients may have been JCV naïve (Christakis et al. 2013; Gheuens et al. 2010; Yoganathan et al. 2012). Additionally, polymorphisms or mutations in the noncoding regions and structural genes have recently been detected in some MS patients with PML, however their roles in the pathogenesis of PML is unclear at present (Reid et al. 2011, Gorelik et al. 2011). Differences in outcome were noted early in the AIDS era as 10% of PML patients display a prolonged survival instead of the traditional fatal short bout with the disease, but the risk factors for prolonged survival versus progression were not understood (Berger et al. 1998). However, more recent studies have suggested that while overall CTL responses are dampened in PML patients, those patients who do elicit a CTL response against T-antigen or VP1 may have better clinical outcomes (Du Pasquier et al. 2001; Gasnault et al. 2003; Koralnik et al. 2001; Lima et al. 2010). Comparatively, PML seen in MS patients on natalizumab appears to be associated with a better clinical outcome compared to the course of the disease in HIV-1⁺ PML patients (Vermersch et al. 2011).

While treatment of the underlying immunodeficiency to restore the anti-JCV immune response, through HAART or discontinuation of monoclonal antibody therapy may seem like the best option for PML treatment, the rapid restoration of JCV-specific CTL response often induces inflammation and generation of immune reconstitution inflammatory syndrome (IRIS). For example, HAART-naïve HIV-1 patients diagnosed with PML will paradoxically experience worsening of PML symptoms upon initiating HAART due to IRIS, which occurs upon increase in CD4⁺ T cell counts and subsequent restoration of immune function upon initiation of HAART. While CTL response appears to correlate with the development of IRIS, it is not clear whether the CTL response serves as an initiator (Marzocchi et al., 2009a). The JCV specific CTL response in PML-IRIS can be dampened

with the administration of corticosteroids, indicating this to be a method to prevent the development of IRIS when treating PML (Antonioli et al. 2012). In a similar fashion, a diagnosis of PML in natalizumab patients prompts discontinuation of the drug frequently followed by the development of IRIS, which can be triggered or exacerbated by more rapid removal of residual natalizumab, using plasma exchange or immunoadsorption. Some studies have reported the detection of high numbers of CD4+ T cells, in addition to CD8+ T cells, B cells, and monocytes in IRIS associated with PML lesions of MS patients treated with natalizumab (Aly et al. 2011). In both situations, the phenomenon of IRIS provides clear evidence that an anti-JCV CTL immune response can rapidly and specifically be mounted in these patients. Furthermore, the presence of an anti-JCV CTL response has been associated with better outcome in PML patients (Marzocchetti et al., 2009b).

Factors favoring viral reactivation

Previous studies have elucidated potential mechanisms capable of directly impacting JCV replication, which may, in part, explain, the strong association between HIV-1 and the development of PML. One of the major HIV-1 regulatory proteins, Tat, a potent transactivator of viral transcription from the HIV-1 long terminal repeat (LTR) promoter, has been shown to be produced and secreted by HIV-1 infected cells and can be taken up by neighboring uninfected cells (Li et al. 2009). Intracellular Tat can then bind to and activate the JCV promoter, as well as interact with several transcription factors which enhance activity of the JCV promoter in glial cells (Daniel et al. 2004; Gallia et al. 1998; Krachmarov et al. 1996). Indirect mechanisms exist whereby HIV-1 can enhance JCV transcription in oligodendrocytes through the release of the cytokine TGF- β and Tat, resulting in increased activities of SMAD proteins and activation of early and late JCV promoters. Concordantly, JCV T-antigen can enhance activity of the HIV-1 LTR promoter while the JCV late protein, Agnoprotein, suppresses transcriptional activation of the HIV-1 LTR promoter (Kaniowska et al. 2006; Tada et al. 1990). Accordingly, SMADs 3 and 4, T-antigen, VP1, TGF- β , and Tat proteins have been found in JCV infected oligodendrocyte inclusion bodies at the border of PML lesions, highlighting the viral crosstalk between JCV and HIV (Daniel et al. 2004; Stettner et al. 2009). There may be other viruses in addition to HIV-1 that may cooperate or synergize with JCV, such as EBV in CNS lymphoma and CMV in glioblastomas (Del Valle et al. 2004; Winkhofer et al. 2000).

While the majority of the human population has been infected with and produce circulating antibodies to the JC virus, these antibodies do not prevent PML. As discussed above, evidence has suggested that cell-mediated immunity is the main mechanism controlling JC virus infection (Du Pasquier et al. 2004; Koralnik et al. 2002). Though some drugs that are associated with the development of PML deplete or block B cells, suggesting that B cells play an active role in controlling JC virus infection. In addition, the site of JC virus reactivation is frequently removed from the site of primary disease in patients receiving immunosuppressive drugs (i.e. treatment for Crohn's disease or lupus results in CNS disease) suggesting that systemic events and indirect mechanisms including soluble immunomodulators play a role in JC virus reactivation. Many studies by us and others have described mechanisms of transcriptional control of JC virus via cytokines or other immunomodulators and signal transduction pathways including TNF, TGF, Smads, -catenin, TNF, and NF κ B (for review see Raj and Khalili 1995). Some of these pathways have been shown to be particularly active in the context of HIV-1 and lead to direct induction of the JC virus regulatory region, providing some insight as to why JC virus reactivation and PML may be frequently seen in the AIDS population. For example, a decrease in MCP-1 has been reported to inversely correlate with an increase in JC virus load in the CSF of patients with HIV-1 associated PML and a decrease in IL-6 was observed in the CSF of Natalizumab treated multiple sclerosis patients (Marzocchetti et al. 2005; Møllergaard et al. 2010). Further,

natalizumab therapy also correlates with enhanced the secretion of the immunosuppressive cytokine, IL-10, by CD4+ T cells in the CNS (Perkins et al. 2012). Such findings suggest that the milieu of immunomodulators or inflammatory cytokines present due to the underlying disease process may set the stage for the initiation of JC virus infection in the context of an impaired immune system, where JC virus may reactivate unchecked, thus leading to the development of PML. Despite the increasing frequency of PML in immunosuppressed individuals on a growing number of therapies, it is not currently possible to identify which patients are at risk for JC virus reactivation and the mechanism whereby these immunosuppressive therapies allows JC virus reactivation are unclear. It is possible that particular immunosuppressive drug regimens result in the production of cytokines or combinations of cytokines that can impact on JCV reactivation and thus may be useful to identify at risk populations for possible development of PML or for early non-invasive detection of JC virus reactivation.

Alternatively, there is some evidence that JCV may evoke protective responses to suppress or evade immune surveillance. For example, to prevent a CTL response, JCV may have evolved a mechanism to promote anergy, or immune inactivation, of CD8+ T cells. The program cell death-1 (PD-1) molecule, a marker of immune exhaustion, can be found on CD8+ T cells in HIV-1+ patients with and without PML. Binding of its ligands, either PD-L1 or PD-L2, triggers anergy of T cells. However, ex vivo blockage of PD-1 can restore the JCV specific CTL response (Tan et al. 2012).

Diagnostic considerations in the PML eras

Though the predominant underlying mechanism in the development of PML has changed over time, the pathological hallmarks (i.e. the triad of demyelination, oligodendrocyte inclusion bodies, and bizarre astrocytes) have remained the same. Biopsy continues to be the gold standard for diagnosis, with greater than 98% specificity and a sensitivity of approximately 68–90%, mainly due to potential sampling error during biopsy (Koralnik et al. 1999). The significant risks associated with brain biopsy have led to the reliance on MR imaging and CSF PCR for the presence of JCV DNA as surrogate markers in lieu of biopsy. As with biopsy, CSF PCR for JCV DNA has an extremely high specificity (Cinque et al. 1997). However, it is important to note that while PCR diagnostic technologies have improved over time, this has not led to significant increases in its sensitivity for the diagnosis of PML; for two distinct reasons. First, it has frequently been noted that patients with confirmed PML may test PCR negative for JCV DNA in the CSF (Marzochetti et al. 2005b). Thus, the absence of JCV DNA by CSF PCR does not exclude a diagnosis of PML and it therefore should not be assumed that the CSF compartment provides an accurate representation of infections within the brain parenchyma. Second, JCV DNA viral loads in the CSF have been declining in recent years, from a high in the pre-HAART era of more profound immunosuppression, to more moderate levels in the cART era, and even lower levels in patients on monoclonal antibody therapies such as natalizumab (Berger et al. 2013a; Marzochetti et al. 2005a).

Outlook for PML: still a long way to go

A broad range of drugs, some with potential anti-viral mechanisms of action have been tested and have failed at controlling JC virus replication and halting its progression in PML patients. As JCV is a DNA virus, nucleoside analogs and inhibitors targeting DNA replication have been tried as therapeutics. The nucleoside inhibitors, cytarabine and cidofovir, have shown some efficacy in vitro, but not in vivo (Andrei et al. 1997; De Luca et al. 2008; Hall et al. 1998). A similar discordance between in vitro and in vivo studies was demonstrated with the drugs, topotecan and camptothecin which potently inhibit JCV

replication in cell culture, but are poorly tolerated by patients (Kerr et al. 1993; Royal III et al. 2003). Treatment with IFN, a potent antiviral and immunomodulatory cytokine, has also been tried in PML patients without success (Geshwind et al, 2001; Huang et al, 1998). Most recently, clinical trials testing mefloquine, which was first used anecdotally, have now been halted (Clifford et al. 2013; Tyler 2013). In cell culture, PARP1 and 5HT_{2a} inhibitors and interferon- β 1a have been successful at halting viral replication, but their efficacy in vivo has not yet been evaluated (O'Hara and Atwood 2008; Nukuzuma et al. 2013). Treatment of PML is further complicated due to their CNS localization with poor bioavailability and poor CNS penetrance likely to blame for some of the above noted failures. A candidate drug would need to pass through the blood brain barrier, and any residual drug would need to provide little to no peripheral toxicity. Therefore, future treatments which are more specific to the virus may help to avoid toxicity.

Currently, the only treatment for PML is reduction in immunosuppression or restoration of underlying immune impairment. For instance, successful HAART treatment of HIV-1+ PML patients has been correlated with an enhanced CD4+ T cell count, and enhanced survival. Other immune-mediated strategies may offer more promising avenues at present. For example, ex vivo expansion of JCV specific CD8+ T cells may restore immune surveillance mechanisms in PML patients (Du Pasquier et al. 2005). However, as described previously, the onset of IRIS may worsen the clinical outcome in PML patients thus a fine balance modulating immunosuppression and immune reconstitution would be needed. Should subsets of individuals with reduced JCV immune responses be identified, prophylactic vaccine strategies prior to immunosuppression could prevent the future occurrence of PML (Jelcic et al. 2013). Further understanding of at risk populations, as well as biomarkers and cytokine profiles which favor reactivation of the virus in immunocompromised patients are needed to monitor disease progression for early intervention in restoring immune function in order to halt JCV reactivation and PML development.

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Eras of PML

Classical

Underlying Mechanism:
Lympho/
Myeloproliferative
disorder

Monoclonal Antibody Therapy

Underlying Mechanism:
Reduced trafficking of
immune cells across
the BBB

HIV-1/AIDS

Underlying mechanism:
Depletion of CD4+ T
cells by HIV-1 infection

IRIS

Underlying Mechanism:
Restoration of immune
cells and/or their
trafficking across BBB

PML Lesion

Figure 1. Histopathological features of the PML lesion and the underlying mechanisms of PML development. (PML Lesion)

The histopathological features diagnostic of PML are demyelination, oligodendrocyte inclusion bodies, and bizarre astrocytes. Oligodendrocytes are the primary cell type supporting a productive infection of JCV in the CNS. During the end stages of viral replication, the nuclei of infected oligodendrocytes become enlarged and develop eosinophilic inclusions. Virus release results in the lytic destruction of infected oligodendrocytes which strips axons of their protective myelin sheaths. These demyelinated axons are mainly localized to the core of the lesion. Virus replication occurs at the outer periphery of active lesions where oligodendrocyte inclusion bodies are seen in proximity to bizarre astrocytes, which are abortively infected cells resembling glioblastoma cells. The

most predominant immune cell type associated with the PML lesion is the CD8+ T cells. To a lesser degree, CD4+ T cells, foamy macrophages, and JCV infected CD20+ B cells can be found in the periphery of the lesion, and nearby perivascular cuffs, respectively. (*Eras of PML*) In the classical era, PML was an extremely rare disorder which occurred in patients with lymphoproliferative and myeloproliferative disorders possibly due to a lack of a JCV specific immune response as a result of uncontrolled expansion of other immune cell types due to the cancer. PML became exponentially more prevalent with the onset of the HIV-1 pandemic. HIV-1+ patients with depleted CD4+ T cell counts, especially in the era before antiretroviral therapy, became increasingly vulnerable to the development of PML, suggesting that this cell type plays an important role in immune surveillance of JCV. More recently, cases of PML have been documented in immunocompromised patients undergoing monoclonal antibody therapy. The most common therapy linked to PML development is natalizumab used to treat multiple sclerosis. This monoclonal antibody blocks the migration of CD8+ T cells into the CNS, to prevent auto-immune mediated attack of normal myelin. However, the lack of normal immune surveillance by CD8+ T cells in the CNS allows for the uncontrolled reactivation of JC virus. Current intervention for PML involves restoration of the immune response by antiretroviral therapy in HIV-1+ patients and discontinuation of monoclonal antibody treatment in MS patients, which triggers rapid expansion and enhanced trafficking of immune cells across the blood brain barrier (BBB) and often leads to the development of immune-mediated inflammatory reconstitution syndrome (IRIS) and the temporary worsening of clinical symptoms.

Table 1

The evolution of PML by era

era	years	most frequent underlying disorder	incidence
classical	1958–1980	immunodeficiency syndrome immunoproliferative disorders	very rare
HIV-1 associated			
pre-HAART	1980–1994	AIDS	5% *
cART	1995-present	HIV-1 positive	1.3 to 3.3 per 1,000 patient-years [§]
monoclonal antibody	2005-present	multiple sclerosis and other autoimmune disorders	1 to 11 per 1,000 patient-years (Tysabri) [¶]

* Berger and Concha 1995

[§] Engsig FN et al. 2009[¶] FDA Drug Safety Communication (2012)

Table 2

A comparison of JCV antibody titer obtained by various assays

JCV antibody titers in the human population (pos/total (%))				
Age	Padgett and Walker 1983 [*]	Knowles et al. 2003 [*]	Kean et al. 2009 ^{**}	Egli et al. 2009 ^{***}
1–4	2/20 (10%)	8/72 (11%)	18/112 (16%)	
5–9	16/69 (28%)	23/160 (14%)	30/146 (21%)	
10–14	13/20 (65%)	39/161 (24%)	45/192 (23%)	
15–19	10/20 (50%)	49/223 (22%)	57/271 (21%)	
20–29		117/341 (34%)		50/100 (50%)
30–39		151/389 (39%)	245/718 (34%)	54/100 (54%)
40–49		131/380 (34%)		59/100 (59%)
50–59		159/353 (45%)	176/423 (42%)	68/100 (68%)
60–69	120/157 (75%)	178/356 (50%)	115/264 (43%)	
>70			49/96 (51%)	

^{*} HAI;^{**} recombinant VP1 based ELISA;^{***} VLP based ELISA