Progressive Multifocal Leukoencephalopathy

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Progressive multifocal leukoencephalopathy (PML) was a rare disease until the advent of the HIV/AIDS pandemic. Recent interest in the disorder has been spurred by its appearance in patients treated with the monoclonal antibodies natalizumab and rituximab. Unless the accompanying underlying immune deficit can be reversed, PML typically progresses to death fairly rapidly. Treatment directed against the JC virus has been unhelpful, but an increased understanding of disease pathogenesis may result in effective therapeutic strategies.

Introduction

Interest in progressive multifocal leukoencephalopathy (PML) heightened in 2005 following the recognition of three cases of the disease attending the use of natalizumab, which is a monoclonal antibody directed against $\alpha 4\beta 1$, an integrin used in the treatment of multiple sclerosis and Crohn's disease [1–4]. The introduction of other immunomodulatory agents that prevent entry of cytotoxic T-lymphocytes into the brain has raised concerns that this, and perhaps other rare central nervous system (CNS) opportunistic infections, may attend their use. Prior to the advent of the AIDS pandemic, PML was as a rare disorder. First described in 1958 [5] in three patients with underlying lymphoproliferative disorders, this unique neurologic disorder was characterized chiefly on the basis of a triad of unique histopathologic features that included demyelination, abnormal oligodendroglial nuclei, and giant astrocytes. A comprehensive review encompassing 26 years, from 1958 through 1984, reported only 230 published and unpublished cases, with HIV/AIDS responsible for only 2.1% of the underlying illnesses in that series. In the ensuing two decades, the frequency with which PML was observed changed dramatically, with HIV/AIDS accounting for nearly 90% of the cases [6].

Molecular Biology of the JC Virus

In the year following the seminal description of PML, Cavanagh et al. [7] suggested the possibility of a viral etiology based on the appearance under an electron microscope of inclusion bodies in the enlarged oligodendroglial nuclei. The features seen under the electron microscope suggested a virus in the papovavirus family [8,9], a family of viruses not previously known to cause human CNS disease. Subsequent viral isolation in human fetal glial cell cultures by Padgett et al. [10] from the brain of a patient with PML confirmed that a polyomavirus from the papovavirus family was the responsible agent. Named by the initials of the person from whom it was first isolated, the JC virus (JCV) is but one of two of the 17 known species of polyomavirus currently recognized to cause disease in humans. The other is BK virus, which shares more than 70% nucleotide homology with JCV. SV40, a monkey polyomavirus that entered the human population with contaminated polio vaccines, can infect human cells. Although it has been suggested as a potential etiology of some human tumors, it has yet to be convincingly demonstrated to cause human disease.

JCV, like all polyomaviruses, is a nonenveloped virus with an icosohedral capsid and a double-stranded DNA genome. It is a relatively small virus, measuring 40 to 45 nm in diameter. Its outer capsid consists of 72 pentamers of the major capsid protein VP1, and each VP1 pentamer is associated with either a VP2 or VP3 minor capsid protein on the internal surface of the virion. The fairly simple genome (Fig. 1) consists of 5.1 kb in supercoiled form and is divided into a noncoding control region (NCCR), an early region that codes for the regulatory proteins T and t antigen, and a late region that codes for the regulatory protein agnoprotein and three structural proteins (VP1, VP2, and VP3). The early and late regions of the genome are very stable, but the NCCR region, which contains approximately 200 nucleotide base pairs, is not. The NCCR contains the signals for DNA replication as well as for promotion and enhancement of transcription [11–15], and appears to be responsible for the cellular tropism of

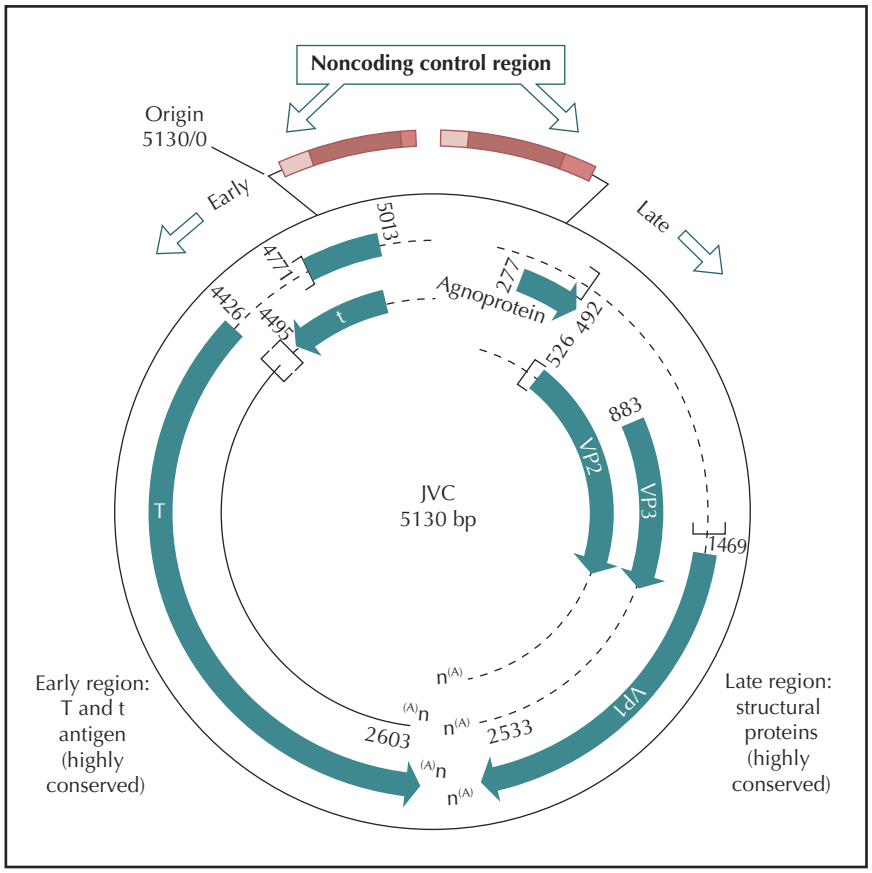

Figure 1. Diagram of the JC virus (JCV) genome. bp—base pair.

JCV [16]. The NCCR demonstrates the most sequence variability in the brains of patients with PML as a consequence of deletions and rearrangements, perhaps acquired during propagation in brain or in extraneural host tissues. The T protein, a nonstructural but multifunctional protein, is a DNA binding protein responsible for initiation of viral DNA replication and transcription of the capsid proteins, which are transcribed from opposite strands of the DNA genome. In some rodent and nonhuman primate cells, JCV T-protein expression is believed to be responsible for malignant transformation or tumor induction, particularly of astroglial cells into astrocytomas. In these cells only the T protein, named for its tumor-promoting function, is expressed. No animal model for PML presently exists. In primate [13] and rodent models [17], JCV may be oncogenic.

Although the initial studies of JC virus in cultures of human fetal brain cells suggested an exclusive neurotropism for glial cells [9,10,18,19], this is not entirely true. JCV infects both oligodendrocytes (productively) and astrocytes (restricted). JCV may also infect the granular cells of the cerebellum [20•]. Loss of cerebellar granular cells in PML was noted even in the pre-AIDS era [21]. JCV may cause an ataxic disorder due to dropout of infected cerebellar granular cells in the absence of the characteristic demyelinating lesions of PML.

JCV uses the serotonin (5-HT) receptor 5-HT2a for binding to the cell surface [22], although this may not be true for all cells, such as brain microvascular endothelial cells [23]. It is quite likely that other receptors, as yet unidentified, may also permit JCV binding. Following binding, the virus enters the cell through clathrin- and eps15-dependent pathways [24,25], where it is transported to the endoplasmic reticulum through caveosomes [26]. From there, it enters the nucleus, where all viral replication occurs. Nuclear DNA binding proteins that selectively interact with the regulatory region of the genome are critical to the tropism of the virus. In particular, binding of nuclear factor 1 (NF-1), a protein that functions in both transcriptional control and replication of DNA, is important to JCV replication. Cells that are not susceptible to JCV infection probably do not have these same protein factors and/or have other proteins that bind the JCV regulatory sequences and block transcription. Oligodendrocyte death appears to occur through apoptotic pathways [27], although necrosis may be the mechanism of cell death in progenitor-derived astrocytes [28].

Pathogenesis of PML

For PML to develop, a number of steps are necessary: 1) infection with JCV; 2) establishment of latency of JCV in extraneural tissues; 3) rearrangement of the NCCR region of the JCV resulting in a neurotropic strain; 4) reactivation of the neurotropic JCV strain from sites of viral latency or persistent expression; 5) entry of JCV into

the brain; 6) failure of the immune system to eliminate the infection; and 7) establishment of productive infection of oligodendrocytes. The initial infection with JCV is either unapparent or unrecognized. Following infection, it is believed that JCV becomes latent in selected tissues, including the tonsils [29], lung, spleen, bone marrow, and kidney [30]. Approximately 5% to 30% of the population excretes JCV in urine that can be detected by either polymerase chain reaction (PCR) or virus isolation, clearly indicating that the kidney is a site of viral latency. However, with respect to some of the other tissues, viral transcripts are detected with only great difficulty. The DNA sequence of the regulatory region of JCV obtained from kidney or urine, referred to as the archetype sequence, is significantly different from the sequence found in the brain of PML patients [31]. It contains 187 nucleotide pairs with no tandem repeats, as is observed in PML brain isolates [19,32]. To convert the archetype sequence to that most often found in PML brain tissue requires gene rearrangement in the NCCR.

A widely held hypothesis regarding PML pathogenesis implicates viral latency in lymphocytes in bone marrow or other lymphoid tissues that are activated during immune suppression, which then enter the peripheral blood and ultimately infect the brain [33,34]. Importantly, several regulatory region sequences have been identified from JCV DNA in the peripheral blood of PML patients that are not related to the archetype but closely related to sequences found in PML brain [35]. Circulating infected lymphocytes may be able to cross the blood-brain barrier and pass infection to astrocytes at the border of vessels, which in turn augments infection through multiplication to eventually infect oligodendrocytes. Using in situ DNA hybridization, JCV-infected cells are frequently found near blood vessels in the brain, in B lymphocytes in bone marrow [34], and in the brain itself [36]. In a report of 19 patients with biopsy-proven PML, over 90% had JCV DNA in peripheral blood lymphocytes [37]. Data derived from other groups of individuals without PML revealed that 60% of HIV-1–seropositive individuals, 30% of renal transplant recipients, and approximately 5% of normal healthy volunteers also had JCV DNA in their peripheral circulation [37]. In relatively immunologically healthy HIV-infected persons on highly active antiretroviral therapy (HAART), the likelihood of finding JCV DNA in circulating lymphocytes appears to parallel that of the normal population [38].

Although as much as 80% to 90% of the population is infected with JCV before early adulthood [39], it is not known whether PML results from a primary infection or a reactivated latent or persistent infection. Three converging lines of evidence support the latter possibilities. First, PML is exceptionally rare in children [40]. Second, whereas the IgG antibody is readily detected, the IgM antibody to JCV is rarely observed in the setting of PML, indicating that the infection is not recent [41]. In one study, only one of 21 patients with PML had IgM specific for JCV in their sera, whereas 20 of 21 patients had IgG antibody specific for JCV [42]. Some investigators have argued that the latter study does not exclude the possibility of PML resulting from acute JCV infection because many of these patients were studied late in the course of their disease [43]. Third, there have been a handful of cases of PML in which JCV isolated from non-CNS tissues long before the development of PML was demonstrated to be genomically similar to that ultimately isolated from the brain [44]. However, it is certainly conceivable that some percentage of patients with PML developed the illness shortly after acquiring JCV infection.

Epidemiology of JCV

The ability of JCV to cause hemagglutination of type O erythrocytes enabled the performance of antibody studies to determine evidence of prior infection. The performance of hemagglutination inhibition studies for JCV is cumbersome and has been supplanted by enzyme immunoassay [45]. As no disease has been convincingly associated with acute infection, the mechanism of viral spread remains speculative. The presence of JCV in tonsillar tissues [29] suggested that saliva and oropharyngeal secretions may be a means of transmission. Recent studies of these fluids in HIV-infected persons and healthy controls indicate that JCV is rarely demonstrated in them and, when present, is there in very low titers [38,46]. Approximately 10% of children between the ages of 1 and 5 years demonstrate antibodies to JCV, and they can be observed in 40% to 60% of the population by the age of 10 years. By adulthood, this figure rises to almost 70% [39]. Seroconversion rates to JCV exceed 90% in some urban areas [39].

Host Factors and Underlying Diseases

PML is rarely observed in otherwise immunologically normal individuals. Typically, the underlying abnormality is one of cell-mediated immunity, more specifically, a general impairment of the Th1-type T-helper cell function [47]. In a large review published in 1984 [48], lymphoproliferative diseases accounted for 62.2% of the cases and were the most common predisposing illnesses. The percentage of other predisposing disorders in that series included myeloproliferative diseases in 6.5% of cases, carcinoma in 2.2%, granulomatous and inflammatory diseases such as tuberculosis and sarcoidosis in 7.4%, and other immune deficiency states in 16.1%. HIV/AIDS was responsible for only 2.1% of the cases, accounting for only five of the 230 cases [48]. Approximately 5% of patients with PML had no recognized underlying disorder, but some immunologic disorders that predispose to PML, such as idiopathic CD4 lymphopenia, had not yet been described.

Until the onset of the AIDS epidemic, PML was a rare disease. For most practicing neurologists, it remained a medical curiosity. The AIDS pandemic changed the inci-

Table 1. Symptoms and signs of AIDS-associated PML in descending order of frequency

Symptoms	Signs
Weakness	Hemiparesis
Cognitive impairment	Gait disturbance
Speech abnormalities	Cognitive impairment
Headache	Dysarthria
Gait impairment	Dysphasia
Visual abnormalities	Hemisensory loss
Sensory loss	Visual field defects
	Ocular palsies

PML—progressive multifocal leukoencephalopathy.
(*Data from* Berger et al. [49].)

dence of PML very rapidly. In a single series collected in south Florida, there were 156 cases during the 14-year interval from 1980 to 1994 [49]. All but two of those cases were associated with HIV/AIDS. There was a 20-fold increase in PML frequency when comparing the interval from 1980 to 1984 with the interval from 1990 to 1994 [49]. As noted, HIV/AIDS is now responsible for approximately 90% of the PML cases in the United States, and the number of patients with PML without a recognized underlying immunosuppressive disorder has fallen to under 1%. The introduction of HAART appears to have decreased the frequency with which PML is observed.

Clinical Disease

The most common symptoms (Table 1) reported by patients with PML or their caregivers are weakness and cognitive disturbances [49]. Other common symptoms include speech abnormalities, headaches, gait disorders, visual impairment, and sensory loss. In one large series, each of these symptoms was seen in more than 15% of patients [49]. In general, these symptoms are similar to those identified in a series of non–HIV-associated PML cases. PML patients with [49] and without [48] headaches were significantly more common in the former, and visual disturbances were more common in the population without AIDS. Seizures are seen in up to 10% of patients. They are usually focal in nature and may secondarily generalize. Seizures may reflect cortical or subcortical disease or, in the HIV-infected patient, be secondary to some other process, including HIV infection of the brain itself [50].

Limb weakness, the most common sign in HIV-associated PML, is observed in over 50% of cases [49]. Cognitive disturbances and gait disorders are seen in approximately one quarter to one third of patients [49]. Visual field loss due to involvement of the retrochiasmal visual pathways is significantly more common than diplopia or other visual disturbances [48,49,51,52]. Optic nerve disease does not occur with PML. Diplopia, reported in about 10% of patients, is usually the consequence of involvement of the third, fourth, or sixth cranial nerves and is typically observed in association with other brain stem findings [49]. Clinically recognized myelopathy occurring as a consequence of PML has not been reported either, although demyelinating lesions have been observed in the spinal cord [53–55], as has the JCV antigen [56]. Lack of clinical involvement of the optic nerve and spinal cord may help the clinician distinguish PML from multiple sclerosis when concern about PML arises due to treatment strategies for multiple sclerosis. PML does not involve the peripheral nervous system.

PML heralds AIDS in approximately 1% of all HIV-infected persons [51]. Therefore, in as many as 20% to 25% of HIV-related PML cases, the neurologic disorder antedates knowledge of the HIV infection. This may lead to significant diagnostic confusion in the otherwise healthy individual with unsuspected HIV infection. A high index of suspicion for underlying HIV infection is required when confronted with unusual neurologic illnesses.

Neuroimaging

In the appropriate clinical context, radiographic imaging strongly supports the diagnosis of PML. CT of the brain in PML typically reveals hypodense lesions of the affected white matter. On CT scan, the lesions of PML exhibit no mass effect and infrequently contrast enhance. A "scalloped" appearance beneath the cortex is noted when there is involvement of the subcortical arcuate fibers [57]. Cranial MRI is far more sensitive than a CT scan to the presence of the white matter lesions of PML [57]. MRI shows a hyperintense lesion on T2-weighted images in the affected regions (Fig. 2) and, as with CT scan, faint, typically peripheral contrast enhancement may be observed in 5% to 10% of cases [57]. The lesions of PML may occur virtually anywhere in the brain. The frontal lobes and parieto-occipital regions are commonly affected, presumably as a consequence of their volume. Lesions in the basal ganglia, external capsule, cerebellum, and brainstem may be seen as well [57]. The lesions of PML may appear identical to those of MS.

Magnetization transfer MRI studies have been suggested as an effective means of monitoring the degree of demyelination in PML. Magnetic resonance spectroscopy reveals a decrease in N-acetylaspartate and creatine, and an increase in choline products, myoinositol, and lactate in the lesions of PML. These changes likely reflect neuronal loss and cell membrane and myelin breakdown consequent to PML. Cerebral angiography is not routinely performed and is not likely to be particularly useful diagnostically. In one study, shunting and a parenchymal blush in the absence of contrast enhancement was observed on magnetic resonance angiography [58]. Pathologic studies suggested that small vessel proliferation and perivascular inflammation were the explanation for these unexpected

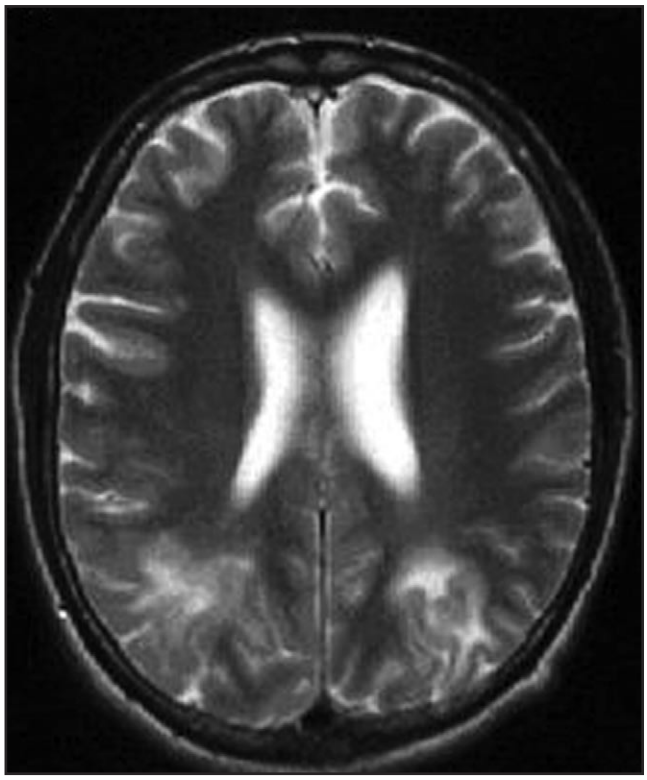

Figure 2. This T2-weighted magnetic resonance image shows hyperintense signal abnormalities in the white matter of the parieto-occipital lobes due to progressive multifocal leukoencephalopathy.

angiographic features [58]. Thallium-201 single photon emission computed tomography (^{201}Tl SPECT) generally reveals no uptake in the lesions of PML, although there is an isolated case report of a contrast-enhancing lesion with a positive ^{201}Tl SPECT.

Laboratory Studies

In the overwhelming majority of HIV-infected patients with PML, severe cellular immunosuppression, as defined by CD4 lymphocyte counts less than 200 cells/mm^3, is observed. In three separate series of AIDS-related PML [49,55,59], the mean CD4 count ranged from 84 to 104 cells/mm^3. However, in the largest series of HIV-associated PML [49], 10% or more of patients had CD4 lymphocyte counts in excess of 200 cells/mm^3.

Examination of cerebrospinal fluid (CSF) is very helpful in excluding other diagnoses. Cell counts are usually less than 20 cells/mm^3 [49]. In one large study, the median cell count was 2 cells/mm^3 and the mean was 7.7 cells/mm^3 [49]. In that same study, 55% had an abnormally elevated CSF protein level, with the highest recorded value being 208 mg/dL (2.08 g/L) [49]. Hypoglycorrhachia was observed in less than 15%. These abnormalities are not inconsistent with that previously reported to occur with HIV infection alone. Several studies [59–61] demonstrate a high sensitivity and specificity of CSF PCR testing for JCV in PML. Many authorities have regarded the demonstration of JC viral DNA, coupled with the appropriate clinical and radiologic features, to be sufficiently suggestive of PML to be diagnostic, thus obviating the need for brain biopsy. Quantitative PCR techniques for JCV in biologic fluids continue to be refined. The sensitivity of CSF PCR testing for JCV in PML is on the order of 75%. Amplification of the virus from the CSF in the absence of PML is very unlikely.

Pathology

The histopathologic hallmarks of PML are a triad of multifocal demyelination, hyperchromatic and enlarged oligodendroglial nuclei, and enlarged, bizarre astrocytes with lobulated hyperchromatic nuclei [5,21]. The cardinal feature of PML is demyelination, which is apparent both macroscopically and microscopically. Demyelination may, on rare occasions, be monofocal, but it typically occurs as a multifocal process, often in a periventricular location, suggesting a hematologic spread of the virus. These lesions may occur in any location in the white matter and range in size from 1 mm to several centimeters [5,21]. Pathologically, examination often reveals that larger lesions may result from the coalescence of multiple smaller lesions, but this is rarely observed radiographically. The astrocytes may be seen to undergo mitosis and appear to be malignant. Infrequently, their presence may lead to a mistaken diagnosis of glioma, as occurred in a patient treated with natalizumab [4]. Examination under an electron microscope or with immunohistochemistry reveals JCV in oligodendroglial cells. The virions measure 28 to 45 nm in diameter and appear singly or in dense crystalline arrays [5,21]. Less frequently, the virions are detected in reactive astrocytes and granular cells of the cerebellum [62]. They are uncommonly observed in macrophages engaged in removing the affected oligodendrocytes. The virions are generally not seen in the large, bizarre astrocytes. In situ hybridization and in situ PCR testing for JCV antigen allows for detection of the virion in the infected cells even in formalin-fixed archival tissue.

Prognosis

Prior to the HAART era, the median survival of AIDS patients with PML was 6 months and the mode was 1 month [49]. In the absence of effective antiretroviral therapy, the survival of AIDS patients with PML is not significantly different than PML occurring with any other immunosuppressive condition. However, even in the pre-HAART era, recovery of neurologic function, improvement of PML lesions in radiographic imaging, and survival exceeding 12 months had been observed in as many as 10% of patients with AIDS-associated PML [49]. Factors that appear to be associated with prolonged survival include PML as the pre-

senting manifestation of AIDS [51], higher CD4 lymphocyte counts (> 300 cells/mm^3) [51], presence of a perivascular inflammatory infiltrate in the PML lesions [63], and contrast enhancement on radiographic imaging [51,64], although another study failed to link any radiologic features with prognosis [65]. Additionally, a correlation between low titers of JC viral DNA load in the CSF and prolonged survival has also been demonstrated. The longest survival has been 92 months from onset of illness [49].

The cellular immune response against JCV appears to be tightly correlated with a favorable clinical outcome in PML [66–68]. The presence of JCV-specific cytotoxic T lymphocytes in these patients is likely related to the presence of inflammatory infiltrates in the PML lesions, which are responsible for the alterations of the blood-brain barrier and marginal contrast enhancement seen on imaging studies. These JCV-specific cytotoxic T lymphocytes are probably instrumental in destroying infected oligodendrocytes, preventing further disease progression, and decreasing CSF JC viral load. The lack of recurrence of PML in some of the patients exhibiting long-term survival and recovery reflects clearance of JCV from CSF [69].

Treatment

To date, there are no successful therapeutic modalities for PML that target JCV. Most of the extant literature consists of anecdotal reports. In the pre-HAART era, zidovudine and other antiretrovirals had been proposed as adjunctive therapy for AIDS-associated PML based on anecdotal evidence, but in vitro assays show no effect of zidovudine on JCV replication [70]. However, HAART, because of its ability to restore failing immune systems, has changed the prognosis of AIDS-associated PML quite considerably. As many as 50% of patients with AIDS-associated PML receiving HAART demonstrate long-term survival (> 12 months). The benefit of HAART in AIDS-associated PML has not been universally observed [71], as the benefit seems to be best for antiretroviral treatment–naive patients [72]. The remarkable success of HAART in the treatment of AIDS-related PML has had its downside, however. A syndrome referred to as the immune reconstitution inflammatory syndrome may develop in tandem with the recovering immune system, giving rise to new or increased neurologic deficits, increased number or size of lesions observed by neuroimaging, contrast enhancement of these lesions, and brain edema [73–78]. Fatal outcomes have been observed and the development of this syndrome with infratentorial PML may be especially dangerous. HAART has no effect on PML unrelated to HIV/AIDS.

Nucleoside analogues have been employed in the treatment of PML because they impede the synthesis of DNA [79]. In vitro studies have clearly demonstrated the ability of cytosine arabinoside (cytarabine) to inhibit JCV replication [70], and anecdotal reports of intravenous and intrathecal administration suggest the value of this therapy in PML [80–85]. Unfortunately, a carefully conducted clinical trial of AIDS-related PML failed to show any value for either intravenous or intrathecal administration of cytarabine when compared with placebo [86]. More effective delivery of the drug to the CNS may prove valuable.

Camptothecin derivatives, such as topotecan, are DNA topoisomerase I inhibitors that result in single strand breaks in DNA. These have been demonstrated to block JCV replication in vitro when administered in pulsed doses in amounts nontoxic to cells [87], but their therapeutic value in PML has been entirely anecdotal [88,89].

Cidofovir (HPMPC; (S)-1-[3-hydroxy-2-phosphonyl methoxypropyl]cytosine) and its cyclic counterpart have demonstrated selective antipolyomavirus activity [90]. The 50% inhibitory concentrations for cidofovir are in the range of 4 to 7 mg/mL, and its selectivity index varies from 11 to 20 for mouse polyomavirus and from 23 to 33 for SV40 strains in confluent cell monolayers [90]. Although a proposal for its use in PML has been supported by anecdotal evidence [91], the largest trial to date with the drug has failed to show any benefit [92]. Additionally, the drug has serious side effects, including ocular hypotony, bone marrow depression, and renal disorders.

Type 1 interferons have been reported to have a salutary effect either subcutaneously [93] or intrathecally [84] when used in conjunction with cytarabine. The antiretroviral activity of the interferons may be the consequence of their ability to stimulate natural killer cells. Unfortunately, limited clinical experience reveals no benefit. The understanding of JCV cellular binding and entry has led to suggestions that drugs that inhibit these processes be used in treating PML, including blockade of the 5-HT2a receptor blocker [94], clathrin-dependent endocytosis, and other pathways such as the nuclear factor of activated T cells [95].

Conclusions

Our appreciation of PML has increased dramatically in tandem with the numbers of affected persons. Recently introduced immunomodulatory therapies have heightened awareness of this formerly rare disorder. Almost certainly, novel strategies for its treatment will emerge with an increased understanding of the molecular biology of the JCV.

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