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REVIEW

Revisiting JC virus and progressive multifocal leukoencephalopathy

Angela Rocchi[1] · **Ilker K. Sariyer**[1] · **Joseph R. Berger**[2]

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Abstract
Since its definition 65 years ago, progressive multifocal leukoencephalopathy (PML) has continued to devastate a growing population of immunosuppressed patients despite major advances in our understanding of the causative JC virus (JCV). Unless contained by the immune system, JCV lyses host oligodendrocytes collateral to its life cycle, leading to demyelination, neurodegeneration, and death. Novel treatments have stagnated in the absence of an animal model while current antiviral agents fail to address the now ubiquitous polyomavirus. In this review, we highlight the established pathogenesis by which JCV infection progresses to PML, highlighting major challenges that must be overcome to eliminate the underlying virus and, therefore, the debilitating disease.

Keywords JC virus · PML · Latency · Immunosuppression · Reactivation · Diagnosis · Treatment

JC virus

At the time of its framing as a distinct disorder in 1958, the etiology of PML remained uncertain (Astrom et al. 1958). With a majority of cases affecting patients with advanced blood cancers (i.e., leukemias, lymphomas, myelomas) and severe inflammatory conditions (i.e., lupus erythematous, sarcoidosis), an early hypothesis proposed that it was the consequence of an opportunistic virus (Richardson 1961, 1974). This was confirmed by Zu Rhein and Chou in 1965, who identified an unknown "papova-like virus" when analyzing PML lesions with electron microscopy (Zurhein and Chou 1965). Six years later, Padgett et al. (1971) successfully isolated the PML-causing strain of JC virus (JCV) from the autopsy of the eponymous patient, JC. By 1976, JCV was identified in the sera of over twenty cases of PML and was the accepted cause of the demyelinating disease (Padgett et al. 1976). Shortly thereafter, Frisque, Bream, and Cannella published the complete genome and associated proteome of the first isolated JCV strain, Mad-1 (Frisque et al. 1984).

JCV is a double-stranded DNA polyomavirus with a circular, supercoiled genome packaged within an icosahedral capsid (Fig. 1A). The non-coding control region (NCCR) regulates the bicistronic genome consisting of two early and four late open reading frames (Fig. 1B). The early NCCR and six coding regions are largely conserved whereas the late NCCR varies across benign and pathologic strains (Fig. 2). Two early proteins, small and large tumor antigen (smtAg and LTAg), act as essential transactivators of the NCCR. In addition to increasing viral transcription by functioning as a helicase (unwinding and unzipping the hypercoiled dsDNA) and enhancer (recruiting host machinery to the promoter), the T antigens interact with housekeeping genes to create a pro-viral environment (Saribas and Safak 2020). Through interaction with E3 ubiquitin ligase, TRIM25, smtAg interrupts the cytokine signaling pathway of the innate immune response (Bollag et al. 2010; Chiang et al. 2021). LTAg inactivates tumor suppressors, p53 and pRb; inhibits the pro-apoptotic protein, survivin, and promotes proliferation through the c-Myc/Wnt pathway (Krynska et al. 1997; Gan et al. 2001) The resultant pro-survival state provides the two early peptides with their "tumor" namesake.

Agnoprotein (Agno), the intermediate or "early-late" protein, hinders early LTAg and promotes late gene expression of additional Agno and the three capsid proteins, VP1-3

✉ Ilker K. Sariyer
isariyer@temple.edu

✉ Joseph R. Berger
Joseph.Berger@pennmedicine.upenn.edu

[1] Department of Microbiology, Immunology and Inflammation, Center for Neurovirology and Gene Editing, Temple University Lewis Katz School of Medicine, Philadelphia, PA 19140, USA

[2] Department of Neurology, Perelman School of Medicine, University of Pennsylvania, 3400 Convention Avenue, Philadelphia, PA 19104, USA

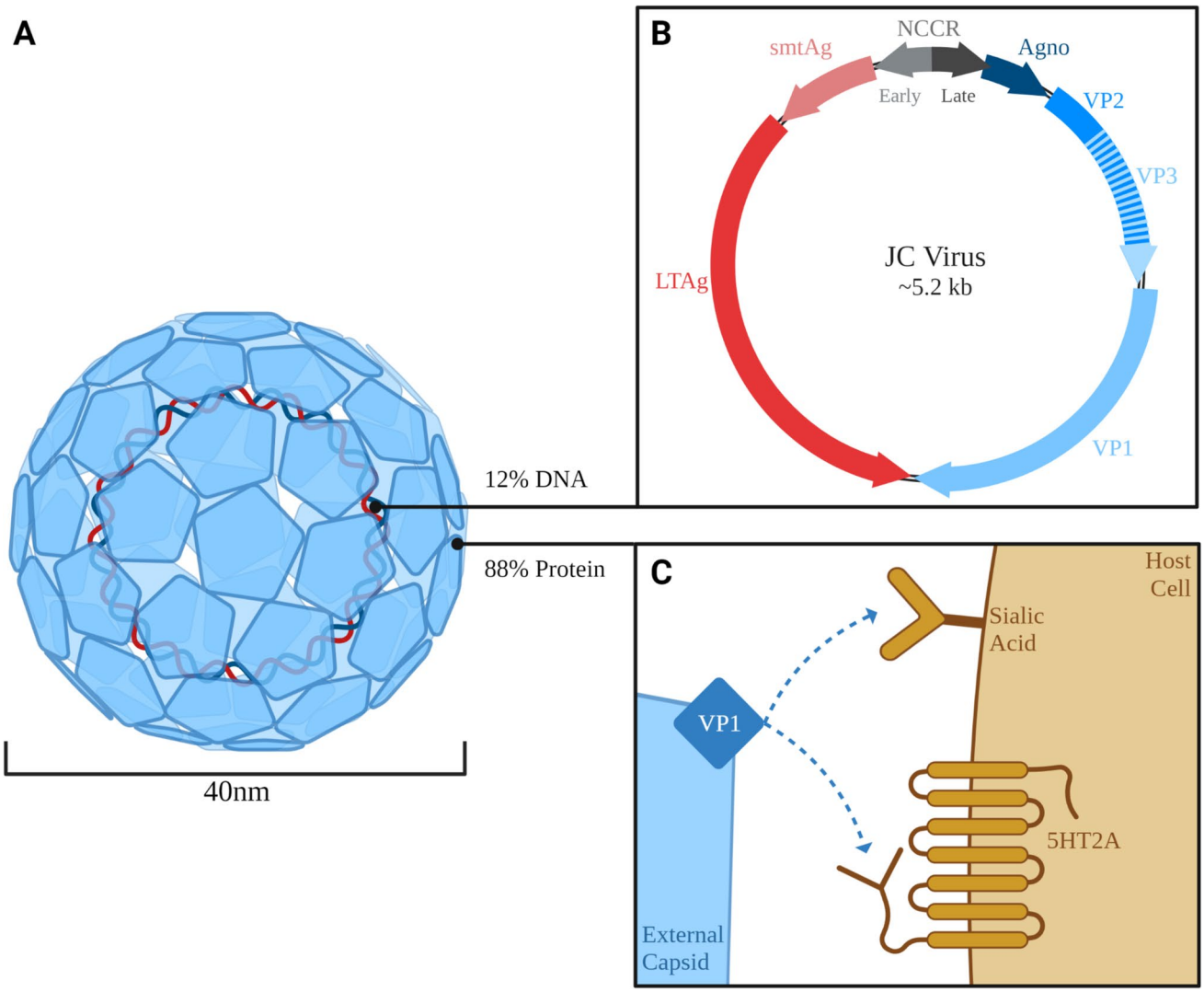

Fig. 1 Overview of JC capsid, genome map, and interaction with host receptors. **A** Cartoonized depiction of the JC virus, which is 12% DNA and 88% protein by weight. The external icosahedral capsid consists of 72 VP1 pentamers organized around an inner capsid of approximately 72 VP2/VP3 proteins (not visible). The circular dsDNA genome is supercoiled within the capsid. **B** The genome is 5.2 kb, including a bicistronic non-coding control region and six open reading frames. The early genome (red) encodes small and large T-antigen; the late genome (blue) encodes agnoprotein and the three capsid proteins. **C** The VP1 proteins of the outer capsid directly bind to sialic acid residues and/or 5HT2A receptors on the host cell surface, initiating endocytosis

(Safak et al. 2001; Akan et al. 2006). Agno exits the host cell prior to cell death and interacts with adjacent cells, priming them for viral infiltration (Saribas et al. 2018). Within the infected cell, it also interacts with mitochondria to directly induce apoptosis of oligodendrocytes, enabling the lytic spread of JCV (Merabova et al. 2008; Saxena et al. 2021).

The external capsid includes 360 VP1 peptides arranged in 72 pentamers, forming an icosahedral capsid with T = 7 symmetry (Fig. 1A). Without an envelope, VP1 directly interacts with the environment, binding sialic acid residues on cell membranes to induce endocytosis (Fig. 1C) (Shishido-Hara et al. 2000; Ou et al. 2001; Kobayashi et al. 2013). Serotonin receptor 5HT2A, in particular, has been shown to interact with VP1, initiating the internalization of the entire virus into clathrin-coated pits (Querbes et al. 2004; Chapagain et al. 2008; Mayberry et al. 2019). The VP1 shell is capable of self-assembly but is stabilized around intrinsic minor proteins VP2 and VP3 (Ou et al. 2001; Shishido-Hara et al. 2004). Each five-VP1 capsomere is paired with an intrinsic VP2 or VP3 monomer, the latter being a truncated version of the former. The absence of either minor protein impairs the nuclear localization of JCV VP1 resulting in a reduction of viral progeny with key roles in nuclear localization and DNA packaging (Shishido-Hara et al. 2004; Gasparovic et al. 2006). The myristoylated VP2 has been identified in the role of uncoating in early infection whereas

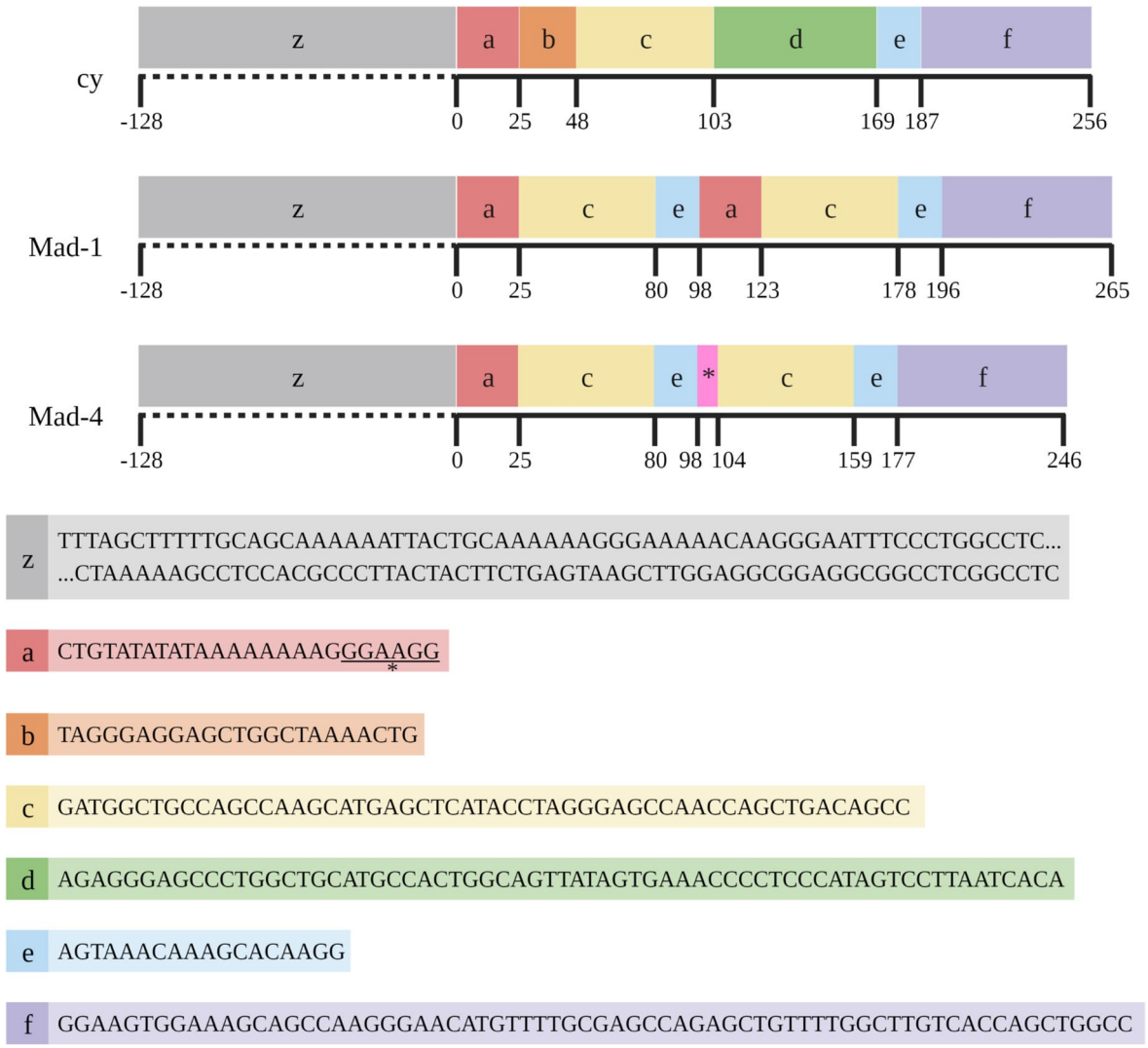

Fig. 2 Arrangements of non-coding control regions of archetype (cy) and PML-type (Mad-1, Mad-4) JCV strains. Depiction of the archetype control region with noted deletions and rearrangements of the late promoter, producing common PML-type strains, Mad-1 and Mad-4. The early promoter (z) is largely conserved and regulates the expression of smtAg and LTAg whereas the late promoter (a–f) is prone to hypervariability and regulates the expression of Agno and VP1-3

VP3 has been shown to inhibit the early promoter of the viral genome (Krauzewicz et al. 1990; Huang et al. 2003). Complexes of VP2 and VP3, together, enhance LTAg binding to the NCCR, promoting viral genome expression (Saribas et al. 2014).

The exact ratio of VP2 to VP3 is unknown; other polyomaviruses, such as Merkel polyomavirus, lack VP3 altogether. Simian virus 40 (SV40) is a primate polyomavirus with a 69% homology to JCV (Deckhut et al. 1991). Its similar external shell of VP1 peptides surrounds a mixed core of 72 VP2 and VP3 monomers of an unspecified ratio (Nakanishi et al. 2006; Gasparovic et al. 2006). A complex of both VP2 and VP3 acts as a viroporin in the endoplasmic reticulum, enabling infection and packaging of viral DNA in the nucleus (Daniels et al. 2006). Given their similarities, it can be extrapolated that the roles of JCV VP2 and VP3 are similar to those of SV40, but the exact numbers remain unestablished for both.

The lifecycle of JCV: the adaptation hypothesis

Using viral isolate from patient samples and hemagglutination-inhibition testing, Padgett and Walker (1973) found 69% of the general population expressed antibodies against JCV, with 14% seropositivity in young children growing to 86% in senior adults. Asymptomatic individuals were identified as carriers as 20% of healthy subjects shed infectious JC virions in their urine (Hogan et al. 1980; Kitamura et al. 1990; Flaegstad et al. 1991). This peripheral strain has been referred to as "archetype"

JCV (cy-JCV). Yogo and colleagues isolated cy-JCV and found it persists within the same, healthy participants over many years without mutations in the genome or decrement in concentrations (Yogo et al. 1990, 1991; Kato et al. 1994; Kitamura et al. 1997). Conversely, hypervariable "prototype" (hyp-JCV, also referred to as "neurotropic") strains were isolated from PML patients, with rearrangements of the late NCCR differing across cases as well as within individuals (Grinnell et al. 1983; Martin et al. 1985; Iida et al. 1993; Ault and Stoner 1993; Yogo et al. 1994).

The "adaptation model" became the accepted theory of the JCV/PML life cycle wherein cy-JCV is spread through contamination of the environment before mutating to hvp-JCV within an immunocompromised host (Fig. 3). This theory is supported by the identification of cy-JCV in sewage and age-graded changes in anti-VP1 seropositivity (Bofill-Mas and Girones 2003; Bofill-Mas et al. 2003). Recent evidence from the novel coronavirus pandemic supports the gastrointestinal route of infection: regions with mask mandates show stable JCV seroconversion rates while viruses known to transmit through respiratory droplets (e.g., coronavirus, influenza, rhinovirus) declined (Cheng et al. 2021; Oh et al. 2021; Leech et al. 2022; Vigiser et al. 2022). LTAg has been implicated in multiple gastrointestinal cancers in the absence of systemic JCV, further supporting the proposed mechanism of infection via ingestion (Enam et al. 2002; Del Valle et al. 2005; Shin et al. 2006; Shavaleh et al. 2020; Querido et al. 2020; Fang et al. 2022).

During immunosuppression, cy-JCV and its hvp-JCV progeny expand to include additional reservoirs, infiltrating lymphocytes and spreading hematogenously from the primary latency site in the kidney to include bone marrow and nervous tissue (Tornatore et al. 1992; White et al. 1992; d'Arminio Monforte et al. 1997; Du Pasquier et al. 2004; Van Loy et al. 2015). In the absence of immunosuppression, the blood–brain barrier successfully prevents the hematogenous spread of JCV, but evidence suggests even temporary immunosuppression can enable infiltration of nervous tissues through B-cell mediated extravasation. Both cy- and hvp-JCV proliferate strongest in glial cells, and both have been identified in the brains and peripheral tissues of PML and non-PML patients (White et al. 1992; O'Neill et al. 2003; Tan et al. 2010). Increased proliferation significantly increases the degree and rate of NCCR mutation, with deletions preceding translocations and variants increasing with viral load (Ault and Stoner 1993; Agostini et al. 1997; Pietropaolo et al. 2003). These mutations have clinical significance, with the number of repeats correlating with poorer PML outcomes (Pfister et al. 2001; Van Loy et al. 2015; Wilczek et al. 2022). The hypervariable prototype strains are not only less common, but less resistant to external stressors which influence the proposed route of transmission (i.e., extremes of pH) (Bofill-Mas et al. 2001, 2003; Bofill-Mas and Girones 2003). Particular rearrangements (e.g., Mad1, Mad4) promote the rate of replication more than cy-JCV and other, less common, hvp-JCV isoforms, supporting their convergent evolution in separate hosts (Daniel et al. 1996; Sock et al. 1996; Ault 1997; Elsner and Dörries 1998; Fedele et al. 2003; O'Neill et al. 2003). Mutations in coding regions, such as the late capsid proteins, are less commonly reported (Stoner and Ryschkewitsch 1995). Phenotype-altering mutations of the early (sm- and LTAg) and early-late proteins (Agno) significantly impaired viral propagation by removing the necessary recruitment of host polymerases (Okada et al. 2001). Similarly, alterations of the capsid proteins (VP1-3) altered the stability and infectivity of the virions. Alterations in these capsid proteins may alter the need for sialic acid binding for cellular invasion and, therefore, favor infection (Gorelik et al. 2011).

Latency and reactivation: the role of immune modulation in JCV pathogenesis

The mechanism by which an immune system promotes JCV latency is unknown, with several different mechanisms of immunosuppression resulting in reactivation (Du Pasquier et al. 2001; Iannetta et al. 2019). Flaws in both humoral and cell-mediated pathways have been implicated in PML pathogenesis, while reductions in pro-inflammatory markers have shown a correlation with JCV proliferation. Nuclear regulators, such as alternative splicing factor SF2/ASF, also change the capacity of a cell to maintain JCV levels at a low, unchanging level (Sariyer and Khalili 2011; Uleri et al. 2013; Piu et al. 2020). In addition to immunosuppression, certain external factors can enhance JCV replication including comorbid viruses and pollution (Dolci et al. 2018).

During the human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) epidemic, PML became significantly more common. The overall death rate of PML quadrupled, and 5–7% of individuals with HIV/AIDS developed PML (Holman et al. 1991). Although immunosuppression of HIV/AIDS increases risk, the incidence of PML was far greater than in other immune disorders (e.g., transplant recipients, lymphoma patients). With 80% of PML cases affecting patients with HIV/AIDS, clinical and epidemiological evidence hinted at a compounding relationship between human immunodeficiency and JC viruses (Wortman et al. 2000; Daniel et al. 2001). A complex of HIV transactivator, Tat, and cellular transcription factor, purα, was found to significantly increase the expression of JCV late genes by interacting with its promoter region. Other viruses have since shown cross-activation of the JCV late promoter with human T-lymphotropic virus type I acting through its tax protein and cytomegalovirus through IE2 (Okada et al. 2000; Winklhofer et al. 2000). Host proteins, including Bag1, NFκβ, YB-1 Sμbp-2, and Spi-B increase expression whereas c-Jun decreases it (Chen et al. 1997; Devireddy et al. 2000; Ravichandran et al. 2006; Marshall et al. 2010).

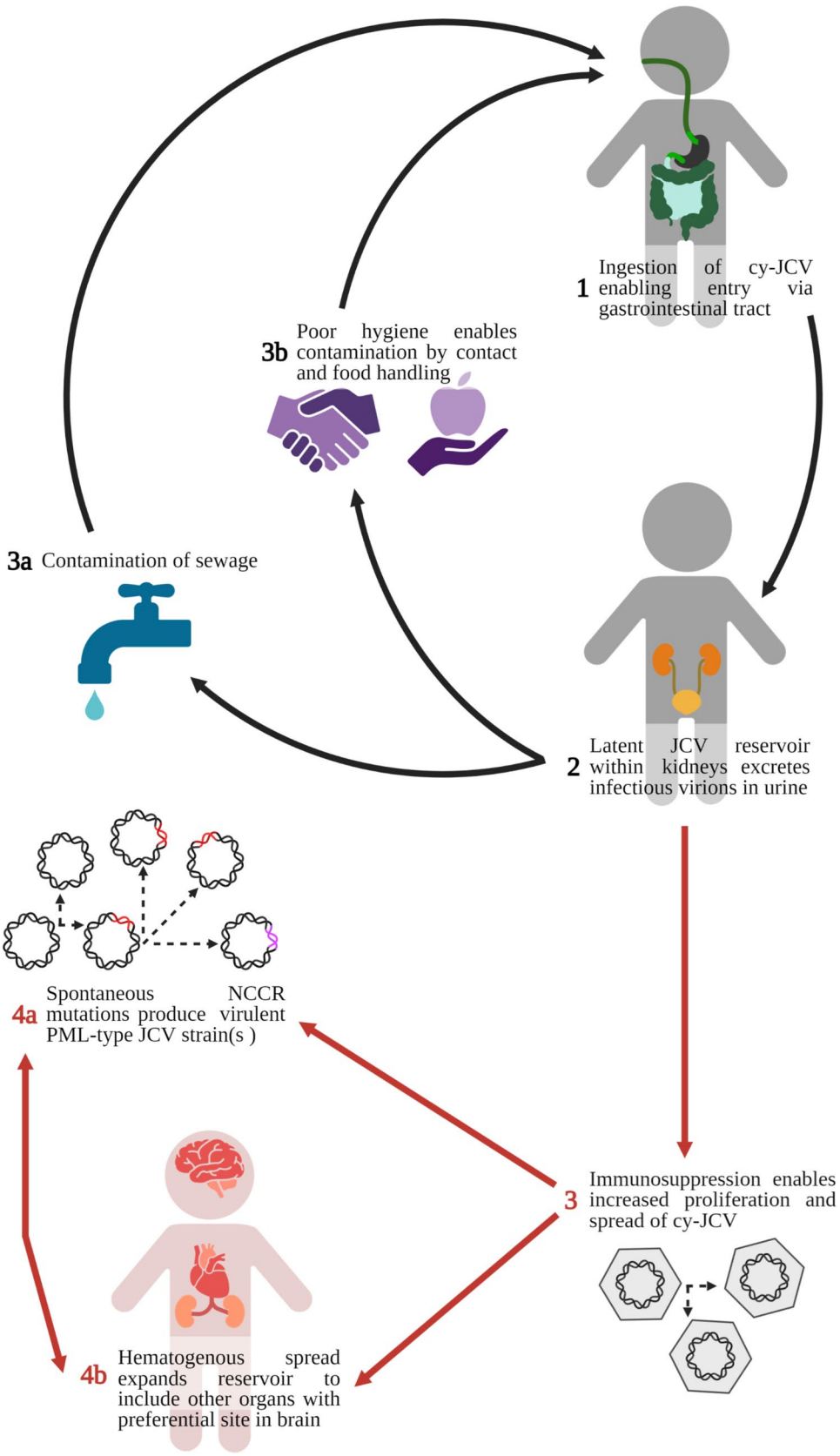

Fig. 3 Transmission of archetype JCV (cy-JCV) in the environment with latent reservoirs in an immune-competent host (black arrows) and pathogenesis during immune compromise (red arrows). Archetype JCV is ingested (1) and passed asymptomatically in urine with persistently low levels of replication in kidney tissue (2). Contamination of sewage (3a) and food products (3b) occurs secondary to poor hygiene, enabling spread to additional hosts through ingestion (1). During immune compromise, disinhibition of latent JCV increases proliferation (3). High proliferation rates increase the spontaneous rearrangements of cy-NCCR into PML-type strains (4a). Disinhibition and mutation enable the spread to additional organ reservoirs with preferential replication in glial cells of the brain (4b)

This interrelationship between HIV and JCV confounded the investigation of JC-specific antiviral agents. PML mortality peaked as the first protease inhibitor, saquinavir, was approved by the FDA in 1995. Within a year, HAART became widely available. The three-drug treatment drastically improved T-cell counts of HIV/AIDS patients, bringing a significant reduction in many HIV-associated diseases, including PML (Christensen et al. 2010). The resultant decline in PML-associated deaths was due to the improvement in predisposing immunosuppression and reduction in the co-promotional HIV. JC itself, however, was not treated. Improved outcomes were observed after the addition of cidofovir to HAART in patients with both HIV/AIDS and PML (De Luca et al. 1999; Brambilla et al. 1999; Jiang et al. 2010). However, as with other potential antivirals for PML, cidofovir has shown no benefit in clinical trials and is not recommended for the treatment of PML (Marra et al. 2002; De Luca et al. 2008).

In 2005, three simultaneously published case reports described the development of PML in patients treated with natalizumab (Tysabri) (Kleinschmidt-DeMasters and Tyler 2005; Langer-Gould et al. 2005; Van Assche et al. 2005). Natalizumab, a selective adhesion molecule inhibitor of α4β1 and α4β7 integrins prevents leukocyte entry into CNS via VCAM and gastrointestinal tissues via MadCAM (Ghosh et al. 2003; Miller et al. 2003). It was approved by the FDA for the treatment of multiple sclerosis and Crohn's disease. The inhibition of α4β1 integrin is responsible for the heightened risk of PML in patients treated with natalizumab through not only impaired immunosurveillance, but also through the release of JCV-infected premature B cells from bone marrow stores and likely other currently unrecognized mechanisms (Berger and Koralnik 2005; Frohman et al. 2014). Proliferating within oligodendrocytes, JCV lyses to propagate its life cycle, and demyelination results as the source of myelin is destroyed. The risk of PML was calculated at 1:1000 after 18 months of natalizumab treatment (Yousry et al. 2006). In addition to altering the levels of natalizumab, studies show altered T-lymphocyte morphology, increasing the risk of JCV reactivation further than general immunosuppression alone (Iannetta et al. 2016; Zingaropoli et al. 2018). After a temporary recall, natalizumab returned to the American market in 2006 with the introduction of "Touch," a mandatory program wherein natalizumab-treated patients underwent regular monitoring with seropositivity and/or PML symptoms indicating immediate cessation of treatment (Sheridan 2006). Four long-term observational studies were also initiated (STRATIFY-2, STRATA, TOP, and TYGRIS). A combined sample of over 37,000 patients revealed significant benefit from annual screening of the anti-JCV antibody index in the peripheral blood (Ho et al. 2017). JCV antibodies in peripheral blood and JCV DNA in urine correlate with previous exposure to JCV and, therefore, indicate an increased risk of natalizumab-induced PML.

Epidemiology: JCV prevalence and PML risk

The prevalence of JCV is consistently reported as 60–80% of the general population, citing the initial hemagglutination-inhibition studies by Padgett and Walker (1973). Their results were updated in 2009 by Egli et al. who observed anti-JCV antibodies by ELISA assay in 58% of healthy blood donors with notable age-graded seropositivity and uniform cy-JCV typing (Egli et al. 2009). A more recent 2018 metanalysis using anti-JCV ELISA found a mean seropositivity of 57.1% in patients with multiple sclerosis or neuromyelitis optica (Paz et al. 2018). Although hemagglutination-inhibition and anti-JCV antibody ELISA do suggest previous exposure to JCV, the serological tests do not equate to prevalence. A 2013 study found 37% of patients with multiple sclerosis who tested negative anti-JCV antibodies were positive for JCV viruria; this high false negative rate suggests a significant underestimation of JCV prevalence in the general population (Berger et al. 2013a, b).

Despite the consistently high levels of JCV, PML remains a rare disorder. Estimated annual incidence rates are on the order of about 1 in 1,000,000 persons, and in one population-wide study from Canada, the incidence of the disease was roughly one-half of that of Creutzfeldt Jakob disease (Bakal et al. 2021). As discussed above, the prevalence of the PML has changed over time to reflect the underlying prevalence of predisposing disorders. This change over time has been referred to as the "Epochs of PML" (Berger and Hartung 2023). These epochs were largely, although not exclusively, derived from the onset of the AIDS pandemic in 1981 and monoclonal antibody–associated PML in 2005. Prior to the AIDS pandemic, PML was predominantly observed in patients with underlying hematological malignancies, chiefly, B cell malignancies (Astrom et al. 1958). The prevalence of PML increased markedly in 1981 with the onset of the AIDS pandemic as 5–10% of all HIV-infected persons would develop PML (Berger 2014). Following the introduction of effective antiretroviral therapy in 1996, the prevalence of PML in the HIV-infected population began to decline; however, AIDS continues to be the most common predisposing cause for PML in the USA (Anand et al. 2019). Immunotherapies became a significant contributor to the prevalence of PML in 2005, after the first 3 cases of PML with natalizumab were reported (Kleinschmidt-DeMasters and Tyler 2005; Langer-Gould et al. 2005; Van Assche et al. 2005). To date, there are over 850 reported cases of natalizumab-associated PML (Dsilva et al. 2023). A wide range of immunosuppressive agents has been associated with the development of PML, although natalizumab and efalizumab (an LFA-1 monoclonal antibody that is now off the market) have rates orders of magnitude higher (Maas et al. 2016). Other conditions—in addition to AIDS and certain immunosuppressive therapies—which are predisposing to PML

are lympho- and myeloproliferative disorders, carcinomas, primary immune deficiency diseases (e.g., idiopathic CD4 lymphopenia), and granulomatous inflammatory disorders (e.g., sarcoidosis). On rare occasion, no underlying explanation for PML is identified, and its development is believed to be simply a stochastic event.

PML: a clinically unmet challenge

In 1958, Astrom, Mancall, and Richardson defined PML by its unique histopathological features in three patients: two diagnosed with chronic lymphatic leukemia and one with Hodgkin's B-cell lymphoma (Astrom et al. 1958). Over the course of the disease, shared symptoms included weakness, gait changes, slurred speech, vision changes, and cognitive alteration. Symptoms progressed in successive examinations. The cytological features differentiated PML from other demyelinating conditions as there was a characteristic triad of disseminated foci of demyelination, hypertrophy of astrocytes into "bizarre gigantic forms," and oligodendrocytes with enlarged, round nuclei that stain darkly basophilic. Using this unique triad, they were able to reassess five historical cases for which histology was preserved: two undiagnosed patients in 1930, one proposed to be lymphogranulomatosis in 1941, and two cases presented as Schilder's disease in 1945 and 1955. The description of PML from 65 years ago remains relevant today as challenges in diagnosis, treatment, and preclinical modeling have gone unmet.

Diagnosis

Brain biopsy remains the gold standard for diagnosis, with qPCR of cerebral spinal fluid (CSF) failing to meet its accuracy, instead functioning as an exclusionary screen (d'Arminio Monforte et al. 1997; Berger et al. 2013a, b; Ikeda et al. 2017). Because the screening procedure for a lumbar puncture is invasive, significant *irreversible* damage must occur and incite symptoms to arouse clinical suspicion and justify the procedure. Although a positive CSF screen is sufficient in the context of symptoms, repeatedly negative CSF PCRs may require a brain biopsy for diagnosis.

The presence of JCV in CSF in the absence of symptoms has a low sensitivity for predicting PML (Swinnen et al. 2019). The seropositive status of anti-JCV antibodies in the periphery does not equate to PML risk, as the prevalence of JCV is so extensive and the incidence of PML is so rare. (Ferretti et al. 2018; Cortese et al. 2021). Similarly, identification of JCV in the peripheral tissues does not translate to PML risk as it does not indicate mutation of cy-JCV to hvp-JCV nor does it reflect penetrance of the blood–brain barrier. An accepted and effective risk mitigation strategy is only currently available for natalizumab, with previous exposure to JCV (measured by anti-JCV antibodies) increasing the risk of drug-induced immunosuppression reactivating the virus (Ho et al. 2017). In these cases, patients are ineligible for certain PML-causing therapies. Other cases with non-iatrogenic or unavoidable immunosuppression have a dire need for a non-invasive, pre-symptomatic screen for PML-causing hvp-JCV.

Treatment

There is no effective treatment for JCV or PML (Berger et al. 2013a, b). Therapeutics are complicated by the heterogeneous patient population, with myriad subpopulations with different predisposing conditions underlying their immunosuppression. The common cause, the virus itself, is therefore the ideal target. A protein vaccine for polyomaviruses JC and BK is currently being studied in macaques; however, the training of the host immune system to recognize capsids as disease-associated is only valuable in an immune-competent environment (Peretti et al. 2023). Although the exact mechanism by which an immune system suppresses JCV is not established, cell-mediated immunity, especially JCV-specific T-cell responses, is clearly fundamental to the suppression of the disorder (Koralnik 2002). The role of the humoral immune system in preventing the development of PML in JCV-infected individuals remains uncertain but likely plays a role (Ray et al. 2015). Unlike many other viral infections, the presence of JCV-directed antibody is not protective. However, there may be neutralizing antibodies that have a role in the amelioration of the disorder.

No antivirals are recommended for any stage of infection, and many fail to show improvement in larger clinical trials (Gasnault et al. 2001; Jamilloux et al. 2016; Summers et al. 2019). Medications known to have high CNS penetrance and pre-existing FDA approval are often investigated, as their toxicities have been established for other conditions, making the lack of a translational animal model moot. Mefloquine, for example, is an anti-malarial agent with which Brickelmaier et al. significantly reduced JC replication within human glial cells (Brickelmaier et al. 2009). Small clinical studies and case reports showed mixed results on its benefit; however, it failed to improve viral load or clinical outcomes in cohort studies (Epperla et al. 2014; Kurmann et al. 2015; Nambirajan et al. 2017). Mefloquine has been paired with mirtazapine, a 5HT2A receptor antagonist approved for the treatment of major depressive disorder. Metanalyses have shown no benefit of mirtazapine, alone, on outcomes for PML patients (Jamilloux et al. 2016). The value of a combination therapy warrants consideration as the anti-malarial is proposed to reduce the genomic threat of JCV while the tricyclic antidepressant prevents capsid binding and, therefore, cell entry. The need for combination therapy is a mainstay of the sister virus, HIV, with highly active

antiretroviral therapy (HAART) combining three drugs targeting different characteristics: a direct antagonist of reverse transcriptase, an indirect antagonist of reverse transcriptase, and a protease inhibitor preventing virion maturation. Redundancy in treatment will likely improve our targeting of both the JCV capsid/packaging—which is key to spread—as well as the JCV genome—which is key to expression and proliferation—to successfully eliminate the virus or suppress its viral load enough for management by a weakened immune system.

Targeting of the capsid through transfusion of T-cells primed against the major capsid protein VP1 and immune checkpoint inhibitors are appealing treatment possibilities but remain to show demonstratable efficacy (Muftuoglu et al. 2018; Cortese et al. 2019). The former mounts an immune response against the viral capsid; the latter increases the production of immune cells by obstructing anti-inflammatory PD-1, CTLA, or LAG-3. Unfortunately, altering the immune response can be problematic as reductions in viral load occur secondary to T-cell mediated culling of affected cells, advancing PML (Martins et al. 2019). Additionally, many PML patients rely on therapeutically low immune cells (i.e., transplant recipients, patients treated for autoimmune conditions) making them ineligible for the immune checkpoint inhibitors proposed.

Preclinical modeling

Progress in both diagnostics and therapeutics is limited by the absence of a pre-clinical, translational model. Animal analogs, including the murine (MuPyV) and simian (SV40) polyomaviruses, are homologous, but not identical, to JCV (White et al. 2015). While capable of duplicating PML-like conditions, the viruses themselves diverge too much to validate non-human preclinical research targeting JCV (Simon et al. 1999; Dang et al. 2005). JC virus preferentially affects human cells and inoculation with JCV uniformly produces tumors in neonatal hamsters, owl monkeys, squirrel monkeys, mice, and rats (Walker et al. 1973; London et al. 1978, 1983; Gordon et al. 2000; Del Valle and Khalili 2021). Multiple laboratories have produced humanized mice capable of harboring the JC virus (Matoba et al. 2008; Kondo et al. 2014). Matoba et al. engrafted JCV-infected cells from PML patients into mice, maintaining the cell line for 2 weeks prior to successfully suppressing the virus with an siRNA targeting agnoprotein. Kondo et al. introduced human glial progenitor cells into perinatal shiverer mice, which lack endogenous myelin, and achieved human myelination of mice axons. When JCV was injected into the brains of these chimeras, human astrocytes and oligodendrocytes were susceptible to infection; mouse cells were spared. Both models, proposed over a decade ago, reveal the potential to model JCV in vivo through humanization.

Future advancements

To eliminate PML, several translational concepts must, and can, be addressed: the development of JCV-targeting therapies, the creation of an animal model to test said therapies, and the creation of a non-invasive diagnostic screen that will enable effective treatment prior to the onset of irreversible demyelination.

Modern advances in gene editing have great value in the treatment of persistent and evasive viral infections. Although protein vaccines and immune modulators can prevent the entry of virions into cells, and reverse transcriptase and polymerase inhibitors can prevent genome expansion, these efforts are viro-static, rather than viro-cidal. CRISPR/Cas9 targets even latent DNA, curing a host of the virus entirely. The similarly persistent virus, HIV, has been successfully eliminated from T-lymphocytes in vitro, humanized mice, and SIV-analogous macaques and is currently in clinical trials (Datta et al. 2016; Kaminski et al. 2016; Dash et al. 2019; Mancuso et al. 2020). The RNA virus persists following integration into the host genome, making the excision of the viral genes essential to effective, lasting therapies. Other RNA viruses which vary significantly across strains, such as corona- and influenza-viruses, can be addressed en masse through conserved gene segments, as shown by Abbott et al. (2020) who produced a "pan-coronavirus" therapy utilizing six gRNAs to account for 90% of all coronaviruses, including SARS-CoV-2. Gene therapies utilizing CRISPR as a curative antiviral have shown success with notoriously latent herpesviruses (including cytomegalovirus, herpes simplex type 1, and Epstein Barr) which contain a dsDNA genome that permanently infects the host, like JCV (Yuen et al. 2015; van Diemen et al. 2016). CRISPR constructs against JCV have been successful, with Wollebo et al. (2015) completely excising the NCCR-LTAg span of the JCV genome in vitro, terminating the viral life cycle. Unfortunately, the absence of an animal model for JCV stalls such therapies at the preclinical stage.

Disease modeling has improved significantly in recent years with the production of multicellular human organoids and the xenotransplantation of said organoids into animal surrogates. Barreras et al. (2022) successfully propagated the JC virus within human cerebral organoids, a three-dimensional system containing neurons, oligodendrocytes, and astrocytes. The system not only produced myelinated axons, but it also hosted JCV infection and displayed associated demyelination. This is a monumental step toward the creation of a JCV model when considered in the context of xenotransplantation foreshadowed by the teams of Matoba and Kondo. A research "pocket" of xenotransplanted cerebral organoids could better reflect the multicellular environment of JCV-driven PML while borrowing the metabolism and blood–brain barrier of host mice, bridging the gap between basic science research and clinical investigations.

In researching xenotransplantation, Dong et al. (2021) and Bao et al. (2021) successfully injected human organoids into murine brains. The human cells formed synaptic and angiogenic connections with their murine neighbors, producing an in vivo model with a more accurate human. Ex vivo infection of human organoids and their incorporation into mouse brains may, therefore, produce a humanized model, a necessary stage for assessing the safety and bioavailability of new JCV therapies and diagnostics prior to clinical trials.

Outside of neuroprotectants or neuro-regeneration, the only true treatment for PML is prevention, as neurodegeneration and central nervous system demyelination are irreversible. Agnoprotein has been proposed as a promising target, as cells actively producing JCV release the early-late protein into the environment prior to cell lysis (Otlu et al. 2014). Potentially acting as a viroporin primer for the anticipated release of virions, agnoprotein interacts with adjacent uninfected cells (Suzuki et al. 2010; Saribas et al. 2018). The ability to detect the 5kD agnoprotein prior to its triggering of apoptosis is complicated by the absence of a preclinical model and the patency of the blood–brain barrier. Highly sensitive diagnostics, like sandwich ELISA and RT-LAMP, have been validated for identifying minute levels of viral proteins and RNA (Morioka et al. 2014; Zai et al. 2018; Huang et al. 2020; Tanimoto et al. 2022). Targeting agnoprotein in the blood may now be possible for early identification of JC-reactivation and pre-symptom PML screening.

Advancements in JCV research have well exceeded those of PML, despite the polyomavirus being discovered nearly a decade after the disease's definition. There is a distinct pathologic link between the infection and its disease, but no such link has entered the clinical sphere diagnostically or therapeutically with the exception of very specific iatrogenic prevention. We have the technology, today, to improve patient outcomes by developing realistic screens, translational preclinical models, and JC-targeting therapies, all necessary tools to better aid individuals of the heterogenous PML patient population.

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Declarations

Ethical approval Not applicable.

Consent to participate Not applicable.

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