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Progressive Multifocal Leukoencephalopathy: Endemic Viruses and Lethal Brain Disease

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Abstract

In 1971, the first human polyomavirus was isolated from the brain of a patient who died from a rapidly progressing demyelinating disease known as progressive multifocal leukoencephalopathy. The virus was named JC virus after the initials of the patient. In that same year a second human polyomavirus was discovered in the urine of a kidney transplant patient and named BK virus. In the intervening years it became clear that both viruses were widespread in the human population but only rarely caused disease. The past decade has witnessed the discovery of eleven new human polyomaviruses, two of which cause unusual and rare cancers. We present an overview of the history of these viruses and the evolution of JC polyomavirus–induced progressive multifocal leukoencephalopathy over three different epochs. We review what is currently known about JC polyomavirus, what is suspected, and what remains to be done to understand the biology of how this mostly harmless endemic virus gives rise to lethal disease.

INTRODUCTION

General Biology

JC polyomavirus (JCPyV) belongs to the family *Polyomaviridae*. This fast-growing virus family now includes 73 species that are taxonomically divided into four genera: *Alphapolyomavirus*, *Betapolyomavirus*, *Gammapolyomavirus*, and *Deltapolyomavirus* (1). Polyomaviruses infect a wide range of host species including both New and Old World monkeys, bats, horses, humans, elephants, mice, badgers, sea lions, birds, cattle, fish, dolphins, and raccoons (1). The natural history of virus infection in each of these species, though far from understood, is generally one in which the virus establishes lifelong and asymptomatic persistent infections (2). The human polyomaviruses have now expanded to include thirteen members, four of which cause disease (**Table 1**) (3–14). Two of these are associated with dysregulated cell growth. Merkel cell polyomavirus causes Merkel cell carcinoma, and trichodysplasia spinulosa–associated virus causes trichodysplasia spinulosa (9, 15, 16). The two originally discovered human polyomaviruses, BK polyomavirus (BKPyV) and JCPyV, both establish persistence in the kidney and urogenital tract of the majority of people worldwide and cause disease in immunocompromised or immunomodulated patients. BKPyV causes polyomavirus-associated nephropathy in kidney transplant patients and hemorrhagic cystitis in bone marrow transplant recipients (17). JCPyV causes a debilitating and often fatal central nervous system disease known as progressive multifocal leukoencephalopathy (PML) (18).

The polyomaviruses are nonenveloped and have capsids composed of one major protein (VP1) and two to three minor proteins (VP2, VP3, and sometimes VP4) (**Figure 1**) (19). The genomes are double-stranded closed circular DNA ranging in size from 4,697 bp (cattle) to 5,722 bp (African elephants). JCPyV has a genome of 5,130 bp. The genomes are packaged as minichromosomes

Table 1 Currently known human polyomaviruses

Full name	Abbreviation	Source of isolation	Year of discovery	Disease caused (if known)
BK polyomavirus	BKPyV	Urine	1971 (3)	Polyomavirus-associated nephropathy, hemorrhagic cystitis
JC polyomavirus	JCPyV	Urine, brain	1971 (4)	Progressive multifocal leukoencephalopathy
Karolinska Institute polyomavirus	KIPyV	Nasopharyngeal tissue	2007 (5)	None
Washington University polyomavirus	WUPyV	Nasopharyngeal tissue	2007 (6)	None
Merkel cell polyomavirus	MCPyV	Skin lesions	2008 (7)	Merkel cell carcinoma
Human polyomavirus 6	HPyV6	Skin	2010 (8)	None
Human polyomavirus 7	HPyV7	Skin	2010 (8)	Pruritic rash (?)
Trichodysplasia spinulosa–associated polyomavirus	TSPyV	Skin lesions	2010 (9)	Trichodysplasia spinulosa
Human polyomavirus 9	HPyV9	Blood, skin, urine	2011 (10)	None
Malawi polyomavirus	MWPyV	Stool	2012 (11)	None
St. Louis polyomavirus	STPyV	Stool	2012 (12)	None
Human polyomavirus 12	HPyV12	Liver, cecum, rectum, stool	2013 (13)	None
New Jersey polyomavirus	NJPyV	Muscle, skin	2014 (14)	Muscle and ocular damage (?)

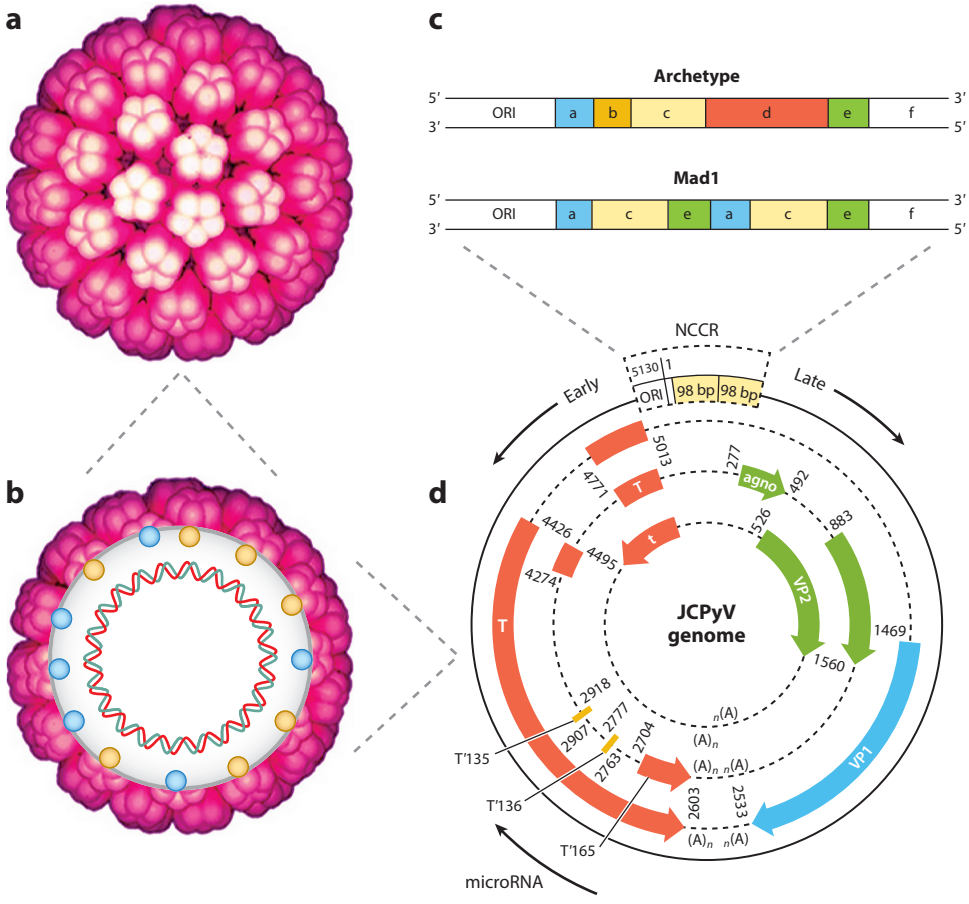

Figure 1

(*a*) Representation of the overall structure of the JC polyomavirus (JCPyV) capsid showing the arrangement of VP1 pentamers. (*b*) Representation of a cutaway view of the capsid showing the closed circular double-stranded DNA genome and positions of the minor capsid proteins VP2 (*blue*) and VP3 (*yellow*). (*c*) A schematic of the noncoding control region (NCCR) that contains the origin of replication (ORI) and transcription factor binding sites, comparing the archetype with the rearranged Mad1 progressive multifocal leukoencephalopathy variant. (*d*) Schematic of the early and late transcriptional units of JCPyV indicating start and stop sites for transcription of viral proteins. Dashed lines represent introns. Solid colored arrows represent protein-coding regions. The solid black line indicates the position of the microRNA encoded on the late strand with complementarity to regions of small t antigen and large T antigen mRNA. Adapted with permission from Reference 19.

with host cell histones H2A, H2B, H3, and H4 (20). The linker histone H1 is generally missing (20). Some groups have reported that the histones play a role in regulating virus gene expression, but others have suggested that their major role is in genome packaging (21–23).

The JCPyV genome is divided into early and late transcriptional cassettes separated by a noncoding control region (NCCR) that includes an origin of replication and one to two enhancer elements that bind numerous transcription and replication factors (**Figure 1**) (24). The NCCR is the most variable portion of the viral genome, and viruses containing different NCCR sequence variants can be found in a single infected person (25–27). Based on the structure of the NCCR,

JCPyV can be referred to as having an archetype or a rearranged NCCR. The archetype NCCR is characterized by six blocks named a, b, c, d, e and f (**Figure 1**). The origin of replication and the f region are conserved among the different variants, whereas the blocks in the middle are highly variable. The prototypical JCPyV strain, named Mad1, was the first to be isolated from the brain of a PML patient, in Madison, Wisconsin; it has a rearranged NCCR composed of a, c, e, a, c, and e (**Figure 1**). Blocks b and d are deleted and blocks a, c, and e are tandem repeats. Such duplications and deletions generate more transcription factor binding sites that confer growth advantages to JCPyV. For example, the archetype NCCR has fewer binding sites for Oct-6/SCIP, Spi-B, and the DDX-1 proteins, which results in less efficient viral gene transcription when compared with the rearranged NCCRs. DDX-1 can act as a coactivator to enhance NF-κB-mediated early and late transcription, and Spi-B can enhance viral gene transcription in B cells and primary astrocytes (28–30). Additionally, the repeats of the c region increase the number of NFI binding sites, which are important for viral gene transcription in the brain and in lymphoid tissues (31, 32).

The PML-type regulatory region is thought to evolve from the archetype regulatory region by a series of rearrangements and duplications (33, 34). The PML-type virus is rarely seen in urine, and the archetype strain is rarely seen in tissues other than those of the renourinary tract. This has led to a consensus in the field that the virus in the urine is the transmitted form and that the virus that emerges in PML brain does so only after significant genetic alterations in immunosuppressed patients. The cellular site of these rearrangements is not clear, but some speculate that the rearrangements take place in B cell precursors in bone marrow, and others argue that the rearrangements take place over long periods of time following persistent infection in the central nervous system (32, 35).

Regardless of the site of rearrangements, the NCCR of the PML-type virus drives early and late viral gene expression and viral DNA replication (36). The first genes to be expressed are the early genes encoding large T antigen, small t antigen, and several small splice variants referred to as T′135, T′136, and T′165 (**Figure 1**) (37, 38). Each of these proteins is produced from a single primary RNA transcript that is differentially spliced. The JCPyV large T antigen is a multifunctional phosphoprotein that is critical for stimulating both host cell and viral DNA synthesis (39, 40). In the absence of lytic replication, the expression of large T antigen can lead to cellular transformation. Small t antigen binds PP2A and cooperates with large T antigen to effect replication and transforming activities. Expression of both large T and small t is required for efficient viral replication and transformation. The role of the T′ proteins is not understood, but they are hypothesized to play auxiliary roles in both lytic replication and transformation (37, 38). Following early gene expression the viral DNA is replicated bidirectionally from a well-defined origin of replication. This activity requires large T antigen and several human-specific host cell replication factors. The inability of rodent replication factors to recognize the JCPyV origin and cooperate with JCPyV T antigen is a major block to the establishment of animal models of JCPyV-induced disease (41).

Viral DNA replication in permissive cells results in the accumulation of large numbers of viral DNA templates from which mRNAs encoding late structural proteins are produced. The structural proteins of JCPyV are VP1, VP2, and VP3 (**Figure 1**). VP1 is the major capsid protein and represents 70% of the total viral protein in the virion (42). Three hundred and sixty VP1 monomers spontaneously assemble into 72 pentamers, which are further assembled into the icosahedral shell of the virus (**Figure 1**). Exposed residues on the surface of each VP1 monomer make specific contacts with sialic acid–containing receptors to facilitate infection (43). VP2 and VP3 together represent 30% of the total viral protein in the virion and associate with each VP1 pentamer on the inner face of the capsid. The virus is able to self-assemble into icosahedral capsids in the absence of VP2 and VP3, but these capsids are not as structurally stable without the minor capsid proteins

(44). VP2 is unique in that it is modified at its amino terminus with myristic acid. This sequence is critical for efficient infection, and it may play a role in the penetration of host cell membranes at some stage during entry (45, 46). The late region also encodes the agnoprotein (**Figure 1**). Agnoprotein is a highly basic nonstructural regulatory protein that binds to numerous host cell and viral proteins, including PP2A, large T antigen, and small t antigen, and cooperates with these proteins to sustain the lytic cycle (47). Polyomaviruses, including JCPyV, have recently been found to encode microRNAs (48). Two mature microRNAs (miRNAs), JC-miRNA-3p and JC-miRNA-5p, are encoded by the late transcript and target the early mRNA region shared between large T antigen and small t antigen. JC-miRNAs downregulate large T antigen expression and, as a consequence, their own expression, generating a negative feedback loop (49, 50). They have also been reported to target the stress-induced ligand ULBP3 to escape immune elimination (51). JCPyV-encoded miRNAs have been found in extracellular vesicles shed from JCPyV-infected cells, suggesting that they play an active role in modulating the cellular environment during virus infection (52). The best study to date of the role of polyomavirus miRNA is one where the role of the BKPyV miRNA was investigated in archetype and rearranged BKPyV genomes (53). The archetype genome favored late promoter activity over early promoter activity and drove high levels of the miRNA that further restricted early mRNA expression. The opposite pattern emerged in rearranged variants, limiting the expression of the miRNA and favoring T antigen expression and lytic replication. It is currently unclear whether the same is true for JCPyV.

Cellular Receptors

Our understanding of the role of receptors in driving JCPyV infection is evolving. Currently the JCPyV receptor is described as a complex involving both attachment and entry components (**Figure 2*a***) (43, 54–57). The major attachment receptor for JCPyV is the carbohydrate motif lactoseries tetrasaccharide c (LSTc) (43). LSTc contains a terminal α2–6-linked sialic acid residue that interacts directly with a sialic acid binding pocket on the major capsid protein of JCPyV, VP1. Mutation of residues in VP1 that make crystal contacts with sialic acid drastically inhibits both virus binding to cells and virus infection (55, 58). The presence of LSTc alone on cells, while sufficient for binding, is not sufficient for entry and infection. Entry and subsequent infection require one of three serotonin receptors belonging to the 5-HT_2 receptor subgroup (56, 57). Two of these, 5-HT_{2A} and 5-HT_{2C} receptors, were identified during studies of viral entry when drugs that interfere with these receptors were found to inhibit endocytosis of the virus (56). Subsequent work found that 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors were not important for initial virus binding but rather facilitated entry by clathrin-dependent mechanisms (57). The presence of these receptors alone is not sufficient for infection, and virus can infect some cells that lack these receptors (59). The discovery that 5-HT_2 receptors are important components of a JCPyV receptor complex led to the use of serotonin receptor blockers to treat patients with PML (60–62). The most widely used drug is mirtazapine, and it has shown some benefit in some patients, but an extensive review of cases showed no correlation between the use of mirtazapine and clinical outcomes (63). A clinical trial was initiated using the antimalarial drug mefloquine, which also has anti–serotonin receptor activity, and no benefit was found (64). As the virus is capable of infecting cells using any of the three 5-HT_2 receptor isoforms, a more broadly acting drug might provide more benefit (57).

Role of Cellular Receptors In Vivo

In human tissue sections, labeled JCPyV binds to kidney tubules, to microglia, to neuroendothelium, to choroid plexus epithelium, and to leptomeninges (65). Virus attachment is mediated

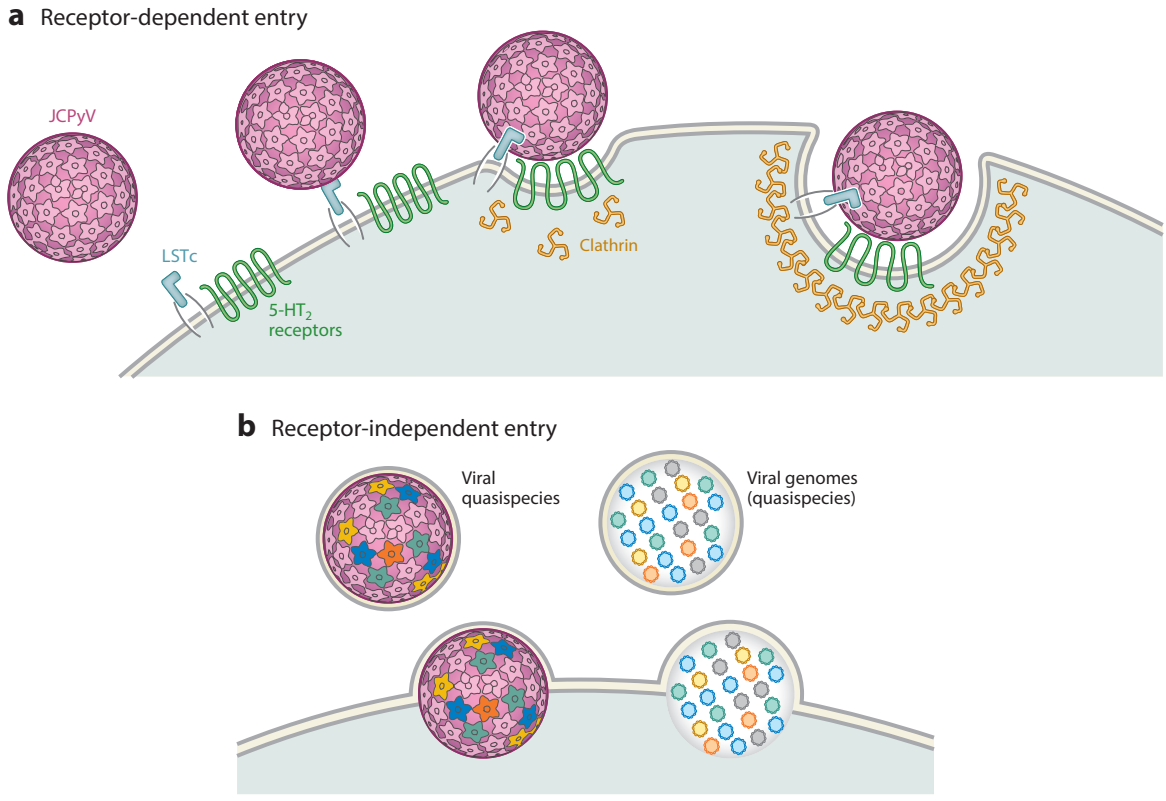

Figure 2

(*a*) Representation of receptor-dependent entry. JC polyomavirus (JCPyV) first attaches to the sialic acid–containing motif lactoseries tetrasaccharide c (LSTc) at the cell surface and then engages serotonin receptors (5-HT_2 receptors). This interaction likely induces molecular signals leading to the formation of a clathrin coat that then drives endocytosis to completion. (*b*) Representation of receptor-independent entry. It is hypothesized that viral quasispecies (*different colors*), represented as virions and as genomes, are released from infected cells in exosomes that can directly fuse with uninfected cells to spread the infection. Virions may also contain mixtures of wild-type and mutant VP1, allowing for immune escape while maintaining some dependence on receptors (represented by the virion with mixed-colored pentamers).

specifically by LSTc in these tissues, as neuraminidase treatment or competition with soluble LSTc eliminates virus binding (65). Virus does not bind to LSTc-negative oligodendrocytes or astrocytes, the major targets of virus infection in the brain, although these cells express the serotonin receptors required for viral entry (65). Human kidney tubules, choroid plexus, and leptomeninges all express both LSTc and serotonin receptors, and virus binding colocalizes with these receptors (65). These data, coupled with in vitro studies, make it clear that virus first engages LSTc in the kidney and in the choroid plexus and leptomeninges, and the presence of serotonin receptors facilitates entry and infection. Engagement of LSTc in the absence of serotonin receptors does not lead to entry or infection, as microglial cells have never been reported to be infected with JCPyV. In contrast, a recent report described a patient with meningitis who was found to have JCPyV in the choroid plexus and the leptomeninges (66). Moreover, choroid plexus epithelial cells and leptomeningeal cells have recently been shown to be susceptible to virus

infection, opening up a new chapter in the biology of JCPyV (W.J. Atwood, unpublished results). The lack of attachment receptors on oligodendrocytes and astrocytes and the fact that serotonin receptors do not by themselves facilitate infection suggest that infection of these macroglia by JCPyV proceeds by an alternative, receptor-independent mechanism. An attractive hypothesis is that virus can spread directly from cell to cell in extracellular vesicles. The evidence to date for this hypothesis is that infected cells produce increased amounts of extracellular vesicles, that these vesicles are associated with virus and can transmit the virus to naive cells, and that drugs that inhibit vesicle production reduce viral spread even under conditions when all receptor components are present (W.J. Atwood, unpublished observations). These data also help to explain the observation that virus genomes isolated from the cerebrospinal fluid and from brain parenchyma of patients with PML have mutations in the sialic acid binding pocket that render the virus unable to bind to sialic acid–containing receptors such as LSTc (67–70). Several studies have found that these sialic acid binding mutants are noninfectious in tissue culture cell lines, but other investigations have reported that mutant virus-like particles (VLPs) bind to cells and mutant pseudoviruses can transduce some cancer cell lines (55, 58, 71). Using pseudoviruses, Buck and colleagues (71) were able to demonstrate that sera from healthy donors neutralized both wild-type and variant viruses. Sera from PML patients, however, were able to neutralize wild-type virus but were not able to neutralize the variants, indicating that these variants are antibody escape mutants. Mice vaccinated with wild-type VLPs developed antibodies that could block wild-type pseudovirus but not variant pseudoviruses. Following a booster with wild-type VLPs, the antibody response matured and strengthened and was able to recognize both the wild-type and the variant forms. The suggestion is that a VLP vaccine in a prime-boost regimen should be used to prevent the development of PML (71). In a related paper, Martin and colleagues (72) found that patients who eliminated virus from the central nervous system following the development of immune reconstitution syndrome mount robust antibody responses to wild-type virus and all variants. Using this information, they isolated and cloned reactive antibodies from the memory B cells of one patient who had successfully controlled PML. Several antibodies were found that could strongly neutralize the variant viruses. The authors suggested that these antibodies or cloned fragments of these antibodies (FABs) could be used as therapeutic reagents (72).

This leaves us with a model where cells with both receptor components can be infected directly with wild-type virus. Cells lacking the attachment receptor can be infected with either wild-type virus, mutant virus, a chimeric virus, or wild-type and mutant viral genomes packaged in extracellular vesicles (**Figure 2*b***). Understanding which cells produce the extracellular vesicles, under what conditions, and how these vesicles disseminate virus and viral components throughout the body is a pressing area of research. Developing therapeutics that target both receptor-dependent and receptor-independent mechanisms of infection is of critical importance.

Switch from Kidney to Brain

There are several steps that are hypothesized to occur for JCPyV to switch from a benign virus harbored in the urogenital system to a neuroinvasive and neuropathogenic virus associated with the development of PML. First is that the virus must undergo genetic rearrangements of its NCCR (33, 34). The archetype or nonrearranged variant is harbored exclusively in the kidney (33, 34). JCPyV with an archetype regulatory region grows poorly in human kidney cells in vitro and does not grow at all in human glial cell lines. Rearrangements to the regulatory region lead to a virus that has increased replication capacity in human glia while maintaining low-level expression in kidney. Mutations in the capsid protein VP1 are also seen in patients with PML, and as described

above, these likely represent immune escape variants (65–68). The mutations overlap with antibody recognition epitopes and with T cell epitopes important for mounting a cell-mediated response against the virus. It is not clear whether these mutant viruses contribute to the development of PML or are the result of high-level viral replication associated with PML. Another possibility is that reduced dependence of the mutants on sialic acid binding allows them to break out of the kidney and spread more efficiently to brain. Interestingly, labeled VP1 pentamers or pseudoviruses harboring the most prominent sialic acid binding pocket mutations fail to bind to kidney cells and to brain (W.J. Atwood, unpublished observations). It is important to note that both wild-type and mutant virus is found in cerebrospinal fluid and brain parenchyma of PML patients, so if spread is directly from the kidney to the brain, then both viruses are capable of neuroinvasion. Given the fact that virus promoter/enhancer rearrangements are selected for optimum expression in glial cells and the fact that both wild-type virus and sialic acid binding pocket mutant virus are found in the cerebrospinal fluid of PML patients, a distinct possibility is that the virus establishes persistent infection in brain in a minority of individuals. Over time and under the right conditions, the virus could convert to a more pathogenic form (35). This would explain the rarity of PML even in immunosuppressed and immunomodulated patients. Recent data linking the choroid plexus and meninges to virus infection make it plausible that these tissues could be a site of viral persistence in some individuals. Another hypothesis is that the virus establishes persistent infection in bone marrow and that genetic rearrangements occur in cells that express high levels of recombination enzymes that are responsible for immunoglobulin gene rearrangements (32, 33). Transcription factors that favor viral gene expression in glial cells are also found in B cells, so selection for a glial-tropic virus could occur in these tissues (33). In the absence of tractable animal models, these types of questions will be difficult to answer and to distinguish.

Entry and Trafficking

Following attachment, JCPyV enters cells by clathrin-dependent endocytosis (73, 74). This differs from the entry mechanisms of other polyomaviruses, including BKPyV and simian virus 40, both of which enter by lipid raft– or caveolae-dependent mechanisms (75–79). Interestingly, serotonin receptors enter cells by clathrin-dependent endocytosis following engagement of their natural ligands, so JCPyV likely takes advantage of the normal route of downmodulation of these receptors. Following entry, JCPyV, like other members of the family, is transported to the endoplasmic reticulum, where the virion is acted on by host cell chaperones (80). These chaperones, including PDI and ERp57, isomerize the interpentameric disulfide bonds of murine polyomavirus and simian virus 40, resulting in partial disassembly of the viral capsid and exposure of the minor capsid protein VP2 (81–83). Knockdown of the endoplasmic reticulum chaperones PDI and ERp57 inhibits JCPyV infectivity, suggesting that JCPyV also engages the quality-control machinery present in the endoplasmic reticulum to undergo partial disassembly, leading to exposure of VP2 (84–86). Pharmacological agents that inhibit retrograde transport or endoplasmic reticulum function decrease JCPyV infectivity (85, 87). Compounds that block traffic to the endoplasmic reticulum, such as the dihydroxy quinazolinone retro-2, effectively block initial infection of cells by JCPyV and block subsequent spread of the infection in tissue culture. Retro-2 has been shown to be relatively nontoxic in mice, and it has chemical properties associated with blood-brain barrier permeability, so it is an attractive pharmacological agent to treat or prevent PML (88). Following endoplasmic reticulum transport, the virus then moves to the host cell nucleus for viral replication. It is unclear how the virus enters the nucleus, but current hypotheses suggest that a partially disassembled virion is transported across the nuclear pore.

PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY

The First Epoch: A New Disease

The disease PML has been evolving since its first description in the literature nearly eighty years ago (89). PML as a distinct disease entity was not known until Astrom, Mancall, and Richardson (89) reported two patients with leukemia and one patient with Hodgkin's disease who presented with unusual and rapidly progressing neurological symptoms. Postmortem brain examination of the three cases showed widespread focal lesions of demyelination with extensive destruction of oligodendrocytes, enlarged bizarre astrocytes, and numerous phagocytic cells and microglia within the lesions and at the borders of the lesions. The authors described a continually evolving process of expansion of existing lesions and appearance of new lesions. They ruled out several other known diseases, including Schilder's disease, acute disseminated encephalomyelitis, and glioblastomatous diffuse sclerosis. In their review of the literature, they identified several cases with similar if not identical findings; in three instances they were able to obtain slides and confirm that these were indeed PML. They concluded that PML was a rare complication of leukemia or malignant lymphoma. They stated that the cause of this disease was unknown but that based on their findings it was almost certainly not caused by any of the treatments patients received for their underlying disease. In 1961, Richardson expanded his study to include a total of 22 cases (90). In this landmark paper, he hypothesized that the causative agent was a virus. He based his hypothesis on the cytopathic changes he observed in oligodendrocyte and astrocyte nuclei and on the fact that the condition occurred as a complication of diseases that weakened the immune system.

Identification of the Virus

Richardson's hypothesis was supported when viral particles were observed in the brains of patients with PML (91, 92). His hypothesis was confirmed when Padgett, Walker, and colleagues (4, 93) succeeded in isolating the virus from PML brain tissue. Isolation was achieved by inoculating cultures of primary human fetal glial (PHFG) cells with extracts made from PML brain. Brain extracts were homogenized by mortar and pestle, sonicated, treated with deoxycholate and trypsin, and finally subjected to low-speed centrifugation. Supernatants from the low-speed centrifugation were diluted and used to inoculate the PHFG cell cultures. The PHFG cell cultures contained mixtures of astrocytes and "spongioblasts," and each preparation gave rise to different ratios of spongioblasts to astrocytes. After 30 days in culture, some cells showed enlarged and bizarre nuclei, but cytopathic effects were minimal. This phenotype could be transmitted by passage of the supernatants to fresh cultures, but it still required 30 days before the appearance of bizarre and enlarged cells. Electron microscopy revealed virus particles that resembled papovaviruses, and these were identical to the viral particles seen in PML brain. The infected cells did not react with antisera against any of the known papovaviruses, including simian virus 40, human papillomavirus, and mouse polyomavirus. Sera from the patient did not react to the virus in the infected cultures, and the authors speculated that this was not surprising as the patient was a long-standing Hodgkin's disease patient and sera from these patients often did not react with many common viruses. Supernatants from the affected cultures were also used to challenge cells that were known to be susceptible to other papovaviruses. These supernatants did not produce cytopathic effect in primary mouse embryo cells or in African Green monkey kidney cells (BSC-1, Vero, or CV1). Primary human embryonic kidney cells, human follicular lymphoma cells, and a human embryo cell line also failed to support infection with this new agent. The authors therefore

concluded that they had isolated a novel virus, and they named it after the patient from whom it was isolated, J.C.

In these early investigations, inoculation of primary fetal brain cultures that contained higher proportions of spongioblasts resulted in more rapid development of cytopathic effect either when fresh PML brain extract or supernatants from affected cultures were used. The spongioblasts were likely a population of rapidly dividing glial and neuronal progenitors (94). Subsequent work showed that both fetal and adult astrocytes could support JCPyV replication, and cell lines derived from these cells became the standard cell culture model for studies on the biology of JCPyV (95–98).

Lessons can be learned from these early descriptions of PML and of the isolation of the virus. The case reports and the neuropathological descriptions of these early patients became the standard for diagnosis of PML postmortem. The methods of virus isolation from infected cultures are also remarkably similar to protocols still in use, including sonication steps and treatment of cells with deoxycholate. The discovery that JCPyV used sialic acid as a receptor led to the introduction of so-called receptor-destroying enzyme (or neuraminidase) in the isolation procedures. It also led to the widespread use of hemagglutination to quantify viral particles. The poor reactivity of patient sera to JCPyV was also observed when PML emerged as a life-threatening complication in patients diagnosed with AIDS (see below). The dogma that emerged from these early experiences was that humoral immune responses to the disease were not important for control, as antibody titers typically did not rise during the disease process and the antibodies that were made failed to neutralize virus. This made sense until PML arose in large numbers of immunologically non-suppressed patients being treated with immunomodulators to combat autoimmune disease (99, 100). Patients under these conditions that succumb to PML often show "holes" in their antibody response to specific variants of JCPyV that arise during the disease process (71, 72). The few patients that control PML make antibodies that are broadly neutralizing. These new data are leading to the development of prophylactic and therapeutic vaccine strategies to control PML and to the development of specific human antibody–based reagents to treat PML (71, 72).

The Second Epoch: PML and AIDS

After Richardson's landmark paper, PML remained extremely rare for two decades until it emerged as a fatal complication of HIV-1 infection in the early days of the AIDS pandemic. The first cases of what would become known as AIDS were diagnosed in San Francisco and New York City in 1980 and 1981, respectively (101–103). The first documented cases of PML in AIDS patients were reported in January of 1982 in New York and in February of 1983 in Miami (104, 105). The descriptions of PML in these cases were identical to the early descriptions by Richardson. A large review by Brooks & Walker (106) in 1984 examined 230 cases of PML that occurred between 1958 and 1984. Of these cases, the underlying causes were lymphoproliferative diseases (62.2%), myeloproliferative diseases (6.5%), carcinoma (2.2%), tuberculosis and sarcoidosis (7.4%), and other immune impairments (16.1%) (106). AIDS was included in this category but only comprised 2.1% of cases (106). As HIV infection and AIDS reached epidemic proportions, the incidence of PML soared (107, 108). The prevalence of PML in AIDS patients, who were mostly men between the ages of 20 and 50, was estimated to be 5%, and these patients constituted the largest group at risk for developing PML (109). A review from 1980 to 1994 in Florida of 156 cases of PML showed that 154 (98.7%) were associated with HIV/AIDS (109, 110). In 1993, 87% of all recorded cases of PML were associated with HIV/AIDS (111). In a nationwide inpatient sample data review of 9,675 cases of PML between 1998 and 2005, 80% were associated with HIV, 8.4% with hematologic malignancies, 2.83% with solid cancers, 0.44% with systemic lupus erythematosus, 0.25% with rheumatoid arthritis, and 0.26% with other connective tissue disorders

(112). In another independent review of 61 patients with PML seen between 1995 and 2004, 79% had AIDS, 13% had hematologic malignancies, 5% were bone marrow transplant recipients, and 1 patient each had thymoma and dermatomyositis (113).

The implementation of highly active antiretroviral therapy (HAART) to treat patients infected with HIV resulted in a significant decline in the incidence of PML in patients with HIV infection. A decline in mortality from HIV-associated PML between the time periods 1992–1995 and 2002–2005 was attributed to HAART (114). A nationwide analysis of adult patients with HIV infection in Denmark illustrated the effectiveness of HAART, as the incidence of PML declined from 3.3 cases per 1,000 patient-years at risk in the pre-HAART era to 1.3 cases per 1,000 in the late HAART era. For patients who already had PML, HAART was shown to stabilize the disease and increase survival, but these patients were often left with severe neurologic complications (115). HAART reduced the incidence of PML in HIV patients, but the prevalence of PML in this patient population has not declined to the same extent as other opportunistic central nervous system infections (116). Despite the decrease in prevalence in the post-HAART era, PML remains a significant complication of HIV infection, and HIV infection continues to account for about 80% of all new PML diagnoses (117). For comparison, estimates of PML incidence in the general Western population range from 0.3 to 0.8 per 100,000 person-years during a similar time period (118).

The Third Epoch: PML and Multiple Sclerosis

In 2005, a third group of patients at risk for developing PML emerged (99, 119). These were individuals with autoimmune diseases such as multiple sclerosis (MS) being treated with potent biologicals, such as the monoclonal antibody natalizumab, aimed at decreasing trafficking of leukocytes into inflamed tissue. The risk of developing PML in MS patients depends on several underlying factors including whether the patient is seropositive or seronegative for JCPyV, whether the patient has previously been treated with immunosuppressants, and the duration of the patient's exposure to natalizumab (120, 121). For seronegative patients, the risk is estimated at less than 1 in 1,000. In seropositive patients with no prior immunosuppressant use, the risk is less than 1 in 1,000 with 24 months of treatment and climbs to 6 in 1,000 following 49 months of treatment. In seropositive patients with prior immunosuppressant use, the numbers increase to 1 in 1,000 at 24 months, 12 in 1,000 at 48 months, and 13 in 1,000 at 72 months. This is higher than the incidence of PML in any other patient population, including patients with AIDS (122–123). Aggressive patient monitoring programs are in place to detect signs of PML early, allowing interventions that have resulted in lower morbidity and mortality in these patients (121).

The occurrence of PML in MS patients who are being treated with natalizumab was very surprising, as there had never been a case of PML documented in an MS patient. The reason is that MS is an inflammatory reaction against self-antigens in the brain and is not by itself immunosuppressive. In these natalizumab-associated cases, unlike the early cases described by Richardson, it was clearly the treatment that led to the disease. It soon became clear that drugs that altered normal immune surveillance in the central nervous system were highly associated with the development of PML. The reasons are not clear, but several non–mutually exclusive hypotheses have been put forward to explain PML in these patients. One prominent hypothesis posits that JCPyV persists not only in kidney but also in lymphoid compartments such as the bone marrow and that during drug treatment immature B cell precursors harboring the virus are released into the bloodstream and trafficked to the central nervous system. In support of the hypothesis, virus has been found in the bone marrow of PML patients, JCPyV has been found to replicate in B cell precursors, and this replication is driven by transcription factors known to be upregulated following drug treatment (33, 100, 124–129). These same transcription factors are

also present in glial cells of the brain that support high-level viral replication. This hypothesis remains unproven, however, as virus is rarely if ever found in the bone marrow or in B cells of healthy subjects, and even during the development of PML there has not been a documented viremia to explain trafficking to the central nervous system. It has also been hypothesized that virus, in rare individuals, establishes a low-level persistence in the central nervous system (130–134). It is these rare individuals who when immunosuppressed or immunomodulated develop PML. Evidence for this hypothesis is largely based on polymerase chain reaction, and as the brain is highly vascularized, it is difficult to conclude that the virus footprint came from brain tissue as opposed to from blood. In the absence of a tractable animal model, these hypotheses are unlikely to be proven or disproven anytime soon.

Animal Models

JCPyV productively infects only human cells; therefore, there is a lack of robust animal models to study the pathogenesis of PML. Recent progress in the development of a PML model in mice has been reported (135–137). A mouse with humanized glia was created by grafting bipotential human glial progenitor cells into the brains of neonatal immunodeficient and myelin-deficient (*Rag2*$^{-/-}$ *Mbps*$^{shi/shi}$) mice (136). Over time, the human glial cell grafts colonized the mouse brain and differentiated into astrocytes and oligodendrocytes. Subsequently, intracerebral injection of JCPyV Mad1 led to replication and productive infection of the glial progenitor cells and astrocytes and to focal demyelination. Unlike in human PML, oligodendrocytes were only rarely infected. One interesting observation was that the virus recovered from the brains of infected mice harbored mutations in VP1. Moreover, infection of the chimeric mice with JCPyV mutants derived from human patients also led to viral spread and disease. This latter point is interesting, as mutant virus arising in human patients seems unable to recognize sialic acid–containing receptors for the virus. Limitations of this study were that the mice acquired JCPyV via inoculation of high titers into the brain rather than via the proposed fecal-oral route, and that both the immune cells and immunosuppression in these mice differed from that in humans. While this model is not a direct parallel to the pathogenesis of PML in humans, it nevertheless has potential to further our understanding of disease development.

SUMMARY

PML continues to be a rare disease, but this is no consolation to those affected. The disease remains devastating, and effective treatments have not been developed. A significant barrier to developing treatments is the lack of a robust animal model. The major barrier to development of such a model is lack of human-specific replication factors in rodents that support the virus life cycle (138–140). As these factors become understood in greater detail, one can imagine that a rodent model expressing human replication factors could be developed. Currently the best model involves grafting human glial cells into immunodeficient mice. As the development of PML involves many other organ systems, this model is severely limited. Our understanding of PML and JCPyV is clearly evolving at a rapid pace. The most promising developments arose from studies examining viral sequences in the cerebrospinal fluid and in brain parenchyma from patients with PML. A swarm of wild-type and mutant viruses was discovered that fit with a model of immune escape variants playing an important role in the development of disease. How these viruses infect cells in the brain remains a mystery, but recent work suggests that virus is capable of receptor-dependent and receptor-independent spread, with the latter involving exosomes. A combination of exosome inhibitors and broadly acting receptor antagonists could limit the development and spread of PML in at-risk

patients. A small molecule that inhibits virus trafficking shows very little toxicity in mice and could be further developed to treat PML (88). Most promising, however, is the idea that vaccination in a prime-boost regimen with wild-type VLPs could eliminate wild-type and variant forms of JCPyV and prevent PML. This same strategy has been used very successfully against pathogenic strains of human papillomaviruses. A promising variant of this approach involves the development of specific and broadly neutralizing antibody–based reagents that could be used therapeutically to treat patients who develop disease or prophylactically to prevent disease in at-risk patient groups.

FUTURE ISSUES

1. It may be possible in the near future to develop a VLP-based vaccine for JCPyV that would protect at-risk patients from developing PML.
2. Efforts should also focus on repurposing old drugs and developing new ones, including antibody-based reagents, to prophylactically or therapeutically treat patients.
3. The development of a tractable animal model would be a major advance in the field and would allow economical testing of drug candidates.
4. It will be important to gain a better understanding of the mechanisms that contribute to viral persistence with a focus on the role of epigenetics, miRNA, and innate immunity.
5. It will be important to identify the sites of viral persistence and the sites of viral DNA rearrangements that are critical for the switch from a benign virus to a neuroinvasive and neuropathogenic virus.
6. Efforts should also focus on distinguishing receptor-dependent and receptor-independent mechanisms of spread so that strategies can be developed to inhibit both.
7. Understanding the role of choroid plexus and leptomeninges in the pathogenesis of PML should be a major focus of future experimentation.

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