Traffic of JC Virus from Sites of Initial Infection to the Brain: The Path to Progressive Multifocal Leukoencephalopathy

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Progressive multifocal leukoencephalopathy (PML) is a demyelinating disorder of the human brain caused by infection with the human polyomavirus, JC. Up to 80% of humans express serum antibodies to JC virus (JCV), yet considerably fewer people develop PML—predominantly those under immunosuppressive conditions. Recent research showed JCV infection in multiple tissues throughout the body, suggesting sites for viral latency. These observations allow the proposal of pathways that JCV may use from sites of initial infection to the brain. Results from investigations into cell-surface receptors, intracellular DNA-binding proteins, and variant viral regulatory regions also suggest mechanisms that may regulate cellular susceptibility to JCV infection. Together, these data elucidate how JCV may establish infection in various cell types, persist latently or become reactivated, and ultimately reach the brain to cause PML.

In 1958, Astrom et al. [1] described an unusual neuropathologic disorder that bore no resemblance to any recognized disease. Yet they found strikingly similar descriptions in the literature dating back to 1930: Each case presented with progressive dementia, motor dysfunction, and vision loss with death occurring within months [2]. Moreover, postmortem examinations revealed demyelinated white matter lesions in the brain, giant astrocytes, and enlarged oligodendroglial nuclei. Compiling these observations and citing the apparent uniqueness of this disorder, the illness was termed progressive multifocal leukoencephalopathy (PML). The diagnosis of PML remained relatively rare for several decades thereafter.

Early evidence for a specific etiology came soon after initial clinical and pathologic description of PML. Inclusion bodies in the nuclei of injured oligodendrocytes from a PML patient suggested the presence of a virus [3]. Electron microscopy revealed virus-like particles in the nuclei of abnormal oligodendrocytes [4]. Ultimately, a polyomavirus was isolated through the inoculation of primary human fetal glial cells with extracts from a PML-diseased brain [5]. This virus was termed “J.C.” after the patient from whom it was isolated.

PML is a disorder of the brain induced by the lytic infection of myelin-producing oligodendrocytes by JC virus (JCV). Initial demyelination occurs as multiple foci sparsely distributed in the subcortical white matter. With disease progression, each focus grows as virus spreads from cell to cell. Ultimately, microscopic areas of necrosis can become macroscopic plaque lesions up to centimeters in diameter. The three main pathologic features of PML are demyelinated lesions accompanied by abnormally large oligodendroglial nuclei and bizarre enlarged astrocytes (figure 1). Virions are less frequently detected in astrocytes than in oligodendrocytes, and neuronal loss is rare, with sparing of demyelinated axons [6]. Macrophages are often present in PML lesions; their likely role is the removal of myelin breakdown products or damaged oligodendrocytes. Further immune system involvement may occur as shown by lymphocytic infiltration. This can lead to periods of remission or protracted disease [7].

The multifocal nature of JCV infection results in a variety of clinical symptoms including visual impairment or blindness, motor dysfunction or weakness, and dementia and other cognitive abnormalities, such as personality change, memory loss, or emotional lability [8]. These symptoms can vary in order of presentation and severity, but most patients develop all three. Less common complications include vertigo, headache, seizures, sensory deficits, and Parkinsonism [8]. Although disease progression is usually rather rapid, with death less than a year after diagnosis, some patients survive with PML for several years [7]. While PML occurs predominantly in immune-suppressed persons, the underlying immunosuppressive disorder (i.e., AIDS) or treatment does not alter the presenting symptoms.

PML was rarely diagnosed when JCV was first identified, yet serum antibodies against JCV are detected worldwide [9] in up to 80% of humans [10, 11]. The AIDS pandemic has led to a much higher incidence of PML in the last 20 years; about 5% of all AIDS patients develop the disease. The question of how JCV can be ubiquitous yet not cause more widespread clinical illness is complex. The answer may be along the pathway of JCV pathogenesis, from sites of initial infection to the development of PML. It is important to analyze what determines cellular susceptibility to JCV and how the virus exploits this in...
order to reach the oligodendrocyte. A prerequisite to this is an understanding of JCV biology.

**JCV Biology**

*Viral characteristics.* JCV is a member of the polyomavirus family based on its capsid size (diameter, ~40 nm), icosahedral structure, and double-stranded, supercoiled 5.1-kb genome [12]. This genome codes for several nonstructural but multifunctional proteins (T, t, T_{135}, T_{136}, T_{165}), 3 capsid proteins (VP1–VP3), and a protein known as agnoprotein. Large T mRNA is produced prior to viral DNA replication and from the complementary strand of the capsid and agnoprotein genes, which are transcribed after DNA replication (figure 2). Cellular splicing of the large T RNA results in the small t and T RNA transcripts.

T protein is a DNA-binding protein necessary for regulation of viral DNA replication and transcription. The small t protein may not have a role in viral multiplication or pathogenicity in humans, but like T protein, it may be involved in transformation of infected rodent and nonhuman primate cells [13, 14].

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Figure 1. Three main pathologic features of progressive multifocal leukoencephalopathy (PML) and its etiologic agent JC virus (JCV). A, Magnetic resonance image of patient with PML shows lesions of subcortical white matter. B, Dense enlarged oligodendroglial nuclei in biopsy tissue from patient with PML. By in situ hybridization utilizing diaminobenzidine (DAB) as the chromophore, JCV DNA is a brown precipitate. C, Bizarre and enlarged astrocyte from biopsy tissue of a patient with PML. Hematoxylin stain identifies nuclei in unaffected cells. In situ hybridization with DAB was used to detect JCV DNA. D, Electron micrograph of JC virion particles visualized by uranyl acetate–negative staining. Each virion particle is ~40 nm in diameter.
The characteristic of prototype-like JCV strains. Segment of the regulatory region shown is a tandem 98-bp repeat regulatory proteins that modulate DNA replication and transcription.

The T proteins contribute to JCV DNA replication and interact with several cellular proteins [15]. Capsid proteins comprise the virion particle; the largest protein, VP1, is responsible for forming the icosahedral structure, with VP2 and VP3 functioning as minor structural components. VP1 also contains the epitopes for antibody induction and recognition. The agnoprotein directly interacts with T protein and can down-regulate both T modulated and basal viral DNA replication and transcription [16]. It is also involved in capsid assembly [12].

The regulatory region of JCV is composed of about 200 bp located between the two coding sequences. This region is considered to be the primary modulator of viral activity and cellular tropism [17, 18]. It also represents the most sequence variability in the genome, possibly due to rearrangements, replications, or deletions acquired during viral propagation [19]. The resulting sequence variations have distinct effects on JCV DNA replication, transcription [19, 20] and, consequently, tropism. JCV variants can be distinguished by differences in regulatory region structure [17].

**JCV tissue and cellular tropism.** Based on early experiments, JCV was originally described as a strictly neurotropic virus [21–23]. However, several studies have since provided evidence of JCV infection in a variety of other cell types. JCV virions have been detected in human tonsillar stromal cells and B lymphocytes isolated from tonsil tissue [24]. This suggests the spread of JCV via respiratory inhalation with the tonsils serving as a potential site for initial infection. Virus can also infect CD34+ hematopoietic progenitor cells and related cell lines, KG-1 and KG-1a, in addition to kidney cell lines and primary cultures of vascular endothelium and amnion cells [12]. Viral protein has been detected in all of these cell types and tissues by immunohistochemistry. Polymerase chain reaction, in situ hybridization, Southern blot, and/or restriction enzyme digest patterning have been used to detect JCV DNA in most of these cell types as well.

The complexity of JCV infection is augmented by the observation that different JCV genotypes are found in different tissues. These variants are distinguished by regulatory region structure, suggesting that control of JCV host range is regulated at the molecular level. Studies in this area are helping to elucidate how JCV can differentially infect cells and how it travels from the site of initial infection to the brain.

**Regulation of JCV infection.** With many other human viral pathogens, susceptibility to infection is determined at least partly by viral attachment to cellular receptors. No such clear association has been found for JCV, however. JCV binds to various receptors, but not all result in internalization. Viral entry is mediated by an N-linked glycoprotein with α(2-6)-linked sialic acids, a ubiquitous receptor [25]. One study demonstrated JCV binding to a variety of cell lines regardless of their susceptibility to infection, suggesting no role for receptors in determining infection [26]. However, the same study revealed much more specific binding with primary cells: JCV bound only to cells known to be susceptible to infection. Thus, there may be some receptor specificity—and therefore a contribution to infection—in vivo that does not exist in vitro in tumor cell lines.

What has become increasingly clear is the critical role of cell type-specific factors in regulating cellular susceptibility to JCV infection by the modulation of viral DNA replication and transcriptional activity [27]. DNA-binding proteins that have been implicated include NF-κB [28], YB-1 [29], Pura [30], Tst-1 [31], c-Jun [32], and NF-1 [33] (figure 2). Evidence has consistently been directed toward a regulatory role for NF-1 (nuclear factor-1). The NF-1 transcription factor family has four class members (A, B, C, and D or X) with conserved DNA-binding domains but distinct transcriptional activation domains. Differential splicing of RNA transcripts results in several subclasses of NF-1. NF-1 has several binding sites in the JCV regulatory region and studies suggest a number of functions for NF-1 and its binding sites [27].

Particular interest has focused on the NF-1 class X protein, which is consistently detected at higher levels in cells susceptible to JCV infection than in nonpermissive cells. Human fetal glial cells, among the cells most permissive to JCV, express NF-1 X at levels much higher than nonsusceptible HeLa cells [34]. The hematopoietic progenitor cell line, KG-1, is also susceptible to infection and expresses NF-1 class X. However, when these cells differentiate to macrophage-like cells upon phorbol ester treatment, NF-1 class X expression is down-regulated and the cells concurrently lose susceptibility to JCV. A recent study trans-

**Figure 2.** Organization of JC virus (JCV) genome including some regulatory proteins that modulate DNA replication and transcription. Segment of the regulatory region shown is a tandem 98-bp repeat characteristic of prototype-like JCV strains.
affected an NF-1 class X expression vector into these differentiated KG-1 cells and consequently restored their susceptibility to infection [35]. Expression vectors for the other NF-1 classes did not restore susceptibility.

Another protein of note is c-Jun of the activator protein-1 (AP-1) family. c-Jun binding sites are commonly found adjacent to NF-1 binding sites in the promoters of several brain-specific genes, including glial fibrillary acidic protein, human and mouse myelin basic protein, proenkephalin, and JCV [32] (figure 2). The well-conserved juxtaposition of these binding sites in the regulatory sequences of various genes suggests a functional interaction between NF-1 and c-Jun.

If DNA-binding proteins control regulation of viral infection, variations in the JCV regulatory sequences that are recognized by these proteins will also affect cellular susceptibility. In fact, different JCV genotypes, having different regulatory regions by definition, are consistently found in different tissues. The regulatory region of the prototype Mad-1 variant, consistently found in infected brain or tonsil tissues, contains direct 98-bp tandem repeats (figure 2). Archetype JCV, isolated from the kidney or urine, has only one 98-bp sequence plus certain insertions. Variations in these sequences result in varying availability of binding sites for regulatory proteins that then modulate infection. JCV genotypes containing the prototype-like tandem repeats, for example, contain more NF-1 binding sites than their archetype counterparts. Of interest, a recent study reported poor clinical outcomes in PML patients whose plasma and brain or cerebrospinal fluid (CSF) predominantly contained JCV isolates with tandem repeats [36]. The archetype sequence predominated in the same tissues of PML survivors. Moreover, Daniel et al. [37] demonstrated the failure of archetype JCV to replicate effectively in glial cells due to an inability of the early viral promoter to express sufficient mRNA to support T antigen–mediated DNA replication. Collectively, these studies support the importance of the JCV regulatory region as a critical factor involved in determining host tissue range and the resulting clinical consequences (or lack thereof) of JCV traffic throughout the body.

JCV Traffic from Initial Infection to PML

Research that has led to the current knowledge of JCV regulation can now also produce hypotheses concerning pathways that JCV utilizes to enter the human host and to ultimately reach the brain (figure 3). Traffic of JCV throughout the body is complex, involving at least cellular receptors, DNA-binding proteins, and a variety of viral regulatory regions in multiple target cells.

Initial infection. Since JCV infection is so prevalent in the human population and seroconversion occurs during childhood, a common route may be responsible for introduction of the virus into the body. Respiratory inhalation was thought to be a possible mechanism, but for many years JCV was described as strictly neurotropic. A related human polyomavirus, BK virus, infects lung [38] and tonsillar tissue [39], but no evidence was found of JCV infection in the respiratory tract or that JCV could survive a gastrointestinal infection. Moreover, it had been reported that tonsillar lymphocytes served as a reservoir for Epstein-Barr virus (EBV), another ubiquitous human DNA virus, and participated in dispersal of virus between individuals [40]. Recently, JCV DNA was detected in human tonsil tissue including stromal cells and lymphocytes [41]. Prototype-like genotypes were predominant in all tonsillar components, with archetype DNA isolated from samples of tonsillar lymphocytes. These data reinforce the hypothesis that the tonsils may serve as a site of initial infection. Airborne JCV can access the tonsillar tissue and bind to various cell surface receptors. However,
only virions binding to N-linked glycoproteins containing α(2-6)-linked sialic acids will result in infection. Virions bound to these specific receptors would likely be internalized into a neutralizing antibody-resistant vesicle via clathrin-dependent endocytosis in a manner similar to that discovered in glial cells [42]. Once the genome reaches the tonsillar cell’s nucleus, the aforementioned cellular DNA-binding proteins can regulate JCV DNA replication and transcription. Tonsillar stromal cells express high levels of the NF-1 class X protein, which likely contribute, along with other proteins, to this cell-specific regulation of infection. Tonsillar NF-1 X binds to recognition sites on the JCV regulatory region, possibly adjacent to c-Jun binding sites. T antigen mRNA is produced and followed by viral DNA replication and then by capsid protein mRNAs. Ultimately, JC virions are assembled and can be passed on to circulating tonsillar lymphocytes. B cells adhere to stromal cells through various adhesion molecules, facilitating transfer of virus from stromal cells to lymphocytes [43].

Another factor that modulates viral infection is the hypervariability of the JCV regulatory region. It has been suggested that mutations occurring during JCV DNA replication produce the variants observed throughout the body. Archetype JCV could arise from the Mad-1 sequence, for instance, by the loss of one 98-bp sequence and the addition of a 23-bp and a 66-bp insert. If prototype sequences are responsible for initial infection, this could explain why the Mad-1 variant is found predominantly in tonsillar stromal cells, but the archetype has been found in tonsillar lymphocytes. Tonsillar lymphocytes containing the archetype variant could then carry the virus to the kidney, where the archetype is predominant [44].

JCV was first demonstrated in lymphocytes by the detection of JCV DNA and capsid protein in bone marrow mononuclear cells from 2 PML patients [45]. Since then, several studies have found JCV in lymphocytes isolated from bone marrow, the central nervous system, and peripheral blood [46-48]. B cell lines are also susceptible [49]. Viral entry into lymphocytes may occur in a manner similar to that described for glial cells. Molecular regulation then allows infectious virions to be produced: Lymphocytes express NF-1 proteins by gel shift assays and DNase protection assays with lymphocytic nuclear extracts have revealed protected NF-1 and c-Jun binding sites in the JCV genome [49]. Thus, mechanisms of viral susceptibility similar to those involved in tonsillar and glial cells may regulate infection of lymphocytes that then disseminate the virus throughout the body. In addition, as with EBV, lymphocytes may serve as reservoirs for viral latency. Another possible mechanism of lymphocyte-mediated traffic of JCV is simply virus association with the cell membrane without internalization. A recent study demonstrated that while JCV does infect B cells, most virions remain cell surface attached [26]. This would explain why JCV DNA, but not mRNA, is more often detected in association with lymphocytes. Regardless of the mechanism, the result is the same: JCV is spread throughout the host to sites of pathologic activity (e.g., the brain) or to sites of latency (e.g., kidney and bone marrow).

Viral latency and reactivation. JCV is excreted in the urine of healthy persons and in patients with PML. It has also been detected in renal tissue, including that of healthy persons [50]. These data suggest that the kidney serves as a site of latent infection, but the mechanisms and/or biochemical events that allow this are unclear. When infected lymphocytes or those with membrane-associated JCV circulate through the kidney, JCV can be released into the surrounding tissue. If these virions include sequences containing tandem repeats, mutations occurring during viral DNA replication could result in the archetype structure—the sequence most commonly found in the kidney. It has also been proposed that cells in certain organs may select for the archetype in order to survive JCV infection because the archetype does not produce progeny virions in vitro [20]. The aforementioned study by Daniel et al. [37] demonstrated the inability of the archetype to replicate effectively in glial cells. Of interest, deletion of either or both the 23- or 66-bp archetype regulatory region inserts increased viral replication. Thus, kidney cells may harbor the archetype at least in part because these virions have no lytic activity, unlike other JCV variants.

Bone marrow is another possible site for JCV latency. Susceptibility to infection has been demonstrated in both a CD34+ hematopoietic progenitor cell line, KG-1, and in primary cells [24]. A persistent infection could occur if progenitor cell populations become exposed to JCV via infected circulating lymphocytes. Moreover, if infected hematopoietic progenitor cells differentiate into B lymphocytes, virus could be further disseminated throughout the host. Although the study of KG-1 used the prototype-like Mad-4 JCV variant, it is possible that archetype JCV infects hematopoietic progenitor cells or that mutations occur in vivo during DNA replication to produce archetype sequences. As in the kidney, archetype JCV may be less active in hematopoietic progenitors than prototype variants, favoring the onset of latency.

The reactivation process for JCV has yet to be conclusively described, although immunosuppression is likely a major component. Loss of specific immune cells may allow for active viral replication and infection. Others have suggested that immune suppression creates conditions suitable for changes in the JCV regulatory region, leading to sequences of varying biologic activity. It is also unclear why some immunocompromised persons develop PML while others with similar JCV burdens do not. This observation suggests that critical steps in the spread of JCV and the development of PML are not solely functions of immune status.

Traffic to the brain and PML. Evidence of multiple tissue and organ involvement suggests that JCV is delivered through the body by a hematogenous route. JCV was identified in several organs other than the kidney and brain (e.g., heart, spleen, lung, colon, and liver) [8, 51]. In situ hybridization of brain
tissue has shown high densities of infected cells surrounding blood vessels [45] and JCV-induced PML lesions throughout brain white matter, further supporting this idea. Research is now focusing more on the mechanism by which JCV uses particular blood cell type(s) to reach the brain. Aside from the likely involvement of B lymphocytes in the dissemination of JCV, the virus may be carried to the brain through viremia, but this has not been demonstrated. JCV is likely not disseminated via T lymphocytes: JCV cannot infect T lymphocytes or bind T cell membranes [26].

An important requisite for virus entry into the brain is penetration of the blood-brain barrier (BBB), which lymphocytes can traverse [52]. JCV may use these cells as a Trojan horse to reach the brain tissue. In fact, infected B lymphocytes have been found in multiple PML brain tissue samples [53]. Virions released by lymphocytes would then attach to glial cells and be internalized rapidly—within 30 min via the endocytic mechanism described earlier [42]. In this rather simple and rapid process following viral reactivation, JCV successfully crosses the BBB and penetrates susceptible brain cells. The DNA-binding proteins of infected glial cells can then regulate and promote JCV transcription and DNA replication.

GliaL cells express levels of NF-1 X as high as any cell type; they are also the cells most susceptible to JCV infection. NF-1 X and other proteins would drive the production of virions in affected cells, facilitated by the presence of multiple binding sites on the regulatory regions of prototype-like JCV variants, which are predominant in PML brain tissue. Archetype sequences contain fewer NF-1 binding sites, for example, due to the absence of the 98-bp sequence that is duplicated in the prototype. This may help explain why PML patients with prototype-like JCV DNA in plasma and brain or CSF generally lack antibodies to JCV antigens [40]. Such patients often succumb to PML within several months after initial diagnosis, although some patients survive longer [7, 8].

Summary

Although PML may once have been rather rare, it is much more prevalent now, especially in the context of AIDS. In the 45 years since its initial description and the 30 years since the identification of its etiologic agent, research has greatly increased our knowledge of PML. Future investigations should focus on JCV activity prior to clinical manifestations, namely, the traffic of JCV from initial infection to latency and reactivation to the brain. A better understanding of viral binding, DNA-binding proteins, and the viral regulatory regions would be constructive in the development of therapeutics for PML.

References

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