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Pathogenesis and Molecular Biology of Progressive Multifocal Leukoencephalopathy, the JC Virus-Induced Demyelinating Disease of the Human Brain

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HISTORICAL ASSOCIATION BETWEEN PML AND JCV

Progressive multifocal leukoencephalopathy (PML) is the only demyelinating disease in the human brain in which the basis for the neurological disorder is a well-characterized etiological agent, the human polyomavirus JC virus (JCV), which causes lytic infection of the myelin-producing oligodendrocyte. Although the simian polyomavirus simian virus 40 (SV40) has been implicated in several reports of PML (222, 223), these isolates have not been well characterized. Reexamination of some of these cases by in situ DNA hybridization has revealed JCV in the brain tissues (195).

Several major elements in this infectious neurologic disease are graphically presented in Fig. 1: (A) JC virions as they assemble in the nucleus of an infected cell in the brain; (B) glial cells, cultured from human brain, which are the targets of virus infection; (C) histopathological plaque lesion of white matter, stained for myelin, that results from that infection; (D) hematoxylin and eosin stain of cells in the lesion identified as macrophages (m) and astrocytes (a); and (E) plaques of demyelinated white matter which represent the neurological impairments characteristic of PML, shown here by magnetic resonance imaging (MRI).

The term PML was originally used in 1958 to describe extensive demyelination associated with chronic lymphocytic leukemia (CLL) and Hogdkin's lymphoma (14). However, accounts of similar pathology in patients with dementia were detailed as early as 1930 by Hallervorden (85). A

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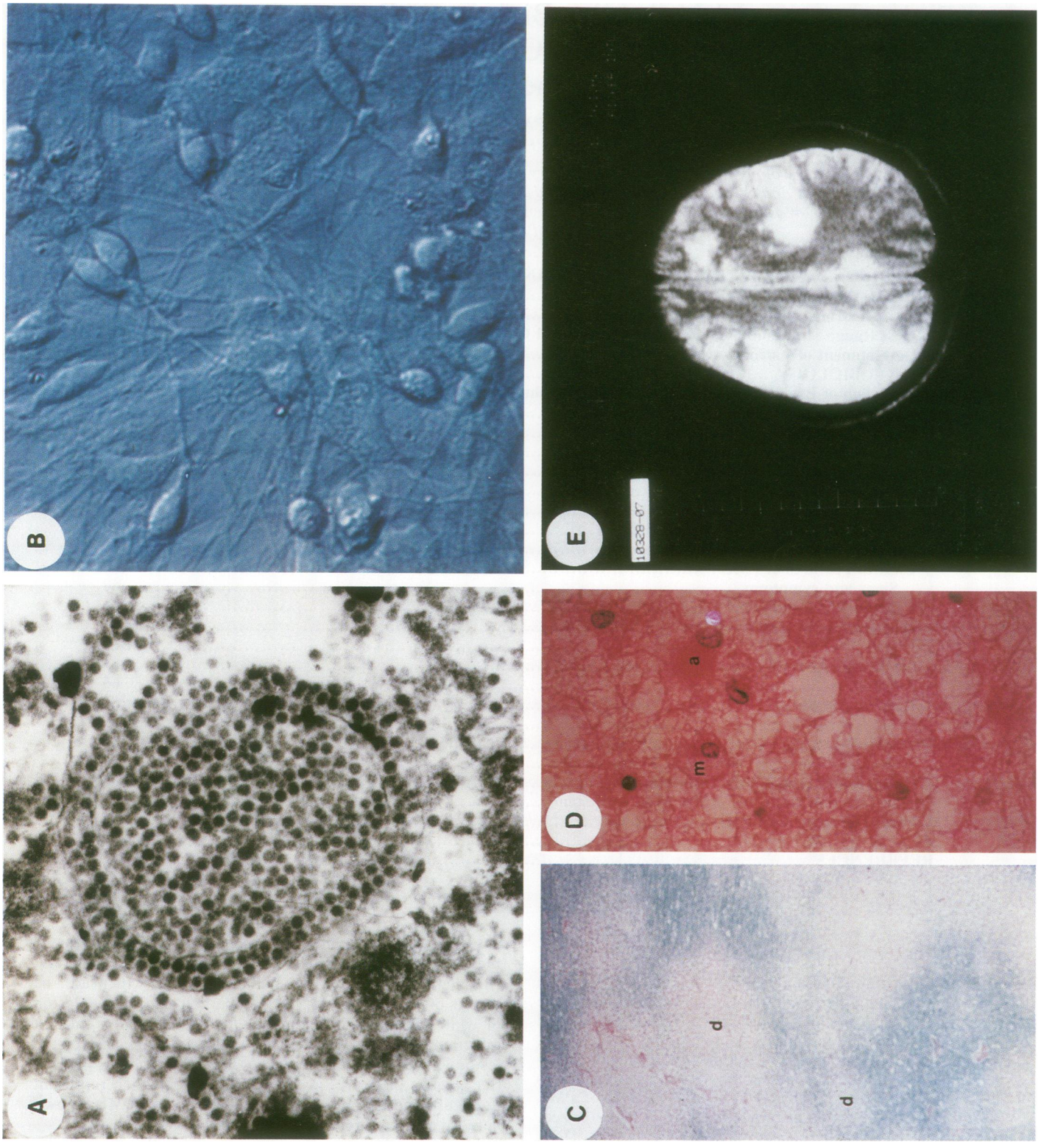

FIG. 1. Representation of the major elements in the pathogenesis of PML. (A) Electron micrograph of brain tissue from a PML patient showing the assembly of JC virion particles in the nucleus of an infected oligodendrocyte. (B) Human fetal neuroglial cells in culture, demonstrating the morphologically heterogeneous population of cells. (C) Luxol Fast Blue stain of the white matter with demyelinated plaque lesions in a patient with PML. (D) Hematoxylin stain of lesion showing the presence of a macrophage (m) and an astrocyte (a). (E) MRI of PML-affected brain with lesions of the subcortical white matter.

historical review by Richardson in 1961 (172) uncovered other early descriptions of possible PML (15, 38, 228). These clinical histories and descriptions of findings at autopsy were consistent with the development of a multifocal distribution of small or confluent white matter plaques in the cerebral hemispheres, basal ganglia and thalamus, cerebellum, and brain stem. The cause of these lesions was not known. The suggestion that a virus might be involved in the pathogenesis of PML came in 1959, when inclusion bodies were seen in the nuclei of damaged oligodendrocytes (34). Several years later, Richardson reported an additional 10 cases of PML in elderly patients and others with underlying diseases such as lymphomas, leukemias, sarcoidosis, and cancer (172). He noted that the distribution of the plaque lesions was consistent with an atypical virus infection. As the pathology of PML became more widely recognized, electron microscopic evidence revealed particles resembling polyomavirus present in the enlarged nuclei of oligodendrocytes with inclusion bodies (234, 235, 237).

Attempts to isolate the suspect virus were not successful until 1971, when brain tissue from a patient with PML was used as a source inoculum in cell cultures derived from human fetal brain. Padgett and her colleagues (161) isolated a human polyomavirus from long-term cultures made up mostly of glial cells. The successful isolation of virus using human fetal brain tissue in primary cultures was the first direct indication that a new "neurotropic" viral agent was associated with the occurrence of PML. The viral architecture (nonenveloped icosahedron) and size, diameter of 40 nm, were similar to those of another virus isolated from a renal transplant patient that was reported at the same time by Gardner et al. (69). Both viruses were named with the initials of the donor patient, i.e., JCV for the patient whose brain was infected with virus and BK virus (BKV) for the renal transplant patient whose urine contained the isolate. Both viruses were also associated with immunosuppressive conditions: most frequently, neoplastic diseases in PML patients with JCV and drug therapy to prevent graft rejection in renal allograft recipients with BKV infection (40). Although both viruses were eventually found in the urine, BKV has been detected far more frequently than JCV in the urine (10, 11, 20, 113) but has never been found in the brain of PML patients.

Unlike the simian polyomavirus SV40, these viruses share the property of hemagglutination of human type O erythrocytes (69, 129, 157). This property has allowed a seroepidemiological survey for viral antibody in patients suspected of infection and in the population as a whole. The seroepidemiological data generated in the mid-1970s using hemagglutination inhibition assays indicated that JCV and BKV were different viruses (67, 68, 156, 157), were distributed worldwide (29), and were present in most of the population, with serum conversion occurring at an early age (178, 217). Immunoglobulin G (IgG) antibody directed against JCV was most often demonstrated and was present in the healthy population, including pregnant women (9, 47). Most PML patients were found to have preexisting antibody in JCV and did not show a rise in antibody titer during the progression of PML. The lack of IgM antibody in the serum and cerebral spinal fluid (CSF) of many seropositive patients suggested reactivation of a latent infection (28, 158, 217). The epidemiological data along with the characteristic of an underlying immunosuppression for the emergence of PML pointed to the ability of JCV and BKV to establish latent infections. This inferred pathogenesis still holds true 20 years after the isolation of these viruses. The fact that JCV was unique among other members of the genus *Polyomavirus* was soon established by its biological, molecular, and genetic characteristics.

BIOLOGY OF JCV

Human Brain Cultures and Virus Growth

The isolation and growth of JCV in human fetal brain cultures in 1971 (161) allowed additional virus isolations from PML-affected tissue. Over the next several years new JCV isolates (159) helped link the etiology of PML with this new species of human polyomavirus (224). Because these first isolations were reported from the University of Wisconsin in Madison, the isolates were serially designated Mad-1 (the prototype), Mad-2, etc. However, the necessity of using human brain cells for successful isolation and growth immediately raised questions about the host range of JCV and began the identification and description of JCV as a new human neurotropic agent. Since this label has been applied to JCV, it has been difficult to alter the concept that JCV multiplies only in human brain. The seroepidemiology of JCV, however, suggested that infection with JCV is common and perhaps recurrent in individuals worldwide. A locus of initial infection in cells more accessible than those of the central nervous system (CNS) appeared likely.

Consequently, the paradox arose of how JCV could be such a prevalent virus in the population and yet appear to infect only a highly specialized neuroglial cell, the myelin-producing oligodendrocyte, and cause a rather poorly recognized demyelinating disease in a select population of patients with underlying immunosuppression. This paradox began to be resolved with the testing of other cell types for susceptibility to JCV.

The initial description of the growth of JCV in primary human fetal brain cultures identified the spongioblast, a bipolar process-bearing cell that was considered the precursor of the oligodendrocyte, as the most susceptible cell (156, 188). The other cell type in these cultures was the astrocyte, which makes a bed layer of strongly adherent cells on which the oligodendrocyte precursors grow (56, 101). These two kinds of cells are the glial cells that constitute the white matter. Multiplication of JCV in this heterogeneous population of cells required many days to weeks before substantial amounts of virus could be harvested, and only subtle cytopathic effects were evident (124, 156). Quantitation of virus was done by using the virion's ability to agglutinate human type O erythrocytes. The data describing the amount of JCV were given in hemagglutination units, usually expressed in logarithmic notation. JCV is an extremely cell-associated virus even after final virion assembly in the nucleus. Consequently, no suitable plaque assay has ever been developed. Very little virus is shed into the cell culture medium. Harvesting of JCV from infected cell cultures, therefore, requires multiple steps of physical disruption such as freeze-thaw as well as chemical treatment with sodium deoxycholate (155). Hemagglutination still remains the most reliable method of quantitating JCV, and hemagglutination inhibition is the only assay used to test for the presence of antibody.

Host Range Studies of JCV in Non-Brain-Derived Cells

By using laboratory measurements of JCV growth such as hemagglutination activity, other cells from human and other species were tested for JCV susceptibility. At first, human embryonic kidney, lung, amnion, liver, and intestine were

reported as negative for JCV Mad-1 growth, as were monkey kidney cell lines such as BSC-1, CV-1, and Vero cells (156). After weeks of culture and subculture, only a few T-antigen-positive cells were found in human kidney tissue. To determine whether host cell restriction was at the level of virion adsorption and penetration, JCV DNA was used to initiate infection by transfection. Four JCV isolates and their DNAs were tested on human embryonic kidney and lung cells with human fetal glial cells as the positive control (65). Only DNA derived from the Mad-4 strain demonstrated some ability to produce the viral early T protein and late capsid protein. DNAs from the other isolates were infectious for glial cells but no others. Consequently, the restricted growth of JCV in other cell types appeared to be dependent not on receptor recognition (adsorption) but on intracellular factors controlling early transcription and replication. A number of other studies documented JCV T-protein production in human vascular endothelial cells (58), human amnion (96, 202), and urine-derived epithelial cells, which also produced small quantities of virus and could be used to isolate JCV excreted in the urine (18).

The limited success of viral synthesis in human embryonic kidney cells (145) was consistent with the detection of JCV in the urine of PML-affected and renal transplant patients and of pregnant women (216). Inoculation of prototype strain Mad-1 in human embryonic kidney cells with subsequent serial passage resulted in an adapted JCV, which was termed JCV-HEK (145). The ability of this adapted virus to grow well in kidney cells resulted from substantial genome deletions and rearrangements in the noncoding sequences of the viral regulatory region (143, 232). This strain appeared as a laboratory artifact of virus subculturing since JCV with a similar sequence in PML tissue has never been identified. This experience provided additional evidence for the role of noncoding regulatory sequences as controlling elements in determining host range. As will be discussed in more detail below, the JCV genome sequences of virus isolated from kidney tissue substantially differ from those of virus isolated from brain tissue (51, 117, 231). These experiments, however, emphasized the fact that JCV grew best in cultures of human glial cells usually derived from fetal brain and, less frequently, adult brain (4, 230).

Development of Astrocyte Cell Lines

Human fetal glial cells are still the most sensitive and most susceptible host for the study of JCV in laboratory cultures. The availability of the primary tissue and the time required for adequate growth and use of these cells severely limited many experiments. To help alleviate this problem, a human fetal glial cell line (SVG) was produced by immortalization of astrocyte cells with a replication-defective SV40 DNA clone that expressed high levels of the SV40 T protein (124). This cell line proved to be susceptible to JCV infection. A similar method was used to produce a human kidney cell line (SV1). The SV1 cells, however, were not susceptible to JCV infection (123). Attempts to immortalize the oligodendrocyte precursor cell, which does not divide rapidly in culture, were not successful. Shortly after the description of the SVG cells, another susceptible human fetal glial cell line (POJ) was produced by using replication-defective JCV DNA expressing the JCV T protein (131). The establishment of the SVG cells as astrocytes, not derived from the "spongioblast" precursor cell, and their continued susceptibility to JCV led to a closer examination of the cell types in human fetal brain cell cultures (56) and their relative susceptibilities to JCV infection.

Both the astrocyte and the precursor cell of the oligodendrocyte allowed JCV growth at passages well beyond primary cultures (127). Because of their increased mitotic activity, astrocytes could produce more JC virions than the previously described spongioblasts and showed no evidence of genome alterations. The identification of JCV-infected astrocytes from PML-affected brain tissue placed in culture confirmed the susceptibility of this glial cell type to JCV (140, 180). Some of the cells in human fetal brain cultures that were described as spongioblasts (156, 215) have been recently identified as neurofilament-containing neurons (194). Their short lives and senescence in JCV-inoculated cultures could be the result of neuronal cell death caused by the lack of stimulation from nerve growth factor (194) rather than the result of infection by virus. These process-bearing cells in human fetal brain cultures do not become infected with JCV, which is consistent with the fact that JCV-infected neurons have never been found (78). Recent tissue culture evidence directly testing neuron susceptibility to JCV came from human fetal dorsal-root ganglion cultures in which only the Schwann cells, the myelin-producing cells of the peripheral nervous system, would support JCV multiplication (13). Neurons did not express any viral protein upon either infection or transfection. The current cell types that are considered susceptible to productive JCV infection are given in Table 1. Also indicated in Table 1 are human tissues or sites in which JC virus or its DNA has been identified or isolated. Further description of these observations is found below in the discussion of the pathogenesis of PML.

TUMOR BIOLOGY

Induction of Brain Tumors in Hamsters and Nonhuman Primates

While studies were being done to determine the lytic host range of JCV, experiments were also conducted to examine the oncogenic potential of this human polyomavirus. JCV was classified in the family *Papovaviridae* because of its physical and genetic similarities to SV40 and the murine papovavirus, polyoma, named for the multiple tumors induced in rodents. For this reason and also because many patients with PML had systemic tumors (lymphatic, not CNS), the ability of JCV to induce tumors was tested shortly after its isolation. Hamsters were chosen as the experimental host for the first studies because of their sensitivity to tumor induction by SV40 and BKV (207).

More than 80% of juvenile hamsters inoculated intracerebrally and subcutaneously with the Mad-1 strain of JCV developed glioblastomas, medulloblastomas, and other unclassified primitive tumors (218, 236, 238). T protein was demonstrated in tumor cells explanted in tissue culture. When cocultivated with permissive human glial cells, the hamster tumor cells released infectious virus, indicating the presence of an entire biologically active JCV genome (218). The Mad-2 and Mad-4 strains produced a high incidence of tumor development, with Mad-4 virus predominantly causing pineal gland tumors (160). Intraocular inoculation of virus in hamsters resulted in abdominal neuroblastomas that metastasized to the liver, bone marrow, and lymph nodes (213). Neuroblastomas and medulloblastomas were also found in animals inoculated subcutaneously and intraperitoneally (238). Hamster brain cells in culture could also be transformed by JCV infection or transfection of JCV DNA

TABLE 1. Identification of human cells or tissues susceptible to JCV

Cell or tissue type	Viral product detected[a]	Result	Reference(s)
Human fetal glial			
Oligodendrocyte-precursor	Virions	Lytic	161
Astrocyte	Virions	Lytic	127, 156
Schwann	Virions	Lytic	13
Astrocyte lines			
SVG	Virions	Lytic	124
POJ	Virions	Lytic	131
POS	Virions	Lytic	121
Human embryonic kidney	Few virions	Poorly lytic	65, 145
Human uroepithelium	Few virions	Poorly lytic	18
Human B lymphocytes			
B-JAB	Few virions	Poorly lytic	122
Namalwa	Few virions	Poorly lytic	122
Brain			
Oligodendrocyte	Virus	Lytic	237
Astrocyte	Virus	Lytic	140
Bizarre astrocyte	DNA[b]	Unknown	5
B lymphocyte	DNA[b]	Unknown	122
Kidney (urine)			
Transitional epithelium (presumptive)	Virus DNA[c]	Presumed latent	35, 147
Bone marrow			
B lymphocyte	DNA[b]	Presumed latent	93
Spleen			
B lymphocyte	DNA[b]	Presumed latent	93
Blood			
Peripheral blood lymphocytes	DNA[c]	Unknown	208, 220
Liver			
Not identified	DNA[d]	Unknown	81
Lymph node			
Not identified	DNA[d]	Unknown	81
Lung			
Not identified	DNA[d]	Unknown	81

[a] Virions, readily detectable amounts of infectious virus are produced in a permissive infection; few virions, barely detectable amounts of virus are produced.
[b] Viral DNA detected by in situ DNA-DNA hybridization, using a biotin-labeled probe.
[c] Viral DNA detected by PCR.
[d] Viral DNA detected by blot hybridization, using a radiolabeled probe.

(66). These transformed hamster cells demonstrated an integrated JCV genome (130) that expressed the viral T protein that could bind the cellular p53 protein. The JCV T protein in transformed hamster cells could also bind another cellular protein associated with oncogenicity, the retinoblastoma protein (54). Another strain of JCV isolated in Tokyo produced cerebellar medulloblastomas in hamsters (149). Explants of these tumor cells expressed the T protein but could not be carried in culture before losing the viral genome. The cells were positive, however, for the intermediate-filament glial fibrillary acid protein, a marker for astrocytes. This Tokyo strain also produced undifferentiated neuroectodermal tumors in the cerebra of rats (153). These tumors also were classified as astrocytomas by the presence of glial fibrillary acid protein.

Although JCV shares oncogenic properties with other polyomaviruses such as SV40 and BKV, it is the only member of this family of viruses that has been shown to induce tumors in nonhuman primates. In an attempt to produce PML in monkeys, JCV was inoculated intracerebrally, subcutaneously, and intraperitoneally into owl and squirrel monkeys (118, 119). These animals were initially screened for antibodies against JCV, BKV, and SV40 and then treated with prednisone to induce an immunosuppressive state. Sixteen months after the inoculation, one owl monkey developed a malignant cerebral tumor similar to an astrocytoma seen in humans. Another owl monkey developed a malignant neuroblastoma 25 months after inoculation. The JCV T protein was identified in these tumor cells but not the V or capsid protein. Animals inoculated with

either SV40 or BKV never developed tumors but did demonstrate antibodies to these viruses (219). Most of the tumors that resulted from JCV inoculation were described as glioblastoma multiforme grade IV or astrocytomas occurring 18 to 32 months after inoculation (141). Figure 2 shows the appearance of such a tumor on necropsy of an inoculated owl monkey 22 months after virus inoculation. The JCV genome was randomly integrated in the tumor tissue, frequently as multiple tandem copies, but was not found in nontumor tissue in the brain (142). One novel transplantable owl monkey astrocytoma (Owl 586) that spontaneously released infectious JCV in cell culture was found (128). The cell cultures of Owl 586 produced a large amount of JCV T protein compared with other owl tumor cells analyzed by immunoprecipitation (125). The Owl 586 T protein was also able to complex with the primate cellular p53 protein. The complex could not be demonstrated in other monkey tumors or cultured cells from them. Several mouse monoclonal antibodies made to the SV40 T protein were found to cross-react with the JCV T protein in monkey or hamster tumors in those regions of homology near the amino terminus of these proteins (125, 128, 196). Even though JCV induces glioblastomas in nonhuman primates and gliomas have been described in PML patients (32, 190), there is no evidence as yet for an association between JCV and human tumors of the nervous system (53).

JCV in Transgenic Mice

Another approach to developing an animal model for the acute demyelination seen in PML-affected brain tissue was the use of transgenic mice. The regulatory and coding sequences for the JCV early T protein were injected into one-cell mouse embryos to generate founder mice (191, 192). Two male founder mice that contained the JCV early region survived to maturity to produce offspring. Approximately 20 to 50% of these offspring contained an intact JCV early region. Some of the offspring demonstrated a mild to severe tremor, which was evident when the mice were in motion. A similar phenotype had previously been observed in myelin-deficient strains of the quaking and jimpy mice (89, 189). In the offspring inheriting the intact JCV early region and exhibiting the tremor, a dysmyelination had occurred in the CNS but not in the peripheral nervous system. High levels of the JCV T-protein mRNA were found in the brains of these myelin-deficient mice. Transgenic mice with the early region of BKV, on the other hand, developed liver and kidney tumors (192). BKV T protein was expressed at low levels in brain, heart, and lung tissues but at high levels in muscle and liver tumors.

To further characterize the dysmyelination in the JCV transgenic mice, Trapp et al. (209) examined the expression of the JCV and myelin-specific genes. An initial study of the brain of JCV transgenic mice revealed an absence of myelin sheaths, although the numbers and diameters of axons did not appear to be reduced. In addition, the levels of myelin basic protein (MBP), proteolipid protein (PLP), and myelin-associated glycoprotein were reduced in the brains of the transgenic mice; however, the expression of *PLP* and *MBP* genes appeared to be normal. Since the JCV T-protein gene appeared to be expressed in the same region of the brain as the *MBP* gene, it was suggested that expression of the JCV T protein altered the levels of proteolipid and myelin basic protein syntheses and the maturation of oligodendrocytes. The mechanism of dysmyelination remains unclear, but there is evidence for the appearance of JCV T protein in PML-affected brain tissue in which clear demyelination (lytic infection of oligodendrocytes) has not yet taken place, suggesting a role for the viral protein in the destruction of myelin (91).

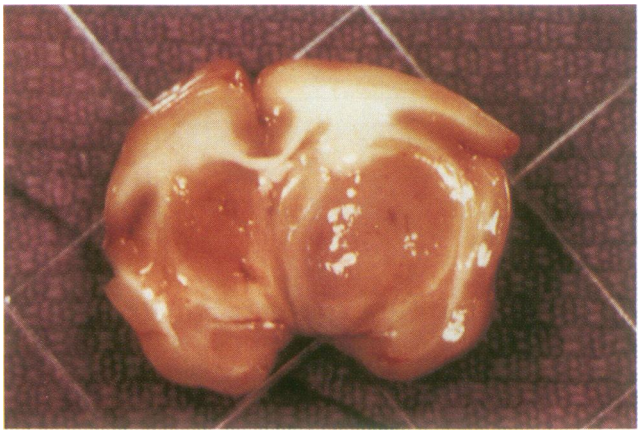

FIG. 2. Owl monkey brain at time of necropsy 22 months after intracranial inoculation with JCV Mad-4. Tumor mass has been identified as an astrocytoma.

MOLECULAR REGULATION

Analysis of the Viral Genome

As more evidence of the neuroglial tropism of JCV was derived from the study of its biological and oncogenic properties, attempts to understand the molecular mechanism behind these observations focused on the structure and regulation of the viral genome. Similar to other polyomaviruses, the JCV genome contains a closed, circular, supercoiled DNA molecule (155). Orientation at its single *Eco*RI restriction endonuclease cleavage site and alignment with SV40 and BKV DNAs following additional digestions revealed that the JCV DNA was unique (112, 134, 135, 155, 170). The first region of the genome to be sequenced was the putative origin of DNA replication, which was compared with that of SV40 (63, 144). Subsequently, the entire sequence of the Mad-1 genome was determined, thus allowing extensive comparisons with SV40 and BKV for function and regulation (64). Analysis of the nucleotide sequence divides the JCV genome into three functional regions: (i) the early region on the proximal side of the replication origin, (ii) the late region located on the distal side of the replication origin, and (iii) the noncoding or regulatory region between the early and late regions (Fig. 3) that contains the origin, promoter, and enhancer signals topographically similar in function to SV40 regulatory sequences (82).

The early-region sequences are transcribed counterclockwise from the *Eco*RI site and encode two proteins, the large T and small t antigens. By analogy with SV40, the large T protein is a nonstructural, multifunctional phosphoprotein that regulates transcription of the early-gene sequences and consequently is autoregulatory. The T protein also defines the switch from early to late transcription (see reference 49 for an extensive review of papovavirus T protein). Besides its effect on transcription, the T protein is required for the initiation of DNA replication and the induction of cellular malignant transformation. Little is known of the function of the small t protein except in SV40 transformation in which it

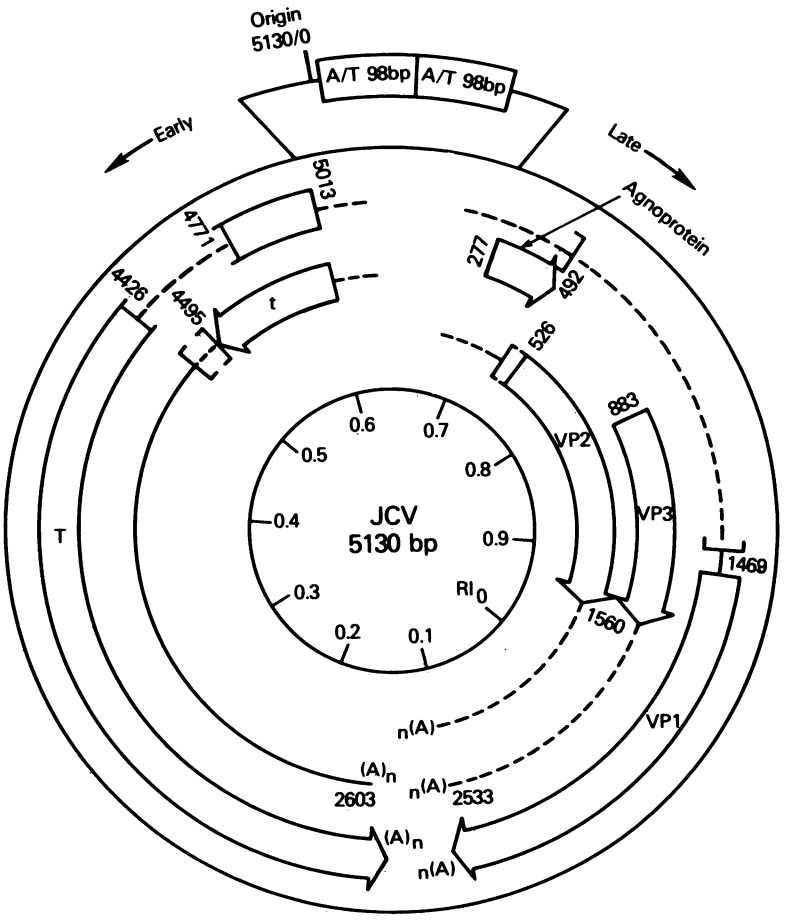

FIG. 3. Schematic diagram of the JCV genome. The circular map shows the beginning and end of the putative open reading frames (64). Two open reading frames (labeled T and t) are shown in the early region on the left side, and four open reading frames labeled agnoprotein, VP1, VP2, and VP3 are shown in the late region on the right side. The relative positions of the origin of DNA replication and the 98-bp repeat units in the noncoding region are indicated at the top.

appears to facilitate the transformation event (146), especially when the concentration of the T protein is limited (22). The small t protein of JCV has been identified in transformed hamster cells (66) but not in lytically infected human glial cells (126) or in monkey tumor cells (125, 128). The open reading frames for both proteins appears to begin at nucleotide 5013, but a differential splicing event generates two mRNA species for the T and t proteins (64). A translation termination signal for the small t protein is found at nucleotide 4495, while the termination signal for the T protein is at nucleotide 2603. Therefore, the T and t proteins share the same 5′ ends but each contains unique 3′ ends. These early open reading frames encode a 688-amino-acid T protein and a 172-amino-acid t protein. The size of the JCV large T protein as determined by migration in sodium dodecyl sulfate-polyacrylamide gels ranged from 83 to 96 kDa (23, 84, 126). These analyses also demonstrated degradation products of the T protein, suggesting its relative instability compared with that of the SV40 T protein.

The late-region sequences are transcribed clockwise from the opposite strand relative to the early genes and, by analogy with SV40, appear to encode four proteins (64). At the 5′ end of the late region is the smallest open reading frame which could encode a polypeptide of 71 amino acids. In SV40, the open reading frame in the leader region encodes a 7.9-kDa protein called the agnoprotein since its function is not known (26). This agnoprotein can bind DNA (98) and may be involved in the viral maturation pathway (94). The largest open reading frame is located near the 3′ end of the late region and encodes the 354-amino-acid VP1 capsid protein, which is the major structural protein functioning in cell adsorption. Between the 5′ and 3′ open reading frames of the agnoprotein and VP1 proteins are two other open reading frames that encode the VP2 and VP3 capsid proteins (344 and 225 amino acids, respectively). The smaller of these two open reading frames, VP3, is a subset of the larger VP2. The number and sizes of the late proteins of JCV have not been analyzed as well as those of the T protein. However, antisera to the capsid proteins have been used to study late gene expression (65, 66, 145, 149, 198). Using immunocytochemistry, for example, Stoner et al. (197, 198) found only the T protein in brain tissues in which the capsid proteins could not be detected. The lack of capsid proteins in these tissues was interpreted as an indication of an abortive or latent infection.

The regulatory region comprises noncoding sequences that lie between the early and late genes. It contains the origin of DNA replication and the *cis*-acting signals for both early and late transcription (63, 64, 144). A comparison of the nucleotide sequence encompassing the JCV replication

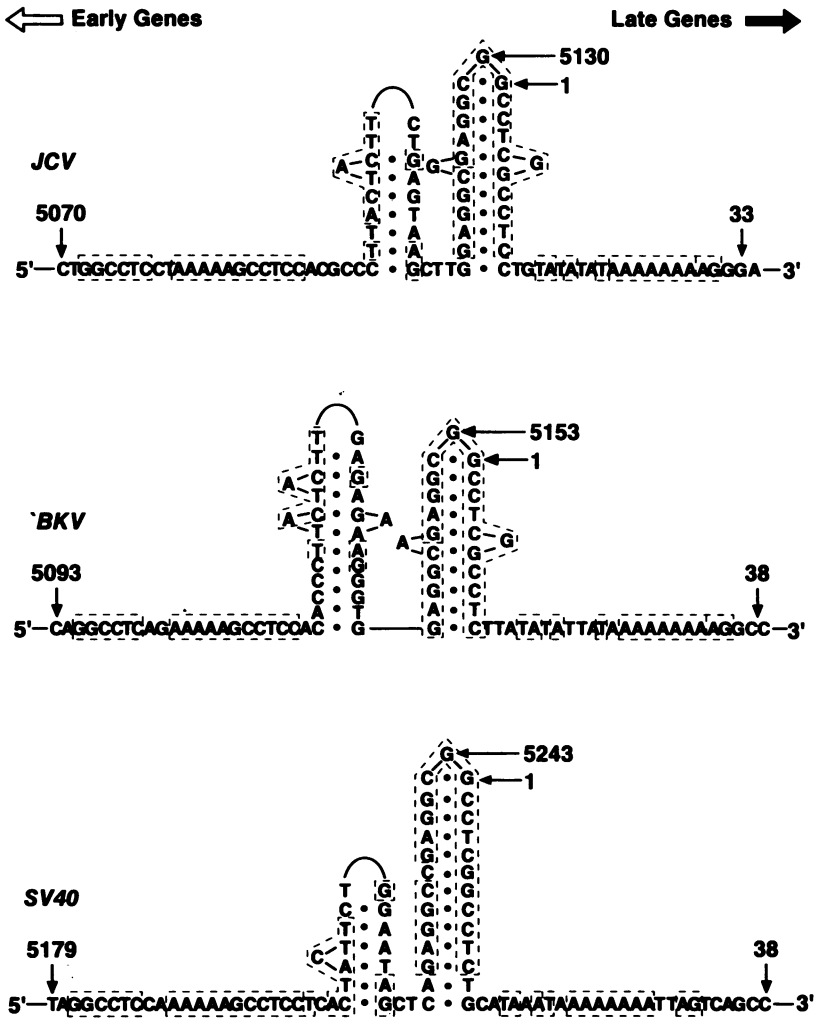

FIG. 4. Similarities between the nucleotide sequences encompassing the origin of DNA replication (49). Shown are the primary nucleotide sequence and potential secondary structure in the region containing the origin of DNA replication from JCV, BKV, and SV40. Nucleotides that are homologous among the three primate polyomaviruses in this region when the nucleotide sequences are aligned are enclosed in open boxes of dashed lines. The secondary structure illustrates the similarity of this region in the three viruses and is not necessarily present in vivo.

origin with those of SV40 and BKV revealed extensive areas of homology (Fig. 4). The presence of two regions of dyad symmetry and a 17-bp palindrome shared by all three viruses reflects the similarities of the organization within this structure. In one region of dyad symmetry, 22 or 23 nucleotides are identical in all three polyomaviruses. In SV40, this latter region contains one of the large-T-protein binding sites (site I), while the second large-T-protein binding site (site II) overlaps the 17-bp palindrome (148). A third large-T-protein binding site present in SV40, however, does not appear to be present in JCV. Binding studies with a hybrid adenovirus-SV40 large T protein demonstrated that it binds to a region of the JCV genome that contains the origin of DNA replication (63).

Besides the origin of DNA replication, there are two 98-bp tandem repeats located on the late side of the noncoding sequences (Fig. 5). Although the organization of the nucleotides in this region of JCV resembles that in SV40 and BKV, there is little sequence homology within the repeat units. The regulatory sequence of the Mad-1 strain of JCV differs from other regulatory sequences in that it has its TATA Goldberg-Hogness box as part of the repeat units. This box is the sequence required for positioning the start site of mRNA synthesis in many genes (73, 75). Each TATA sequence is located on the early side of the individual 98-bp tandem repeats. However, the second TATA box distal to the DNA origin is deleted in many naturally occurring JCV isolates and does not appear to be necessary for virus multiplication (120, 136) or to contribute to the DNA tertiary structure (8). The *cis*-acting nucleotide sequences within these 98-bp repeats have been the focus of much experimental attention as crucial elements in explaining the tropism of JCV.

Alterations in the Viral Genome

Since the first isolation of JCV from brain tissue, the genome has appeared to be heterogeneous in size (65, 79, 95, 135, 145, 155). This heterogeneity could depend on certain factors, including (i) in vitro propagation conditions, (ii)

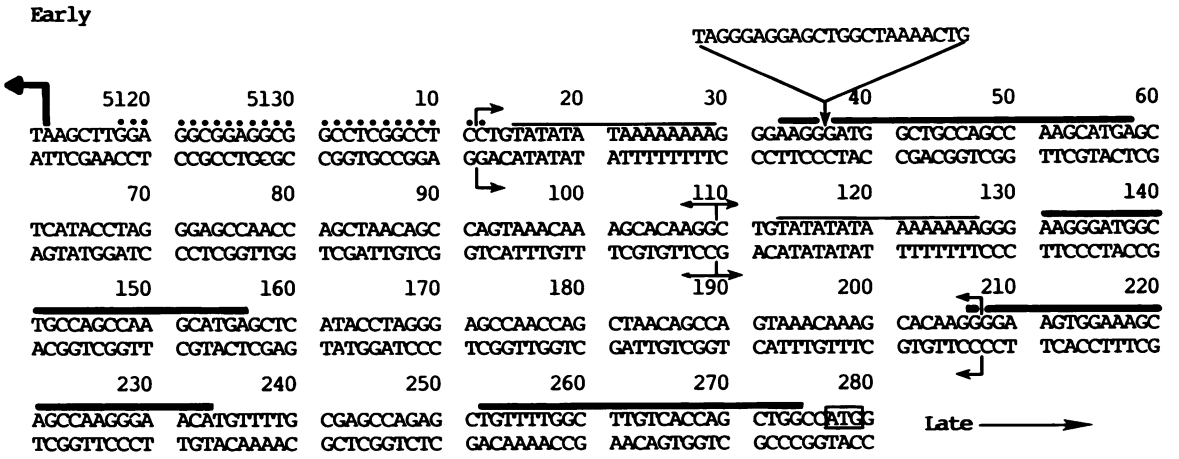

FIG. 5. Nucleotide sequence in the noncoding region of the JCV genome (Mad-1 strain) (64). The brackets with arrows enclose the two 98-bp repeat units, and the thin straight line indicates the putative TATA region. The nucleotide sequence and position of a 23-bp insertion found in variants of JCV (132) are shown above the first 98-bp repeat unit. Nucleotides protected from DNase I digestion by the nuclear protein NF-1 are indicated by the heavy straight line. The heavy arrow at the beginning of the nucleotide sequences signifies a major start site for early-mRNA synthesis (106). Dots over the nucleotides denote the locations of the origins of DNA replication (64, 120). The ATG boxed at the end of the nucleotide sequence is the putative translational start site for the agnoprotein.

source of the JCV genome and the derivation of a molecular clone (57, 79, 80, 138, 170), and (iii) the natural genetic variation of the isolate (137). Alterations in the JCV genome were seen in JCV strains that have been cloned directly from tissue specimens (117, 138) and have not been previously propagated in tissue culture. Although the genomes of these different isolates were not identical, each appeared to be homogeneous. This lends support to the possibility of a natural genetic variation in strains of JCV that could appear in vivo as well as in vitro.

Genomes of JCV found in the urine of non-PML-affected elderly individuals, with no specific signs of immunodeficiency, demonstrated a genotype that contained a 23-bp insertion (see Fig. 5 for detail) that had been previously described in some JCV isolates from PML-affected brain tissue (137). This 23-bp insertion is not found in the Mad-1 strain. A 66-bp insertion between base pairs 80 and 98 of the tandem repeat (231) was also found in these JCV genomes. The sequence arrangement that included both the 23- and 66-bp insertions, in comparison with the Mad-1 strain, was suggested as an "archetypal" genome from which other JCV genomes found either in kidney or brain, for example, may have been derived by alterations and deletions. In fact, the genome of JCV from the kidney of a PML-affected patient contained a sequence arrangement similar to the archetype (117). Polymerase chain reaction (PCR) amplification of JCV regulatory sequences recovered from the urine of AIDS patients and bone marrow transplant recipients also demonstrated the archetypal sequence (61). However, sequences of urine-derived JCV isolates from other bone marrow and renal transplant patients showed no differences from those of the Mad-1 strain found in the brain (147).

Most changes in the genome have been located in these regulatory sequences, with the most notable being the deletion of 19 bp in the second tandem repeat. This deletion eliminates the duplicated TATA box and was first described in the Mad-4 strain (136). A similar genotype was found in several other brain isolates (Her-1, Mad-7, Mad-8, Mad-9, and Mad-11 [136], a strain from Tokyo [138]) and the virus released from the owl monkey astrocytoma (128). In addition to this 19-bp deletion, there is a 23-bp insertion of purine-rich sequences (Fig. 5) between nucleotides 36 and 37 of the Mad-1 strain in the first 98-bp tandem unit (5′-TAGGGAG GAGCTGGCTAAAACTG-3′). These sequences may represent a functional counterpart (136) to the 21-bp repeats in SV40 that are binding sites for transcription factor Sp1 (25). The Tokyo strain also has a 20-bp insertion between the first and second repeat units that is almost identical to a sequence found at the 5′ end of the agnoprotein region.

The functional significance of these alterations in the pathogenesis of PML is still unclear. However, the sequences of the JCV isolates from the kidney and brain of one PML patient (117) raised the following interesting point. The coding sequences of the two isolates appeared identical, which suggested that these viruses arose from a single strain. The noncoding sequences, however, demonstrated extensive and varied alterations. In the regulatory region of the brain isolate, the tandem 98-bp repeats found in the Mad-1 strain have been truncated at the 3′ end of the repeats, although they are still tandemly arranged (two repeats of 63 bp each). Between these two sets of repeating units there was a 12-bp insert almost identical to an insertion found in the 3′ end of the second 98-bp repeat unit of the Mad-8a isolate (136). The first of the 63-bp repeats contained the 23-bp insertion found in the regulatory region of some JCV Mad isolates, while the second repeat had lost the TATA sequence and contained only 16 of 23 bp of the insertion found in the first repeat. The brain isolate also has a 93-bp tandem repeat unit closely following the first 63-bp tandem repeats which includes a duplication of 69 bp. The kidney isolate, on the other hand, shared some features with the isolate from the brain. It had only one copy of the Mad-1 98-bp repeat unit, with several insertions that resembled the archetypal sequence (231). The kidney isolate also had short sequences inserted in its 98-bp repeat unit that resembled SV40 and adenovirus E1A core enhancer elements (64) and sequences from the JCV-HEK strain. There are a number of differences in the coding sequences between the kidney and brain isolates, but usually most of them do not alter the amino acid composition. In 10 isolates from brain tissue of PML patients, small variations were reported in the capsid protein sequences (80).

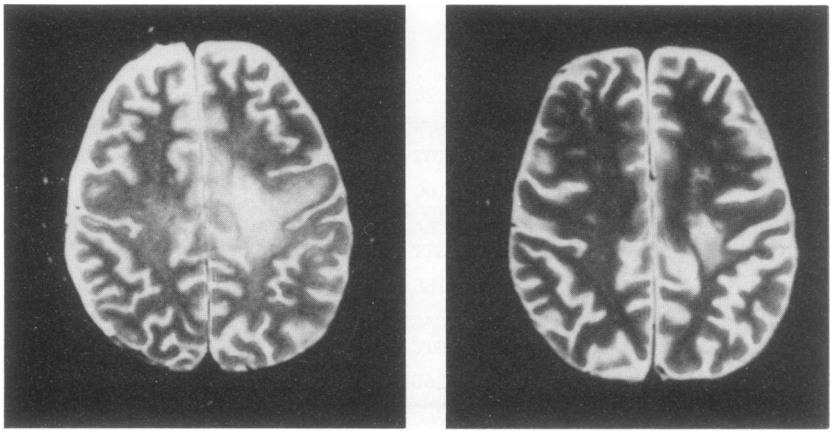

FIG. 6. (Left) T2-weighted MRI of the brain of an HIV-1-infected patient with PML showing bilateral white-matter lesion that are quite extensive on the left side. (Right) Repeat T2-weighted MRI obtained following 7 months of 9×10^6 to 12×10^6 U of alpha interferon given subcutaneously daily, showing clear resolution of the white-matter lesions. (Figure provided courtesy of Justin McArthur, Johns Hopkins Hospitals and School of Medicine.)

It should be noted that JCV DNA sequences are usually compared with the sequence derived from the first JCV isolated, Mad-1, which has been considered the prototype strain. New data on other strains or isolates indicate that the Mad-1 strain may be atypical. Also, it is still unclear whether viral replication in brain or kidney tissues, for example, results in selection of a strain trophic for each tissue or whether there is an archetypal strain that fosters the virus that finally enters the nervous system. Dynamic alterations of JCV DNA perhaps during multiplication in host tissues has added another degree of challenge in determining the critical factors controlling the glial cell tropism and the pathogenesis of JCV.

Expression of the Viral Genome

The physical description of the JCV genome helped researchers design experiments to determine the functional significance of the noncoding sequences. In studies directly testing the regulatory sequences for host range control, Kenney et al. (104) examined the ability of the 98-bp tandem repeats to act as a promoter-enhancer to stimulate synthesis of the chloramphenicol acetyltransferase (CAT) gene. Two important observations were made. First, the regulatory region was able to stimulate the expression of CAT when the region was cloned in an orientation with the first TATA sequence proximal to the CAT gene. Second, this stimulation appeared to be relatively cell specific, 20-fold higher in human fetal glial cells than in HeLa cells and 7-fold higher than in CV-1 cells. The promoter-enhancer sequences of SV40 and BKV showed significant activity in most cells tested (76, 177). In HEK cells, the JCV regulatory sequences produced only minimal levels of CAT, reflecting the very limited ability of JCV to propagate in kidney cells. When the orientation of the promoter-enhancer sequences was reversed, CAT activity was still highest in human fetal glial cells. The tissue specificity and low activity exhibited by the regulatory sequences were confirmed by others (60, 200).

In a series of studies set to determine which nucleotides influence the lytic and transforming properties of JCV in cell cultures, hybrid genomes of JCV and SV40 and of JCV and BKV were constructed (23, 39, 84). In one study (39) in which the regulatory region of JCV was replaced with that from either SV40 or BKV, the new hybrid constructs were not viable in human fetal glial cells. In the reverse hybrid constructs, however, the regulatory region of JCV drove synthesis of either the SV40 or BKV T proteins in human fetal glial cells. These results suggest that SV40 and BKV T proteins could positively interact with the JCV regulatory sequence but that JCV T protein, although shown to bind the SV40 DNA, could not functionally substitute for the homologous T protein. Along this line of experimentation, researchers constructed hybrid T-protein viruses that demonstrated (23, 84) that JCV T protein was also weak in its ability to induce transformation. From these data, it appeared that the JCV T protein played a significant role in determining efficient gene expression in a cell-type-specific manner.

In another study, JCV regulatory sequences were altered following transfection of a DNA construct that placed the SV40 21-bp triplicates and the 72-bp repeats at the distal end of the JCV 98-bp repeats that did not interrupt the coding sequences for the JCV T protein or the capsid proteins (211). A series of cell-induced deletions were made following transfection of the constructs into either human fetal glial or embryonic kidney cells. An infectious viable virus was isolated with a regulatory region that contained almost one complete 98-bp repeat from JCV and one and one-third SV40 72-bp repeats. The other JCV 98-bp and the SV40 21-bp triplicates were consistently deleted whenever transfections were started with the parent construct. This virus produced JCV T protein that was not altered except that its concentration was higher than that of prototype JCV T protein in human fetal glial cells. The host range of this chimeric virus was expanded to include lytic activity in human and simian kidney cells, but it remained restricted to primate cells for growth (211). These data suggested that the JCV regulatory sequences were responsible for the host range phenotype and the glial cell tropism. Although still a subject for experimentation, the regulatory region sequence for JCV does appear to be the predominant factor in controlling growth properties of JCV.

Nuclear DNA-binding proteins that interact with these sequences are being identified as important proteins for transcription. Nuclear proteins from human fetal glial cultures and HeLa cells, which serve as a control for binding

proteins that are probably not functional, could bind a set of oligonucleotides that span the JCV regulatory region located at the 5′ and 3′ ends of the 98-bp repeats (107). In addition, binding with an oligonucleotide homologous to the central portion of the 98-bp repeat was observed. Several sizes of protein were identified by using cross-linking gel assays. Two of the proteins that bound internal regions of the 98-tandem repeat were specific to human glial cells.

Four nucleotide domains for binding were found as a result of other experiments. Two domains located in the TATA region of the 98-bp repeat and one outside the second 98-bp repeat seemed to downregulate transcription in nonglial cells, perhaps serving as negative regulatory factors (200). A 45-kDa protein from glial cells that recognizes sequences in the central part of the 98-bp repeat was found to positively regulate transcription (1). In another reported set of experiments, a protein factor recognized four specific sites in the promoter-enhancer region that were identical to the recognition sites for nuclear factor 1 (NF-1) (6, 7). By examining the binding of NF-1-like factor by DNase I protection assays, one binding site in each of the 98-bp repeat units at nucleotides 33 to 58 and 131 to 156, one site at nucleotides 207 to 233 just outside the second 98-bp repeat, and another site further toward the late region were identified (Fig. 5). The latter NF-1-binding site differed from that within the 98-bp repeat, and it exhibited a weaker binding for the protein. In the Mad-4 strain in which the TATA region in the second 98-bp repeat is deleted, the binding of the NF-1-like factor to the adjacent nucleotide sequences did not appear to be affected. From the results of competitive binding assays, NF-1 appeared to be similar to the multifunctional NF-1 protein that is involved in the initiation of DNA replication and transcription in adenovirus 2 but not to the protein factor that interacts with the adenovirus major late promoter, the CCAAT transcription factor (37, 169). Tamura et al. (203) reported similar binding results with the regulatory region of JCV from mouse brain extracts.

The data implicate at least three or four proteins that participate in transcriptional control. One of these is an NF-1-like factor that may be different in brain than in other tissues, suggesting a family of such proteins that recognize partially homologous sequences in various genes (35, 37, 50, 169). Analysis of these experiments would indicate that there are both *cis*- and *trans*-acting elements that control viral gene transcription. Glial cell-specific proteins perhaps positively regulate transcription, whereas non-glial cell proteins act to downregulate transcription efficiency.

Since the Mad-1 strain has two tandem repeat units in its promoter-enhancer region, with a TATA sequence within each repeat, experiments were conducted to determine which site is used to initiate transcription. By S1 nuclease analysis, the 5′ termini of early JCV mRNAs harvested 5 days postinfection from human glial cells mapped within the second TATA box (103). In transformed hamster brain cells both the Mad-1 and Mad-4 early-mRNA start sites mapped to nucleotides 5115 to 5124, approximately 25 bp downstream from the first TATA box (130). Two other minor start sites closer to the TATA sequence and the one close to the second TATA sequence in Mad-1 were also identified. Similar mRNA start sites were described in a human fetal glial cell line, POJ, transformed by a replication-defective JCV (131). Primer extension assays revealed similar start sites in primary human fetal glial cultures (106). Three to five days after infection, two early-mRNA sites were detected downstream from the first TATA sequence at nucleotides 5122 and 5082. Ten days after infection, start sites were found at different locations, i.e., at nucleotides 35, 5012, 5037, and 5047. By this time in the viral growth cycle, DNA replication had begun. These series of studies indicate that the major early-mRNA start sites are downstream from the first TATA sequence and that the second TATA sequence plays a negligible role. In addition, once viral DNA replication begins, there is a shift in the start sites of the early mRNA as can be seen also in the transcription of SV40 (179).

The initiation sites for late JCV mRNAs were examined by Kenney et al. (102) in infected human glial cells. S1 nuclease analysis was done on mRNA extracted 17 to 19 days after infection. Four major and three minor sites that spanned the JCV regulatory region were identified. RNA synthesized in vitro with a HeLa cell extract mapped to the same start sites as found in vivo. However, no TATA sequences were seen within 25 nucleotides upstream from these start sites. Two sites, at nucleotides 90 to 98 and 198 to 203, were located approximately 30 nucleotides downstream from the sequence TACCTA. An identical sequence was located 25 to 30 nucleotides upstream from a major in vitro late transcription start site in SV40 (25, 150). Further analysis will have to be performed to determine whether the TACCTA or any other *cis*-acting sequence is involved in specifying the start sites for late transcription. The influence of the T protein on late transcription and the number and exact start sites for late mRNA remain to be determined.

Replication of the Viral Genome

When the nucleotide sequence of the JCV genome was established, the sequences for the origin of DNA replication were easily recognized by their homology to the sequences of other polyomaviruses (Fig. 4) (64). The sequences responsible for T-protein binding to the *ori* region resemble those of SV40 DNA. Early experiments demonstrated that the SV40 and JCV T proteins did bind to at least two of the three sites with the sequence GAGGC (63). A third site present in SV40 may not be required for JCV replication. Unlike the efficient and rapid multiplication of SV40, JCV DNA replication could not be detected until 3 to 5 days following infection, after which time it continued for several weeks (60, 106). Even in many human glial cells in which the JCV T protein is present, replication does not begin or cannot be detected for several more days (124). The necessity of a functional JCV T protein was demonstrated by Mandl et al. (131) by showing that JCV could not replicate when there was a mutation in the coding sequences for T protein. It was also shown that the SV40 and BKV T proteins could functionally interact with the JCV *ori* sequences and result in a lytic infection (39).

The cell type restriction for JCV growth had already been documented at the level of early transcription (60, 104). However, there was yet another level of restriction for replication (60). JCV DNA could replicate in immortalized primate cell lines that contained an SV40 T protein such as SVG (human glial), SV1 (human embryonic kidney), and COS-1 (monkey kidney) but not in HJC (hamster brain cells with expressing JCV T protein), CV-1 (monkey kidney), or HeLa. In these experiments, JCV replication occurred in HeLa and CV-1 cells only when the SV40 T protein was present. In the hamster cells, replication could not take place even in the presence of T protein. Conclusions drawn from these series of experiments were that primate cells contain necessary factors for replication when the T protein is present but that rodent cells do not. Therefore, it appeared

that transcription of the JCV genome is restricted by cell-specific factors functioning best in glial cells and that replication is restricted by species-specific factors functioning best in primate cells (60).

Recently, Lynch and Frisque (120, 121) determined that sequences to the late side (nucleotides 38 to 30 containing the repeat sequence AGGGA) of the second T-protein-binding site on JCV DNA had a strong influence on the efficiency of JCV T-protein-dependent replication activity. This region in the regulatory sequences has been shown to adopt a non-B conformational tertiary structure (8) and to contain a binding site for NF-1 (6). Mutations introduced into this region by deletion or conversion greatly reduced the replication function of the JCV T protein. Its proximity to the A+T sequences in the exact *ori* site for initiation of replication suggested that the JCV T protein may require a particular DNA conformational structure to function effectively. In other experiments testing JCV DNA replication in a variety of cell lines, JCV T protein had a lower specific DNA binding activity than SV40 T protein and was generally much less efficient (111). This inefficiency in driving replication may be due to weak interactions between the JCV T protein and its target DNA and to the need for the JCV T protein to interact with host cell proteins for replication to occur. Consequently, the restricted nature and unusually long duration of viral multiplication can be attributed in large measure to the replication machinery needed for successful viral multiplication. These elements include the viral T protein, the viral DNA sequences that are recognized by this protein, the conformational structure probably required for successful binding, and the cooperation exhibited by the protein factors in the appropriate host cell.

PML AS AN INFECTION OF THE BRAIN

Pathology of PML

Following infection by JCV, oligodendrocytes undergo cytolytic destruction that results in loss of myelin. At its simplest, this is the central observation in understanding the pathology and subsequent clinical features of PML. The principal function of the oligodendrocyte is to myelinate the axons that project from the neuronal cell bodies of the overlying cortex. In gross sections, cortical neurons appear to be grey, hence the term cortical grey matter. In contrast, the underlying oligodendrocytes and their myelin sheaths appear to be white, and they are described as subcortical white matter. Both the cerebral hemispheres and the cerebellum are stratified in this manner. Lytic infection of oligodendrocytes in patients with PML can therefore be recognized as destruction and demyelination of the subcortical white matter.

Foci of demyelination initially are microscopic and asymmetrically distributed in space. As the disease progresses, areas of demyelination enlarge and these foci may coalesce, making them visible on gross examination in cut sections of the brain. In areas of active demyelination, there is a progressive attrition of oligodendrocytes from viral cytolysis, and while they may be found throughout the lesions, oligodendrocytes are more prominent at the borders of demyelinated areas (97). Moreover, some of the remaining oligodendroglial nuclei may be two- to threefold larger than normal, may be strongly basophilic with effacement of the chromatin pattern, and may contain intranuclear inclusions (14, 172). As seen by electron microscopy, the intranuclear inclusions consist of a dense array of crystalline and filamentous JCV particles (139, 234, 235). In both the cerebral and cerebellar hemispheres, viral DNA and antigen can also be detected by in situ hybridization and immunocytochemistry in what appear to be normal oligodendrocytes (91, 97). These normal oligodendrocytes are most frequently seen in areas with no demyelination.

In addition to the oligodendroglial pathology, greatly hypertrophied giant astrocytes may be observed in areas of demyelination in 80% of cases (175). These astrocytes are pleomorphic and sometimes described as bizarre. They have basophilic nuclei that frequently contain mitotic figures and are irregularly lobulated. While viral DNA can be detected in these astrocytes by in situ hybridization (2), they rarely express late viral proteins (3). Although the morphology of the astrocytes suggests a neoplastic process, few cases of intracranial tumors have been associated with PML (32, 55, 74, 88, 172). Reactive astrocytosis, a nonspecific finding in many neuropathological processes, is also seen with PML.

The demyelinated axon typically is spared in areas of demyelination, but rare neuronal loss in grey matter has been reported (114, 151). Since some oligodendrocytes are found in the cortical ribbon as well as in the grey matter of the basal ganglion and cerebellum, infection of these cells then probably leads to demise of the adjacent neurons. Alternatively, loss of the myelin sheath leads to axonal injury and subsequent retrograde degeneration of the cell body in the grey matter. Neurons are not infected by JCV (13). Neuronal death is probably secondary to an as yet unrecognized process. Some cases of grey matter involvement have been seen in brain cells coinfected with the human immunodeficiency virus (HIV), suggesting that HIV-caused encephalitis may further contribute to neuronal death.

In areas of demyelination, lipid-laden macrophages are frequently found in the center of the lesion, probably recruited into the CNS to phagocytize the myelin breakdown products. Detection of viral DNA in macrophages by in situ hybridization has not been reported. In some cases of PML associated with AIDS (see below), large numbers of HIV-infected macrophages are found in extremely extensive, necrotic lesions (171, 183, 226). JCV infection may recruit HIV-infected macrophages into a demyelinated lesion, which then leads to a localized HIV encephalitis. Alternatively, uninfected macrophages may become infected by HIV after they are recruited into the nervous system. It is unclear whether the extent of these lesions can be explained by some degree of cytolytic synergy occurring with coinfection of the brain by both JCV and HIV (214). Except for the macrophage ingress, inflammatory infiltrates are seldom seen in patients with PML. When lymphocytic perivascular cuffing and parenchymal accumulation are present, the course of the disease tends to be protracted, with periods of remission (105, 110, 174). This suggests that these patients have some degree of immunocompetence that allows for clearance of the virus. It has recently been demonstrated that viral DNA and antigen may be found in perivascular B cells as well as in the Virchow-Robin spaces of PML-affected brains (93, 122). The role of these B cells in the pathogenesis of PML will be discussed in a subsequent section.

Clinical Features of PML

Signs and symptoms. Since PML involves the subcortical white matter, it follows that the clinical correlates of these lesions may manifest as a wide variety of neurological disturbances. Brooks and Walker (28) reviewed 69 patholog-

ically confirmed and 40 virologically and pathologically confirmed cases of non-AIDS-associated PML and categorized the neurological signs and symptoms at onset and during disease progression. Visual deficit was the most common presenting sign, present in 35 to 45% of cases. Of the visual deficits, homonymous hemianopsia (loss of vision for one-half the visual field in each eye) was the most common. Interestingly, 6 to 8% of the patients were cortically blind at the time of diagnosis, indicating bioccipital pathology. Motor weakness was the initial sign in 25 to 33% of cases, though by the time the diagnosis was made, hemiparesis or hemiplegia was present in nearly all patients. A change in mentation, which included personality change, difficulty with memory, emotional lability, and frank dementia, was the presenting sign in approximately one-third of cases and eventually involved most patients.

Berger et al. (20) also found that hemiparesis, visual impairment, and altered mentation were the three most common initial manifestations as well as the most frequent signs during the course of AIDS-related PML. In their series of 28 patients, motor weakness, found in almost half of the cases, was the most common presenting symptom. Almost all patients went on to develop a spastic hemiparesis. Visual loss and mental status changes each accounted for approximately 25% of the initial manifestations. A small number of patients present with signs and symptoms referable to the posterior fossa, i.e., ataxia, dysmetria, and dysarthria, which usually indicate involvement of the white matter of the cerebellum and brainstem (100, 162). Other signs and symptoms associated with PML include headache, vertigo, seizures, sensory deficits, parkinsonism, aphasia, and neglect syndromes (20, 28). In some cases, the coexistence of HIV encephalitis could have accounted for some of the symptoms. Spinal cord involvement is rare (16).

Diagnostic testing. Neuroimaging is by far the most useful tool in investigating a patient with PML. On a computerized tomography (CT) scan, the demyelinating lesions appear as subcortical hypodensities with a propensity for the parieto-occipital area that respect the grey-white junction of the cortex and do not follow a vascular distribution (24, 43). In general, no mass effect is noted, although rare exceptions have been reported on CT scanning and angiography (46, 166). Single-dose intravenous contrast and delayed, double-dose contrast CT scanning as a rule usually fail to enhance lesions. Exceptions to this have been reported (181). Contrast enhancement is evidence of disruption of the blood-brain barrier and should prompt the clinician to consider an alternative diagnosis or consider the presence of a second pathologic process in the vicinity of the demyelination. In a representative case, Shafran et al. (186) described a patient with CLL who had multiple hypodense contrast-enhancing lesions on CT scan. At autopsy, the hypodensities corresponded to areas of demyelination consistent with PML, while the contrast-enhancing areas coincided with a dense perivascular leukemic infiltrate apparently related to invasion of the brain by CLL. There are rare cases of slight homogeneous contrast enhancement at the periphery of a demyelinating lesion not associated with a second pathologic process (83). This is probably the CT scan equivalent of the small artery and vein dilatation seen on cerebral angiograms of some patients with PML (111). The vascular dilatation remains unexplained.

MRI has proved to be very sensitive in detecting the demyelinating lesions of PML (83, 115, 132) which have altered signal characteristics in comparison with the surrounding white matter. MRI is superior to CT scanning in demonstrating not only the number but also the extent of the lesions (165). Occasionally, the MRI scan will clearly demonstrate pathology when the CT scan is normal. MRI is vastly superior to CT scanning in detecting infratentorial lesions which are now more frequently recognized in patients with PML. Several cases of grey-matter involvement have also been recognized antemortem by using MRI. Gadolinium enhancement of lesions seen on MRI is distinctly rare in patients with PML.

There is one case of PML studied with positron emission tomography which revealed cortical hypometabolism of glucose in the same portion of a hemisphere in which there was a subcortical white-matter lesion (108). While this case raised several interesting points on the relationship between white-matter pathology and its effect on grey-matter metabolism, positron emission tomography scanning at this time has no role in the diagnostic workup of a suspected PML patient.

Electroencephalography is both insensitive and nonspecific for PML, but it may corroborate the presence of a lesion seen on neuroimaging. Early in disease presentation, focal slowing in the theta to delta range may correspond to focal lesions seen by a CT or MRI scan. As the lesions spread and become multifocal, the electroencephalogram becomes diffusely slow (59). Evoked potentials are sensitive to demyelinating processes, as has been extensively demonstrated with multiple sclerosis. There is little information on evoked potentials in the context of PML, but one case report found that brainstem auditory-evoked and somatosensory-evoked potentials could accurately localize a lesion in the posterior fossa that could not be seen on CT scan (116).

CSF findings are nonspecific, with most patients demonstrating a normal profile. A mild lymphocytic pleocytosis, which is rarely if ever over 25 leukocytes per ml, may be seen in 15% of cases. Total protein is mildly elevated in approximately 20 to 30% of cases, while myelin basic protein, IgG, and IgG/albumin indices have been reported to be increased in a few cases (152). The primary utility of lumbar puncture in the setting of possible PML is to exclude the presence of other illnesses, including treatable infections.

The most reliable and accurate method for the diagnosis of PML remains brain biopsy in order to demonstrate the pathological hallmarks described. The techniques of in situ hybridization and immunocytochemistry add a further degree of specificity when the biopsy material is examined. In situ DNA-DNA hybridization is a method of annealing JCV DNA to complementary strands either in paraffin-embedded tissue or in frozen sections from biopsy samples. The probe may be either radiolabeled (52) or biotinylated (2, 3, 5, 92, 187), which in either case allows for the morphologic identification of the infected cells. In immunocytochemistry, antibodies to both T antigen and the common polyomavirus capsid antigen are used to detect cells undergoing productive viral infection (31, 72, 77). A colorimetric method is used to identify the infected cells. Cells that are positive by in situ hybridization are in a stage of active viral replication since several hundred copies of the viral genome must be present for the colorimetric reaction to be visualized. Cells positive by immunocytochemistry that are expressing viral capsid antigens are in a stage of viral transcription and translation, i.e., undergoing productive infection. In addition to their utility in confirming a diagnosis of PML, these techniques have demonstrated the presence of JCV in perivascular locations and at sites distant from foci of demyelination,

adding new insight into the natural history and pathogenesis of PML as discussed above.

PCR is an emerging technology that may have a role in the diagnosis of PML. Several groups have reported detecting JCV in brain biopsies, CSF, and in some cases the peripheral lymphocytes of patients with PML by using PCR (11, 87, 206, 220). We have found that more than 80% of patients with brain biopsy-proven PML will have JCV DNA in their lymphocytes as detected by PCR. In contrast, none of a group of Parkinson's disease patients had circulating JCV DNA.

Interestingly, one prolonged (more than 4 years) survivor of PML who has had a stable course still has JCV genome present in his peripheral blood lymphocytes (208). In addition to supporting the theory that JCV infection is spread hematogenously, PCR may also have some clinical utility as a noninvasive method of confirming the diagnosis of PML. We are currently pursuing this possibility. A summary of the clinical characterization of PML is outlined in Table 2.

While the prognosis of patients with PML is very poor, i.e., an average survival of 9 months (158, 199), the course of the disease appears to be related to the severity of the underlying immunosuppression. PML in the setting of the profound immunodeficiency seen with AIDS is usually rapidly and relentlessly progressive, leading to death in 2 to 4 months from the time of symptom presentation (20). PML developing in the setting of immunosuppression with cytotoxic agents to prevent graft rejection may have a prolonged course lasting several years if the cytotoxic agents are withdrawn or decreased (181). In addition, there have been several cases of PML that stabilized without medical intervention (86, 162, 185). In some cases, no clear immunodeficiency could be documented (233), while three cases involved patients with AIDS (21). In most cases, a return to immunocompetence apparently led to clearance of the virus from the CNS. In one case of prolonged survival, the pathology at the time of death no longer involved oligodendroglial or astrocytic infection (167), again suggesting that the virus had been cleared from the CNS. These patients offer hope that JCV infection may be abrogated by effective antiviral or immunomodulatory therapy.

TABLE 2. Clinical characteristics of PML

Pathological Features
Hyperchromatic, enlarged oligodendroglial nuclei
Contain detectable inclusion bodies
Express JCV capsid protein by immunocytochemistry
Demonstrate JCV DNA by in situ hybridization
Bizarre astrocytes with enlarged nuclei contain JCV DNA by in situ hybridization
Macrophages in the center of demyelinated lesions with perivascular cuffing seen occasionally
Clinical Signs and Symptoms
Classic triad (dementia, hemiparesis, hemianopsia)
May present with any one of these three
Lesions at all levels of the neuraxis reported
Laboratory and Diagnostic Features
CT scan shows nonenhancing, subcortical hypodensities
MRI scan shows altered signal from subcortical lesions
EEG shows focal slowing corresponding to white-matter lesions and generalized slowing with advancing disease
CSF is usually benign but may see mild elevation of protein or an increased cell count
Brain biopsy may demonstrate pathology noted above and demonstrate viral DNA by in situ hybridization, viral antigens by immunocytochemistry, virions by electron microscopy
PCR can detect circulating viral DNA in peripheral blood lymphocytes and CSF

Host Factors in the Development of PML

Approximately 10% of children between the ages of 1 and 5 years demonstrate antibody to JCV. By age 17 years, 65% of adolescents will have seroconverted, and the adult population in some urban areas shows as high as 92% seroconversion (217). Primary exposure is not associated with any known clinical manifestations. Prior to the AIDS epidemic, the peak incidence of PML occurred during the sixth decade. This suggests that primary exposure to JCV occurs in most people during the first two decades of life and then is reactivated in those who later develop PML. A lack of IgM antibody directed at JCV in the presence of a preexisting IgG antibody to JCV is found in most cases of people with PML, again suggesting that the virus that causes demyelination originates from reactivation of a latent infection rather than from primary exposure (23, 217). In most cases, the JCV-directed IgG titers do not rise during the course of the illness and are absent from the CSF (99, 215), although exceptions have been reported (109). The vast majority of patients with PML have some degree of underlying cellular immunodeficiency that apparently allows for reactivation of infection. Prior to 1980, Hodgkin's disease and CLL were the underlying conditions most frequently associated with PML (173). Other processes included autoimmune diseases, granulomatous processes, myeloproliferative disorders, soft tissue malignancies, and immunosuppression acquired either during organ transplantation or secondary to the cytotoxic effects of chemotherapy. Since 1980, the number of cases of PML has increased dramatically, an increase directly attributable to the AIDS epidemic. It is now accepted that 55 to 85% of recent cases of PML have AIDS as the underlying pathology, again supporting the theory that reactivation of JCV is associated with an immunosuppressed state (20, 111). Up to 3.8% of all cases of AIDS will develop PML, a rate higher than that for other immunodeficient states (20, 111, 196). This rate may reflect the more profound immune suppression associated with AIDS. There has also been the suggestion of a possible direct interaction between HIV type 1 and JCV proteins (70, 201). Polyclonal gammopathies are frequently seen with HIV infection and reflect the loss of T-cell modulation of B cells. The possible role of B-cell activation in the pathogenesis of PML is discussed below.

In a study of seven patients with PML, in vitro lymphocyte proliferation in response to mitogen stimulation was blunted, suggesting a generalized depression of cell-mediated immunity as expected (227). Cellular immunity directed specifically against JCV was assessed by measuring the production of leukocyte migration inhibitory factor from lymphocytes in response to JCV antigens. Production of this factor was normal in response to JCV antigens in non-PML patients. In PML patients with cellular immunodeficiency, the factor was absent, suggesting that in addition to the generalized immunodeficiency, there is a selective deficiency in the cellular response to JCV in patients who develop PML. The further observation that both pregnant women (41) and patients who have been immunosuppressed (67) (but who do not have PML) may shed JCV in their urine suggests that loss of immunocompetence predisposes an

individual to the reactivation of a latent infection but does not necessarily lead to CNS infection.

In what cell type is JCV latent? This issue remains unresolved, but current research has questioned whether JCV could be latent in brain cells from healthy individuals. Brains from immunosuppressed and nonimmunosuppressed patients without PML do not demonstrate early or late viral antigens by immunocytochemistry, nor do they demonstrate the presence of viral genome by in situ hybridization (90). Recently, PCR technology has been used to demonstrate the presence of viral DNA in brain tissue from PML patients (12, 206). However, in two studies, PCR did not amplify the JCV DNA in healthy brain tissue or tissue of subjects with other neurological processes (87, 206). In total, these observations suggest that JCV remains latent outside the CNS and then gains access to the nervous system some time during immunosuppression. As noted above, JCV may be shed in the urine of pregnant women and immunosuppressed renal transplant patients, which suggests that the virus is latent in the genitourinary tract. While one study found that 10% of healthy subjects have nonintegrated JCV DNA in renal tissue (36), a second study found no evidence of JCV DNA in kidney tissue from non-PML-affected patients (81). Using dot blot hybridization, Grinnell et al. (81) found viral genomes in the kidney of five of seven patients. In the same study, they also found JCV DNA in lung, liver, lymph node, and spleen cells in three of these patients, two of whom were children with combined immunodeficiency syndromes, which suggests that widespread dissemination of the viral genome can occur in the setting of severe dysimmunity. None of the organs examined in this study expressed viral antigens that would have indicated a productive phase of infection. Greenlee and Keeney (77) also reported that extraneural tissue from PML patients did not express viral antigens. The work of Houff et al. (93) has identified JCV DNA in B cells of the spleens and bone marrow of two patients with PML. Using immunocytochemistry, they found that these B cells expressed JCV antigens. Similar experiments have demonstrated that JCV-infected B cells are present in the brain parenchyma of PML patients in a perivascular location as well as in the Virchow-Robins space (122). This suggests that JCV infects B cells which may then remain latent in the marrow. With the loss of an integrated immune response in cases of cellular dysimmunity, B-cell populations may polyclonally expand, as seen with HIV-1 infection when the CD4 count decreases. Activated B cells, now in the active phase of viral replication and transcription, cross the blood-brain barrier and seed the CNS.

Hematogenous spread to the CNS is supported by the multifocal nature of the demyelinating lesions as well as their location near the grey-white junction where the end arterioles of the cerebrovascular tree are found. In our laboratory, we have used PCR to demonstrate the presence of JCV genome in peripheral lymphocytes of PML-affected subjects, again supporting the notion of hematogenous spread of the virus via lymphocytes.

TREATMENT OF PML

Effective therapy of PML, whether specific antiviral therapy directed at JCV or attempts to enhance cellular immunity, remains elusive. On the basis of anecdotal reports and a small series of reported cases of PML, a variety of treatment regimens have been proposed (Table 3). However, no therapeutic regimens have been studied in a randomized, double-blind fashion. The need for a large formal study of any treatment regimen suggested for this illness is highlighted by the observations that PML may remain quite stable for long periods and may even remit in the rare patient (21, 57, 105, 158, 167, 190). Before the recent increase in the prevalence of PML as the result of HIV-1 infection, the disease was sufficiently rare to tender a study of this nature impractical. With an estimated prevalence of 3.8% in patients with HIV-1 infection (20), some institutions with large populations of patients with AIDS may treat large enough numbers of patients with PML to perform such studies without having to resort to a multi-institutional study.

TABLE 3. Proposed therapies for PML

Proposed therapy	Route of administration
Cytosine arabinoside	Intrathecal or intravenous
Adenine arabinoside	Intrathecal or intravenous
Iododeoxyuridine	Intrathecal
AZT	Oral
Alpha interferon 2A (recombinant)	Subcutaneous
Beta interferon	Intrathecal
Transfer factor	Intravenous
Heparin	Subcutaneous
Corticosteroids	Oral or parenteral

Nucleoside Analogs

Nucleoside analogs interfere with the synthesis of DNA and have established efficacy in the treatment of some viral diseases. Among these nucleic acid base analogs are adenine arabinoside, iododeoxyuridine, and acyclovir for herpes simplex virus, ganciclovir for cytomegalovirus, and zidovudine (AZT), dideoxycytidine, and dideoxyinosine for HIV-1 infection. These agents often have antiviral activities and may be incapable of inhibiting the replication of other viruses in the same family. Several nucleoside analogs have been used in the treatment of PML with various degrees of success (Table 4).

Despite earlier failures (33), therapeutic trials with cytosine arabinoside, a drug chiefly used in the treatment of myeloproliferative disorders, suggested that the drug is effective (17, 42). Bauer and colleagues (17) reported the use of cytosine arabinoside administered intravenously at 60 mg/m^2/day and intrathecally at 10 mg/m^2 in a 36-year-old man with PML complicating CLL. Within 48 h of the initiation of therapy, improvement in his dysarthria and hemiparesis was noted. Improvement continued during 10 months of follow-up. Marriott et al. (133) described marked improvement within 6 weeks and continued improvement over 18 months after the initiation of cytosine arabinoside at 2 mg/kg/day five times every 3 weeks in a 52-year-old woman whose PML complicated sarcoidosis. Buckman and Wiltshaw (30) noted a resolution of neurological symptoms in a 46-year-old woman with Hodgkin's disease and suspected PML (never proven by biopsy) who received both intravenous and intrathecal cytosine arabinoside. O'Riordan et al. (154) described minimal neurological improvement in a 51-year-old woman with PML complicating non-Hodgkin's lymphoma that was in complete remission. She received intravenous cytosine arabinoside at 2 mg/kg of body weight per day for 5 days repeated every 21 days. However, when

TABLE 4. Nucleoside analogs in the treatment of PML[a]

Nucleoside analog	Age of patient (yr)	Sex of patient	Underlying disease	Therapy	Outcome	Reference
ARA-C	58	M	CLL	40 mg/m^2/day	Death	33
	36	M	CLL	60 mg/m^2/day for 6 days (i.v.), 10 mg/m^2 for 2 days (i.t.)	Improved within 48 h	17
	54	M	CLL	100 mg/m^2 for 1 day, 30 mg/day for 2 wks (i.v.), 100 mg for 3 days (i.t.)	Improved over 24 h, deterioration and death in 2 wk	42
	52	F	Sarcoidosis	2 mg/kg/day for 5 days every 3 weeks for 18 mo	Improved in 6 wk, continued for 1.5 yr	133
	45	F	None	60 mg/m^2/day for 5 days (i.v.), 10 mg/m^2 for 2 days (i.t.)	Equivocal improvement followed by deterioration over 20 mo	176
	32	M	Hodgkin's disease	60 mg/m^2/day for 6 days (i.v.), 10 mg/m^2 for 2 days (i.t.)	Gradual improvement over 3 wk	163
	32	M	Sarcoidosis	60 mg/m^2/day for 18 days, 100 mg/m^2/day for 8 days (i.v.)	Deterioration	193
	51	F	NHL	2 mg/kg/day for 5 days (i.v.) every 21 days, 3 times; 50 mg/m^2 for 6 days (i.t.)	Clinical recovery	154
	57	F	Unknown	30 mg/m^2 for 5 days (i.v.), 100 mg/m^2 for 1 day (i.t.), corticosteroids, ACTH, transfer factor	Continued deterioration	212
	35	M	HIV	2 mg/kg for 5 days (i.v.) every 4 wk	Clinical and MRI improvement	164
	44	M	HIV	2 mg/kg for 5 days (i.v.) every 4 wk	Clinical and MRI improvement	164
	57	M	HIV	2 mg/kg for 5 days (i.v.) every 4 wk	Clinical and MRI improvement	164
ARA-A	59	M	CLL	20 mg/kg/day for 2 wk		
	49	M	CLL	20 mg/kg/day for 2 wk	Continued deterioration	229
	46	M	CLL	6 mg/kg/day for 2 wk	Continued deterioration	229
	69	M	CLL	20 mg/kg/day for 2 wk	Continued deterioration	229
	44	F	None	ARA-A dose not stated and beta interferon, 3 × 10^6 U/day for 4 days (i.v.); ARA-C, 90 mg/day for 7 days and 10 mg/day for 2 days (i.v.); and beta interferon at 10^6 U/wk for 19 wk (i.t.)	No improvement	168
Iododeoxyuridine	46	M	Sarcoidosis	2 mg/kg/12 h for 7 weeks (i.t.) and prednisone	Worsening until i.t. beta interferon initiated	205
AZT	26	M	HIV	200 mg every 4 h	Continued deterioration with death Improved during brief follow-up	204 44

[a] ARA-C, cytosine arabinoside; ARA-A, adenine arabinoside; M, male; F, female; i.v., intravenous; i.t., intrathecal; NHL, non-Hodgkin's lymphoma; ACTH, adrenocorticotropin hormone.

weekly intrathecal cytosine arabinoside at 50 mg/m^2 was added to the regimen, she demonstrated a remarkable recovery (154). Conomy and colleagues (42) described a patient with CLL who experienced a dramatic neurological improvement over 24 h when 100 mg of intravenously administered cytosine arabinoside was followed by 30 mg of the same drug intravenously daily and 100 mg given intrathecally on three separate occasions over 1 week. However, rapid deterioration over the ensuring 2 weeks ended in death. Portegies et al. (164) noted both clinical and MRI improvement in three patients with AIDS-related PML (not pathologically confirmed) following treatment with cytosine arabinoside at 2 mg/kg given intravenously for 5 days every 4 weeks. These investigators suggest that long periods of therapy with cytosine arabinoside may be required before a favorable response is detected. Less convincing neurological improvement associated with treatment with cytosine arabinoside was noted by Rockwell and colleagues (176) in a patient without an identified underlying risk for PML. Peters and colleagues (163) reported a gradual improvement over 5 weeks in a 32-year-old man with PML complicating Hodgkin's disease who received cytosine arabinoside at 60 mg/m^2/day for 5 days and 100 mg/m^2 intrathecally on one occasion. Some case reports suggest a lack of efficacy of cytosine arabinoside administered either solely intravenously (33, 193) or in combination with intrathecal therapy (212).

Other nucleoside analogs appear to have met with less success than cytosine arabinoside in the treatment of PML. Wolinsky et al. (229) noted failure of a 14-day course of adenosine arabinoside (at 20 mg/kg/day) in two patients with PML. Similar failures of adenosine arabinoside therapy in

the treatment of PML have also been described (168, 215). Tarsy and colleagues (204) had no success with a combination of prednisone and intrathecal iododeoxyuridine administered at 2 mg/kg of body weight/12 h for 7 weeks in a 46-year-old man with PML complicating sarcoidosis.

Interferons

The interferons have been proposed as potential therapeutic agents in the treatment of PML because of their antiviral activity. Their antiviral response appears to be directly proportional to their ability to stimulate natural killer cells (210). Alpha interferon has established efficacy in the treatment of other papovaviruses and has become an accepted form of therapy for resistant genital warts that result from infection with various human papillomaviruses (HPV) (221). Alpha interferon has also been demonstrated to be effective against HPV whether administered topically, intralesionally, or systemically (221). Certain strains of HPV, such as HPV 16 and 18, appear more resistant to alpha interferon than other strains (184). Alpha interferon has also demonstrated efficacy in the treatment of laryngeal papillomatosis, a disorder that also results from HPV infection. While the genetic homology between HPV and JCV is slight, alpha interferon has also shown activity against SV40, which is closely related to JCV and has been implicated in several rare cases of PML (223), although the identification of SV40 rather than JCV in these cases is not unequivocal. Pretreatment of African green monkey CV-1 cells with alpha interferon inhibits the accumulation of SV40 mRNAs and SV40 T protein. Not surprisingly SV40-transformed mouse embryo fibroblasts, C57BL/6, are susceptible to lysis by natural killer cells (62).

In light of these observations regarding alpha interferon and the selective defect in alpha interferon production by HIV-1-infected monocytes (71), an open label trial of the safety and efficacy of recombinant alpha interferon 2A has begun in the treatment of PML associated with AIDS. In HIV-1-seropositive patients with PML confirmed by brain biopsy, we administer 3×10^6 U of recombinant alpha interferon 2A subcutaneously daily, with a gradual increment (typically, by 3×10^6 units every third day). A daily dose of alpha interferon 2A of 18×10^6 units was maintained following the initial titration. AZT (100 mg every 4 h) was also administered. If AZT could not be tolerated, dideoxyinosine was administered. Nine patients have been treated with this regimen at the University of Miami School of Medicine, and a similar number have been treated at collaborating institutions. The small number of patients and the short follow-up time have not permitted a detailed analysis of the treatment effects. No patient has yet exhibited a dramatic reversal in neurological function, though modest improvement has been noted in several patients. A significant improvement in the MRI was observed in one patient (Fig. 6). At least three patients have remained clinically stable for periods of 6 months following the initiation of alpha interferon 2A. Progression of the disease despite alpha interferon 2A therapy has been observed in four patients. Three of these patients had advanced and rapidly progressive neurological disease when they were enrolled in the experimental protocol.

Tashiro et al. (205) reported a 44-year-old woman with PML whose neurological deficit progressed despite treatment with intravenous adenine arabinoside and beta interferon. A subsequent 7-day course of intravenous cytosine arabinoside at 90 mg/day supplemented with 2 days of intrathecal cytosine arabinoside at 10 mg/day also failed to result in any improvement. She then received intrathecal beta interferon at 10^6 U weekly for 19 weeks and monthly thereafter. During the administration of this regimen, a modest improvement in her clinical picture and MRI has been noted.

Heparin Sulfate

The rationale for the use of low-dose heparin sulfate as an adjunct to other agents in the treatment of PML is based on the model of the pathogenesis of PML suggested by Houff et al. (93) which postulates that PML is the result of activated JCV-infected B lymphocytes crossing the blood-brain barrier and initiating new areas of neurological infection throughout the course of the disease. In animal models, heparin sulfate has been shown to prevent activated lymphocytes from crossing into the brain by stripping the lymphocyte glycoprotein cell surface receptors for cerebrovascular endothelial cells (27). If heparin sulfate has analogous effects on activated B lymphocytes in humans, heparin therapy may prevent new demyelinating lesions from occurring. Eleven nonselected HIV-1-infected PML patients have been treated with 5,000 U of heparin sulfate administered subcutaneously every 12 h. Although our experience is limited, heparin sulfate therapy appears to have a beneficial effect in patients with single demyelinating lesions, CD4 lymphocyte counts of >200 cells/m^3 and no prior history of opportunistic infections. In three of four patients with PML who met these criteria, the disease did not progress for a period of 15, 18, and 27 months after heparin administration. The fourth patient experienced progression of his neurological disease that led to death 4 months after the initiation of heparin therapy. A multicenter study of heparin therapy for PML is now being planned which should clarify the role subcutaneous heparin may have in the treatment of PML.

Other Therapies

Few other agents, used alone or in combination with nucleoside analogs, have been tried in the treatment of PML. Among the trials are the failed attempts of Tarsy and colleagues (204), who administered prednisone in combination with iododeoxyuridine, and of Van Horn and colleagues (212), who administered corticosteroid, adrenocorticotropin, and transfer factor with cytosine arabinoside. Theoretically, a recovery from any underlying immunological disorder should be associated with recovery from PML. Selhorst et al. (185) reported a stabilization of the neurological deficits in a patient with PML complicating renal transplantation whose azothioprine was stopped and who then was treated with tilorone, an immune enhancer. Dawson (48) noted no improvement following the cessation of immunosuppressive therapy in a patient with PML and myasthenia gravis. A recent report by Conway et al. (44) suggests that PML occurring in association with HIV-1 infection may respond to AZT. Their patient developed PML as the presenting manifestation of HIV-1, improved dramatically following the administration of AZT at 200 mg every 4 h, and worsened with a reduction in dose to 200 mg every 8 h that was prompted by the development of granulocytopenia. During a 10-day follow-up after a return to his previous higher dose of AZT, he remained neurologically stable. The value of AZT in the treatment of PML with or without concomitant HIV-1 infection remains questionable. Almost all of the more than 45 HIV-1-infected patients with PML seen by one of us (19)

have been treated with AZT in various doses. No correlation with neurological recovery has been noted. Furthermore, none of our patients who showed dramatic clinical recovery and whose MRI showed resolution of brain lesions were on AZT at the time of recovery.

DISCUSSION

The early observation that JCV caused a common viral infection in the population but could multiply efficiently only in the oligodendrocyte of the CNS created a paradox in understanding the pathogenesis of PML. Over the 20 years since the isolation of JCV in 1971 (161), experiments have been designed to determine the mechanisms that explain how a virus could be so widespread in the population but maintain such a narrow host range and selectively target the specialized, myelin-producing cell in the human brain. What has emerged from these studies are two dominant areas of investigation that have become central to the understanding of this virus-induced demyelinating disease: the molecular control of JCV gene expression and replication and the establishment and reactivation of viral latency.

Molecular Control of Viral Gene Expression

Understanding the effects of host cell-restricted JCV gene expression is essential for understanding the establishment of JCV latency preceding the development of PML. The molecular basis for this restriction centers on the noncoding or regulatory sequences of the viral genome. Variations in the nucleotide sequences are found to occur predominantly within the regulatory region (51, 80, 117, 170, 231). Some differences in nucleotide sequences of the regulatory region are found in JCV isolates cloned from different brains (136). These differences, however, do not seem to affect the ability of the virus to induce PML. Major alterations occur in the nucleotide sequences of the regulatory region of JCV isolates cloned from kidney cells. The differences in the regulatory region sequences between brain and kidney isolates have been shown to occur in virus cloned from the same patient with PML (117). That JCV may have a parent or archetypal DNA sequence that evolves into a brain-specific strain has been suggested by the analysis of regulatory sequences with multiple insertions (231). Following alterations and deletions of these insertions, different viral strains with different cell specificities could have evolved. These observations have yet to be tested. The regulatory region of the JCV strain responsible for primary infection may determine which host cells undergo initial JCV infection. Therefore, the JCV strains responsible for primary infection would determine whether and where JCV latency occurs, e.g., in the kidney or elsewhere. Changes in the nucleotide sequences within the regulatory region during initial viral replication could extend the host range and cell types in which JCV persistence could be established. The role of the regulatory region in JCV latency is being studied by cloning and sequencing PCR amplification products of viral genomes in latently infected cells. Using these techniques, we may soon be able to determine the significance of certain DNA sequences in the regulatory region in establishing latency.

Host cell restriction of JCV expression also occurs at the level of viral mRNA transcription and depends on the ability of host cell transcription factors to recognize elements within the JCV regulatory region (60). When a reporter gene vector, CAT, was used, transcription from the JCV regulatory region occurred only in specific cells (104, 107). Specific binding to nucleotide sequences within the JCV regulatory region has been described for a number of host cell nuclear proteins, including NF-1, which is found in human fetal brain cells in DNA binding experiments (1, 6, 107). The result of protein binding of a combination of both positive and negative nuclear transcription factors that recognize specific nucleotide sequences could affect viral expression.

Primary and Latent JCV Infections

Primary JCV infection appears to be asymptomatic, making prospective studies difficult to perform. An understanding of the primary infection has important implications for understanding JCV pathogenesis, since the cell types initially infected are candidates for virus latency. Viruses not directly injected into the bloodstream by arthropod vectors undergo an initial replication cycle in cells proximal to the portal of entry. Since the portal of entry for JCV is unknown, the cell types supporting initial virus replication remain undefined. We do know that JCV DNA has been found in spleen, lymph node, kidney, and lung cells in two children with combined immunodeficiency disease who developed PML during what was thought to be primary JCV infection (81). Whether primary JCV infection in the immunocompetent host results in systemic spread of the virus is unknown.

Since JCV latency is most likely established during primary infection, determination of the sites of persistence of the viral genome will provide the minimum number of cell types undergoing primary infection. Evidence reviewed here suggests that JCV is latent in two extraneural sites but is probably not present in the brain. JCV has not been detected in neuroglial cells of any healthy brain using a variety of techniques including cocultivation with susceptible glial cells, in situ DNA hybridization with radiolabeled probes, electron microscopy, and PCR amplification (28, 206). These studies suggest that JCV does not reach the brain during primary infection and that spread to the nervous system must follow reactivation at other sites.

Evidence of JCV infection in the kidney and in lymphocytes has been described. JCV has been isolated from the urine of immunosuppressed patients with and without PML, and viral DNA has been detected in kidney cells by in situ hybridization. For several reasons, reactivation of latent JCV in the kidney is of uncertain significance in the pathogenesis of PML. JCV cloned or isolated from brain requires adaptation before significant viral replication will occur in kidney cultures. Nucleotide sequences within the JCV regulatory region differ in viruses isolated or cloned from brain and kidney cells, even in those from the same patient (117). Finally, JCV DNA is not always in the kidney cells from patients with PML. Lymphocytes are a second extraneural site of JCV infection. B lymphocytes containing JCV DNA and virion capsid antigens are present in the bone marrow and brain cells of PML patients. JCV DNA is present in peripheral blood lymphocytes of patients with PML, and JCV replication may occur in B-lymphocyte cell lines (93, 122). Although other sites of JCV latency are unknown, the techniques used in the experiments described above are not, generally, adequately sensitive to detect the low copy number of JCV genomes expected to occur in latently infected cells. PCR amplification technology offers the needed sensitivity. PCR amplification of DNA extracted from extraneural cells of JCV seropositive patients without immunosuppression or PML should provide conclusive evidence of the frequency and sites of viral latency following primary infection.

JCV Infection and Cells of the Immune System

If JCV persists in B lymphocytes, the biology of B-cell growth and development (27, 45, 225) may offer insights into the mechanisms of viral latency and reactivation. Long-lived B-lymphocyte memory cells provide a stable cell line for maintenance of latency. B-lymphocyte growth and differentiation, controlled by immunoregulatory T cells, require synthesis of nuclear transcription factors not present in resting memory cells. With T-cell immunodeficiency diseases, particularly AIDS, T-lymphocyte immunoregulation of B-lymphocyte development is impaired. B cells can become activated, and nuclear transcription factors required for growth and differentiation can be synthesized. The JCV genome which has remained latent as a result of the absence of the required transcription factors could be reactivated as an unintended consequence of B-lymphocyte reactivation. The transcription and DNA-binding experiments with B lymphocytes described above (122) provide support for this hypothesis. Future experiments to determine what inducible factors in B cells are required for JCV multiplication will be required.

Whether or not viral latency actually occurs in B lymphocytes, it appears that JCV can enter the brain in activated B cells (93). The demonstration of JCV DNA in peripheral lymphocytes by PCR analysis (220) and JCV-infected B cells in PML-affected brain tissue adds critical information to the suspected cellular route of infection to the brain. JCV-infected B cells could gain access to glial cells since activated lymphocytes do not require antigen-specific recognition to cross the blood-brain barrier (27, 225). Further studies are needed to determine the frequency of JCV DNA in peripheral blood lymphocytes in PML- and non-PML-affected individuals and to identify those cell types containing JCV DNA.

Macrophages are the predominant cellular immune response to JCV neuroglial infection. The mechanism of macrophage recruitment into demyelinating lesions is unknown and deserves further study. JCV-specific cellular immune studies in a small number of patients suggest that T-cell cytotoxicity is present despite the presence of immunodeficiency. JCV clones produced for molecular biological studies provide a means of obtaining sufficient viral antigens for future studies of JCV-specific cellular immunity in PML patients and controls. The failure of cytotoxic T cells to clear JCV infection may be related to the inability of JCV-infected glial cells to express viral antigens with major histocompatibility complex class 1 and 2 antigens. These antigens are not constitutively expressed in the nervous system but can be induced by viral infection. Further experiments are needed to determine whether JCV can induced major histocompatibility complex antigens on neuroglial cells. Finally, assessing the ability of JCV-infected glial cells to synthesize lymphokines and interferons will provide further insight into immune reactions in PML lesions.

Future Treatment of PML

Successful treatment of PML remains elusive. Cytosine arabinoside and alpha interferon are the only agents of possible efficacy found in previous studies. Our understanding of the pathogenesis of PML as described in this review suggests other means of treatment which would not have been considered prior to the demonstration that JCV-infected B cells are the likely source of glial infection. The use of subcutaneous heparin, which prevents activated lymphocytes from crossing the blood-brain barrier in experimental animals, is one such treatment that has been instituted as a result of our increased knowledge of PML pathogenesis (27). Gamma interferon, which increased major histocompatibility complex antigen expression by virus-infected glial cells, may improve the effector functions of host T cytotoxic cells by enhancing recognition of latent JCV and could be used prophylactically in patients at risk of developing PML. Until antiviral drugs that target JCV in its multiplication cycle become available, immunomodulating agents that reflect our understanding of the pathogenesis of PML appear to offer the best approach to treatment.

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REFERENCES

1. **Ahmed, S., J. Rappaport, H. Tada, D. Kerr, and K. Khalili.** 1990. A nuclear protein derived from brain cells stimulates transcription of the human neurotropic virus promoter, JCV_E, in vitro. J. Biol. Chem. **265**:13899–13905.
2. **Aksamit, A. J., E. O. Major, N. R. Ghatak, G. S. Sidhu, J. E. Parisi, and J. G. Guccion.** 1987. Diagnosis of progressive multifocal leukoencephalopathy by brain biopsy with biotin labeled DNA:DNA in situ hybridization. J. Neuropathol. Exp. Neurol. **46**:556–566.
3. **Aksamit, A. J., P. Mourrain, J. L. Sever, and E. O. Major.** 1985. Progressive multifocal leukoencephalopathy: investigation of three cases using in situ hybridization with JCV virus biotinylated DNA probe. Ann. Neurol. **18**:490–496.
4. **Aksamit, A., and J. Proper.** 1988. JC virus replicates in primary adult astrocytes in culture. Ann. Neurol. **24**:471.
5. **Aksamit, A., J. L. Sever, and E. O. Major.** 1986. Progressive multifocal leukoencephalopathy: JC virus detection by *in situ* hybridization compared with immunocytochemistry. Neurology **36**:499–504.
6. **Amemiya, K., R. Traub, L. Durham, and E. O. Major.** 1989. Interaction of a nuclear factor-1-like protein with the regulatory region of the human polyomavirus JC virus. J. Biol. Chem. **264**:7025–7032.
7. **Amemiya, K., R. Traub, L. Durham, and E. O. Major.** 1991. Binding of NF-1 and c-jun to overlapping sites on the regulatory region of the human polyomavirus JC virus. Fed. Am. Soc. Exp. Biol. **5**:A1169.
8. **Amirhaeri, S., R. Wohlrab, E. O. Major, and R. D. Wells.** 1988. Unusual DNA structure in the regulatory region of the human papovavirus JC virus. J. Virol. **62**:922–931.
9. **Andrews, C. A., R. Daniel, and K. Shah.** 1983. Serologic studies of Papovavirus infections in pregnant women and renal transplant recipients, p. 133–141. *In* J. L. Sever and D. Madden (ed.), Polyomavirus and human neurological disease. Alan R. Liss, Inc., New York.
10. **Arthur, R., A. Beckmann, C. L. Chou, R. Saral, and K. Shah.** 1985. Direct detection of the human papovavirus BK in urine of bone marrow transplant recipients: comparison of DNA hybridization with ELISA. J. Med. Virol. **16**:29–36.
11. **Arthur, R. R., K. V. Shah, P. Charache, and R. Saral.** 1988. BK and JC virus infections in recipients of bone marrow transplants. J. Infect. Dis. **158**:563–569.
12. **Arthur, R. R., S. Dagostin, and K. Shah.** 1989. Detection of BK virus and JC virus in urine and brain tissue by the polymerase chain reaction. J. Clin. Microbiol. **27**:1174–1179.
13. **Assouline, J. G., and E. O. Major.** 1991. Human fetal Schwann cells support JC virus multiplication. J. Virol. **65**:1002–1006.
14. **Åstrom, K.-E., E. L. Mancall, and E. P. Richardson, Jr.** 1958. Progressive multifocal leukoencephalopathy. Brain **81**:93–127.
15. **Bateman, O., G. Squires, and S. Thannhauser.** 1945. Hodgkin's

disease associated with Schilder's disease. Ann. Intern. Med. **22**:426–431.
16. **Bauer, W., W. Chamberlin, and S. Horenstein.** 1969. Spinal demyelination in progressive multifocal leukoencephalopathy. Neurology **19**:287.
17. **Bauer, W. R., A. P. Turci, Jr., and K. P. Johnson.** 1973. Progressive multifocal leukoencephalopathy and cytarabine. JAMA **226**:174–176.
18. **Beckman, A., and K. V. Shah.** 1983. Propagation and primary isolation of JCV and BKV in urinary epithelial cell cultures, p. 3–14. *In* J. L. Sever and D. Madden (ed.), Polyomaviruses and human neurological diseases. Alan R. Liss, Inc., New York.
19. **Berger, J. R.** Personal communication.
20. **Berger, J. R., B. Kaszovitz, M. J. Post, and G. Dickinson.** 1987. Progressive multifocal leukoencephalopathy associated with human immunodeficiency virus infection. A review of the literature with a report of sixteen cases. Ann. Intern. Med. **107**:78–87.
21. **Berger, J. R., and L. Mucke.** 1988. Prolonged survival and partial recovery in AIDS-associated progressive multifocal leukoencephalopathy. Neurology **38**:1060–1065.
22. **Bikel, I., X. Montano, M. E. Agha, M. Brown, M. McCormack, J. Boltax, and D. M. Livingston.** 1987. SV40 small t antigen enhances the transformation activity of limiting concentrations of SV40 large T antigen. Cell **48**:321–330.
23. **Bollag, B., W.-F. Chuke, and R. J. Frisque.** 1989. Hybrid genomes of the polyomaviruses JC virus, BK virus, and simian virus 40: identification of sequences important for efficient transformation. J. Virol. **63**:863–872.
24. **Bosch, E. P., P. A. Cancill, and S. H. Cornell.** 1976. Computerized tomography in progressive multifocal leukoencephalopathy. Arch. Neurol. **33**:216.
25. **Brady, J., M. Radonovich, M. Vodkin, V. Natarajan, M. Thgoren, G. Das, J. Janik, and N. P. Salzman.** 1982. Site-specific base substitution and deletion mutations that enhance or suppress transcription of the SV40 major late RNA. Cell **31**:625–633.
26. **Brady, J. N., and N. P. Salzman.** 1986. The papovaviruses. General properties of polyoma and SV40, p. 1–26. *In* N. P. Salzman (ed.), The papovaviridae, vol. 1. The polyomaviruses. Plenum Press, New York.
27. **Brenan, M., and C. R. Parish.** 1986. Modification of lymphocyte migration by sulphated polysaccharides. Eur. J. Immunol. **16**:423–436.
28. **Brooks, B. R., and D. L. Walker.** 1984. Progressive multifocal leukoencephalopathy. Neurol. Clin. **2**:299–313.
29. **Brown, P., T. Tsai, and D. C. Gajdusek.** 1975. Seroepidemiology of human papovaviruses: discovery of virgin populations and some unusual patterns of antibody prevalence among remote peoples of the world. Am. J. Epidemiol. **102**:331–340.
30. **Buckman, R., and E. Wiltshaw.** 1976. Progressive multifocal leukoencephalopathy successfully treated with cytosine arabinoside. Br. J. Haematol. **34**:153–154.
31. **Budka, H., and K. Shah.** 1983. Papovavirus antigens in paraffin section of PML brains, p. 299–309. *In* J. L. Sever and D. Madden (ed.), Polyomaviruses and human neurological disease. Alan. R. Liss, Inc., New York.
32. **Castaigne, P., P. Roudot, T. Escourolle, J. L. Ribadeau-Dumas, F. Carthala, and J. J. Hauw.** 1974. Leucoencephalopathie multifocale progessive et "gliomes" multiple. Rev. Neurol. (Paris) **130**:379–392.
33. **Castleman, B., R. E. Scully, and B. J. McNeely.** 1972. Weekly clinicopathological exercise, case 19-1972. N. Engl. J. Med. **286**:1047–1054.
34. **Cavanaugh, J. B., D. Greenbaum, A. Marshall, and L. Rubinstein.** 1959. Cerebral demyelination associated with disorders of the reticuloendothelial system. Lancet **ii**:524–529.
35. **Celada, A., M. Suiga, M. Imagawa, J. Kop, and R. A. Maki.** 1988. Identification of a nuclear factor that binds to a conserved sequence of the I-A_b gene. J. Immunol. **140**:3995–4002.
36. **Chesters, P. M., J. Heritage, and D. J. McCance.** 1983. Persistence of DNA sequences of BK virus and JC virus in normal human tissues and in diseased tissues. J. Infect. Dis. **147**:676–682.
37. **Chodosh, L. A., A. S. Baldwin, R. W. Carthew, and P. A. Sharp.** 1988. Human CCAAT-binding proteins have heterologous subunits. Cell **53**:11–24.
38. **Christensen, E., and M. Fog.** 1955. Case of Schilder's disease in adult with remarks on etiology and pathogenesis. Acta Psychiatr. Neurol. Scand. **30**:141–154.
39. **Chuke, W.-F., D. L. Walker, L. B. Peitzman, and R. J. Frisque.** 1986. Construction and characterization of hybrid polyomavirus genomes. J. Virol. **60**:960–971.
40. **Coleman, D. V., S. D. Gardner, and A. M. Field.** 1973. Human polyomavirus infection in renal allograft recipients. Br. Med. J. **3**:371–375.
41. **Coleman, D. V., M. R. Wolfendale, R. A. Daniel, N. K. Dhanjal, S. D. Gardner, P. E. Gibson, and A. M. Field.** 1980. A prospective study of human polyomavirus infection in pregnancy. J. Infect. Dis. **142**:1–8.
42. **Conomy, J. P., N. S. Beard, H. Matsumoto, and U. Roessmann.** 1974. Cytarabine treatment of progressive multifocal leukoencephalopathy. JAMA **229**:1313–1316.
43. **Conomy, J. P., M. A. Weinstein, D. Agamanolis, and W. S. Holt.** 1976. Computed tomography in progressive multifocal leukoencephalopathy. Am. J. Roentgenol. **127**:663–665.
44. **Conway, B., W. C. Halliday, and R. C. Brunham.** 1990. Human immunodeficiency virus-associated progressive multifocal leukoencephalopathy: apparent response to 3′-azido-3′-deoxythymidine. Rev. Infect. Dis. **12**:479–482.
45. **Cooper, M.** 1987. B lymphocytes: new development and function. N. Engl. J. Med. **317**:1452–1456.
46. **Cunningham, M. E., P. R. S. Kishore, S. S. Rengachary, and S. Preskorn.** 1977. Progressive multifocal leukoencephalopathy presenting as a focal mass lesion in the brain. Surg. Neurol. **8**:448–450.
47. **Daniel, R., K. Shah, D. Madden, and S. Stagno.** 1981. Serological investigation of the possible congenital transmission of papovavirus JC. Infect. Immun. **33**:319–321.
48. **Dawson, D. M.** 1982. Progressive multifocal leukoencephalopathy in myasthenia gravis. Ann. Neurol. **11**:218–219.
49. **De Pamphilis, M. L., and M. K. Bradley.** 1986. Replication of SV40 and polyoma virus chromosomes, p. 99–246. *In* N. P. Salzman (ed.), The papovaviridae, vol. 1. The polyomaviruses. Plenum Press, New York.
50. **Dorn, A., J. Ballekens, A. Staub, C. Benoist, and D. Mathis.** 1987. A multiplicity of CCAAT box-binding proteins. Cell **50**:863–872.
51. **Dörries, K.** 1984. Progressive multifocal leukoencephalopathy: analysis of JC virus DNA from brain and kidney tissue. Virus Res. **1**:25–38.
52. **Dörries, K., R. T. Johnson, and V. Ter Meulen.** 1979. Detection of polyoma virus DNA in PML-brain tissue by (in situ) hybridization. J. Gen. Virol. **42**:49–57.
53. **Dörries, K., G. Loeber, and J. Meixensbarger.** 1987. Association of polyomaviruses JC, SV40, and BK with human brain tumors. Virology **160**:268–270.
54. **Dyson, N., R. Bernards, S. Friend, L. Gooding, J. Hassel, E. Major, J. Pipas, T. Vandyke, and E. Harlow.** 1990. Large T proteins of many polyomaviruses are able to form complexes with the retinoblastoma protein. J. Virol. **64**:1353–1356.
55. **Egan, J. D., B. L. Ring, M. J. Reding, and I. C. Wells.** 1980. Reticulum cell sarcoma and progressive multifocal leukoencephalopathy following renal transplantation. Transplantation **29**:84–86.
56. **Elder, G., and E. O. Major.** 1988. Early appearance of type II astrocytes in developing human fetal brain. Dev. Brain Res. **42**:146–150.
57. **Embry, J. R., F. G. Silva, J. H. Helderman, P. Peters, and A. I. Sagalowsky.** 1988. Long term survival and late development of bladder cancer in renal transplant patient with progressive multifocal leukoencephalopathy. J. Urol. **139**:580–581.
58. **Fareed, G. C., K. K. Takemoto, and M. A. Gimbrone, Jr.** 1978. Interaction of simian virus 40 and human papovaviruses, BK and JC, with human vascular endothelial cells, p. 427–431. *In*

D. Schlessinger (ed.), Microbiology—1978. American Society for Microbiology, Washington, D.C.
59. **Farrell, D. F.** 1969. The EEG in progressive multifocal leukoencephalopathy. Electroencephalogr. Clin. Neurophysiol. **26:** 200–205.
60. **Feigenbaum, L., K. Khalili, E. O. Major, and G. Khoury.** 1987. Regulation of the host range of human papovavirus JCV. Proc. Natl. Acad. Sci. USA **84:**3695–3698.
61. **Flægstad, T., A. Sundsfjord, R. R. Arthur, M. Pedersen, T. Traavik, and S. Subramani.** 1991. Amplification and sequencing of the control regions of BK and JC virus from human urine by polymerase chain reaction. Virology **180:**553–560.
62. **Fresa, K. L., H. E. Karjalainen, and S. S. Tevethia.** 1987. Sensitivity of simian virus 40-transformed c57BL/6 mouse embryo fibroblasts to lysis by murine natural killer cells. J. Immunol. **138:**1215–1220.
63. **Frisque, R.** 1983. Regulatory sequences and virus-cell interactions of JC virus, p. 41–59. *In* J. L. Sever and D. Madden (ed.), Polyomaviruses and human neurological disease. Alan R. Liss, Inc., New York.
64. **Frisque, R., G. Bream, and M. Cannella.** 1984. Human polyomavirus JC virus genome. J. Virol. **51:**458–469.
65. **Frisque, R. J., J. D. Martin, B. Padgett, and D. Walker.** 1979. Infectivity of the DNA from four isolates of JC virus. J. Virol. **32:**476–482.
66. **Frisque, R. J., D. B. Rifkin, and D. L. Walker.** 1980. Transformation of primary hamster brain cells with JC virus and its DNA. J. Virol. **35:**265–269.
67. **Gardner, S., E. Mackenzie, C. Smith, and A. Porter.** 1984. Prospective study of the human polyomaviruses BK and JC and cytomegalovirus in renal transplant recipients. J. Clin. Pathol. **37:**578–586.
68. **Gardner, S. D.** 1973. Prevalence in England of antibody to human polyomavirus (BK). Br. Med. J. **1:**77–78.
69. **Gardner, S. D., A. M. Field, D. V. Coleman, and B. Hulme.** 1971. New human papovirus (BK) isolated from urine after renal transplantation. Lancet **i:**1253–1257.
70. **Gendelman, H., W. Phelps, L. Feigenbaum, J. Ostrove, A. Adachi, P. Howley, G. Khoury, H. Ginsberg, and M. Martin.** 1986. Trans-activation of the human immunodeficiency virus long terminal repeat by DNA viruses. Proc. Natl. Acad. Sci. USA **83:**9759–9763.
71. **Gendelman, H. E., R. M. Friedman, S. Joe, L. Baca, I. Turpin, G. Dveksler, M. Meltzer, and C. Dieffenbach.** 1990. A selective defect of interferon alpha production in human immunodeficiency virus-infected monocytes. J. Exp. Med. **172:**1433–1442.
72. **Gerber, M. A., K. V. Shah, S. N. Thung, and G. M. ZuRhein.** 1980. Immunohistochemical demonstration of common antigen of polyomaviruses in routine histologic tissue sections of animals and man. Am. J. Clin. Pathol. **73:**794–797.
73. **Ghosh, P. K., P. Lebowitz, R. J. Frisque, and Y. Gluzman.** 1981. Identification of a promoter component involved in positioning the 5′ termini of simian virus 40 early mRNAs. Proc. Natl. Acad. Sci. USA **78:**100–104.
74. **Gia Russo, M. H., and A. H. Koeppen.** 1978. Atypical progressive multifocal leukoencephalopathy and primary malignant lymphoma. J. Neurol. Sci. **35:**391–398.
75. **Gluzman, Y., J. F. Sambrook, and R. J. Frisque.** 1980. Expression of early genes of origin-defective mutants of simian virus 40. Proc. Natl. Acad. Sci. USA **77:**3898–3902.
76. **Gorman, C., L. Moffat, and B. Howard.** 1984. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2:**1044–1051.
77. **Greenlee, J., and P. M. Keeney.** 1986. Immunoenzymatic labelling of JC papovavirus T antigen in brains of patients with progressive multifocal leukoencephalopathy. Acta Neuropathol. **71:**150–153.
78. **Greenlee, J. E.** 1990. Progressive multifocal leukoencephalopathy. Curr. Clin. Top. Infect. Dis. **10:**140–156.
79. **Grinnell, B. W., J. D. Martin, B. L. Padgett, and D. L. Walker.** 1982. Is progressive multifocal leukoencephalopathy a chronic disease because of defective interfering particles or temperature-sensitive mutants of JC virus? J. Virol. **43:**1143–1150.
80. **Grinnell, B. W., B. L. Padgett, and D. L. Walker.** 1983. Comparison of infectious JC virus DNAs cloned from human brain. J. Virol. **45:**299–308.
81. **Grinnell, B. W., B. L. Padgett, and D. L. Walker.** 1983. Distribution of nonintegrated DNA from JC papovavirus in organs of patients with progressive multifocal leukoencephalopathy. J. Infect. Dis. **147:**669–675.
82. **Gruss, P., and G. Khoury.** 1981. The SV 40 tandem repeats as an element of the early promoter. Proc. Natl. Acad. Sci. USA **78:**943–947.
83. **Guilleux, M. H., R. E. Steiner, and I. R. Young.** 1986. MR imaging in progressive multifocal leukoencephalopathy. Am. J. Neurol. Radiol. **7:**1033–1035.
84. **Haggerty, S., D. L. Walker, and R. J. Frisque.** 1989. JC virus-simian virus 40 genomes containing heterologous regulatory signals and chimeric early regions: identification of regions restricting transformation by JC virus. J. Virol. **63:**2180–2190.
85. **Hallervorden, J.** 1930. Eigennartige und nicht rubriziebare Prozesse, p. 1063–1107. *In* O. Bumke (ed.), Handbuch der Geiteskranheiten, vol. 2. Die Anatomie der Psychosen. Springer, Berlin.
86. **Hedley-Whyte, E. T., B. P. Smith, H. T. Tyler, and W. P. Peterson.** 1966. Multifocal leukoencephalopathy with remission and five year survival. J. Neuropathol. Exp. Neurol. **25:**107–116.
87. **Henson, J., M. Rosenblum, and H. Furneaux.** 1991. A potential diagnostic test for PML: PCR analysis of JC Virus DNA. Neurology **41**(Suppl.):338.
88. **Ho, K.-C., J. C. Garancis, R. D. Paegli, M. S. Gerber, and W. J. Borkowski.** 1980. Progressive multifocal leukoencephalopathy and malignant lymphoma of the brain in a patient with immunosuppressive therapy. Acta Neuropathol. **52:**81–83.
89. **Hogan, E. L.** 1977. Animal models of genetic myelin disorders, p. 489–520. *In* P. Morell (ed.), Myelin. Plenum Press, New York.
90. **Hogan, T. F., B. L. Padgett, D. L. Walker, E. C. Bordon, and J. A. McBain.** 1980. Rapid detection and identification of JC virus and BK virus in human urine by using immunofluorescence microscopy. J. Clin. Microbiol. **11:**178–183.
91. **Houff, S. A.** Personal communication.
92. **Houff, S. A., D. Katz, C. Kufta, and E. O. Major.** 1989. A rapid method for in situ hybridization for viral DNA in brain biopsies from patients with acquired immunodeficiency syndrome (AIDS). AIDS **3:**843–845.
93. **Houff, S. A., E. O. Major, D. Katz, C. Kufta, J. Sever, S. Pittaluga, J. Roberts, J. Gitt, N. Saini, and W. Lux.** 1988. Involvement of JC virus-infected mononuclear cells from the bone marrow and spleen in the pathogenesis of progressive multifocal leukoencephalopathy. N. Engl. J. Med. **318:**301–305.
94. **Hou-Jong, M.-H. J., S. H. Larson, and A. Roman.** 1987. Role of the agnoprotein in regulation of simian virus 40 replication and maturation pathways. J. Virol. **61:**937–939.
95. **Howley, P. M., G. Khoury, K. K. Takemoto, and M. A. Martin.** 1976. Polynucleotide sequences common to the genomes of simian virus 40 and the human papovaviruses JC and BK. Virology **73:**303–307.
96. **Howley, P. M., F. Rentier-DelRue, C. A. Heilman, M. F. Law, K. Chowdhury, M. A. Israel, and K. K. Takemoto.** 1980. Cloned human polyomavirus JC DNA can transform human amnion cells. J. Virol. **36:**878–882.
97. **Itoyama, Y. H. deF. Webster, N. H. Sternberger, E. P. Richardson, Jr., D. L. Walker, R. H. Quarles, and B. L. Padgett.** 1982. Distribution of papovavirus, myelin-associated glycoprotein and myelin basic protein in progressive multifocal leukoencephalopathy lesions. Ann. Neurol. **11:**369–407.
98. **Jay, G., S. Nomura, C. W. Anderson, and G. Khoury.** 1981. Identification of the SV40 agnogene product: a DNA binding protein. Nature (London) **291:**346–349.
99. **Johnson, R. T.** 1982. Viral infections of the nervous system, p. 244–252. Raven Press, New York.
100. **Jones, H. R., T. Hedley-Whyte, S. R. Freidberg, J. E. Kelleher,**

and J. Krolikowski. 1982. Primary cerebellopontine progressive multifocal leukoencephalopathy diagnosed premortem by cerebellar biopsy. Ann. Neurol. **11**:199–202.

101. **Kennedy, P. G. E., and J. Fok-Seang.** 1986. Studies on the development, antigenic phenotype, and function of human glial cells in tissue culture. Brain **109**:1261–1277.
102. **Kenney, S., V. Natarajan, and N. P. Salzman.** 1986. Mapping 5′ termini of JC virus late RNA. J. Virol. **58**:216–219.
103. **Kenney, S., V. Natarajan, G. Selzer, and N. P. Salzman.** 1986. Mapping 5′ termini of JC virus early RNAs. J. Virol. **58**:651–654.
104. **Kenney, S., V. Natarajan, D. Strike, G. Khoury, and N. P. Saltzman.** 1984. JC virus enhancer-promoter active in human brain cells. Science **226**:1337–1339.
105. **Kepes, J. J., S. M. Chou, and L. W. Price, Jr.** 1975. Progressive multifocal leukoencephalopathy with 10 year survival in a patient with nontropical sprue: report of a case with unusual light and electron microscopic features. Neurology **25**:1006–1012.
106. **Khalili, K., L. Feigenbaum, and G. Khoury.** 1987. Evidence for a shift in 5′ termini of early viral RNA during the lytic cycle of JC virus. Virology **158**:469–472.
107. **Khalili, E., J. Rappaport, and G. Khoury.** 1988. Nuclear factors in human brain cells bind specifically to the JCV regulatory region. EMBO J. **7**:1205–1210.
108. **Kiyosawa, M., T. M. Bosley, A. Alavi, N. Gupta, C. H. Rhodes, J. Chawluk, M. Kushner, P. J. Savino, R. C. Sergott, N. J. Schatz, and M. Reivich.** 1988. Positron emission tomography in a patient with progressive multifocal leukoencephalopathy. Neurology **38**:1864–1867.
109. **Knight, R. S., N. M. Hyman, S. D. Gardner, P. E. Gibson, M. M. Esiri, and C. P. Warlow.** 1988. Progressive multifocal leukoencephalopathy and viral antibody titres. J. Neurol. **235**:458–461.
110. **Krempien, B., F.-W. Kolkmann, H. G. Schiemer, and P. Mayer.** 1972. Uber die progressive multifokale Leukoencephalopathie (Beitrag zur Differentialdiagnose mit elektronenmikroskopischen und cytophotometrischen Untersuchungen). Virchows Arch. A **355**:158–178.
111. **Krupp, L. B., R. B. Lipton, M. L. Swerdlow, N. E. Leeds, and J. Llena.** 1985. Progressive multifocal leukoencephalopathy: clinical and radiographic features. Ann. Neurol. **17**:344–349.
112. **Law, M.-F., J. D. Martin, K. K. Takemoto, and P. M. Howley.** 1979. The colinear alignment of the genomes of papovaviruses JC, BK, and SV40. Virology **96**:576–587.
113. **Lecatsas, G., O. W. Prozesky, J. Van Wyk, and H. J. Els.** 1973. Papovavirus in urine after renal transplantation. Nature (London) **241**:343–344.
114. **Ledoux, S., I. Libman, F. Robert, and N. Just.** 1989. Progressive multifocal leukoencephalopathy with gray matter involvement. Can. J. Neurol. Sci. **16**:200–202.
115. **Levy, J. D., K. L. Cottingham, R. J. Campbell, G. K. Moore, F. Gyorkey, T. Ashizawa, and A. M. Goldman.** 1986. Progressive multifocal leukoencephalopathy and magnetic resonance imaging. Ann. Neurol. **19**:399–401.
116. **Lipton, R. B., L. Krupp, D. Horoupian, S. Hershkovitz, and J. C. Arezzo.** 1988. Progressive multifocal leukoencephalopathy of the posterior fossa in an AIDS patient: clinical, radiographic and evoked potential findings. Eur. Neurol. **28**:258–261.
117. **Loeber, G., and K. Dörries.** 1988. DNA rearrangements in organ-specific variants of polyomavirus JC strain G5. J. Virol. **62**:1730–1735.
118. **London, W. T., S. A. Houff, D. L. Madden, D. A. Fuccillo, M. Gravell, W. C. Wallen, A. E. Palmer, J. L. Sever, B. L. Padgett, D. L. Walker, G. M. ZuRhein, and T. Ohashi.** 1978. Brain tumors in owl monkeys inoculated with a human polyomavirus (JC virus). Science **201**:1246–1249.
119. **London, W. T., S. A. Houff, P. E. McKeever, W. C. Wallen, J. L. Sever, B. L. Padgett, and D. L. Walker.** 1983. Viral-induced astrocytomas in squirrel monkeys. Prog. Clin. Biol. Res. **105**:227–237.
120. **Lynch, K. J., and R. J. Frisque.** 1990. Identification of critical elements within the JC virus DNA replication origin. J. Virol. **64**:5812–5822.
121. **Lynch, K. J., and R. J. Frisque.** 1991. Factors contributing to the restricted DNA replicating activity of JC virus. Virology **180**:306–317.
122. **Major, E. O., K. Amemiya, G. Elder, and S. A. Houff.** 1990. Glial cells of the human developing brain and B cells of the immune system share a common DNA binding factor for recognition of the regulatory sequences of the human polyomavirus, JCV. J. Neurosci. Res. **27**:461–471.
123. **Major, E. O., and P. Matsumura.** 1984. Human embryonic kidney cells: stable transformation with an origin defective simian virus 40 DNA and use as host for human polyomavirus replication. Mol. Cell Biol. **4**:379–382.
124. **Major, E. O., A. E. Miller, P. Mourrain, R. Traub, E. De Widt, and J. L. Sever.** 1985. Establishment of a line of human glial cells that supports JC virus multiplication. Proc. Natl. Acad. Sci. USA **82**:1257–1261.
125. **Major, E. O., P. Mourrain, and C. Cummins.** 1984. JC virus induced owl monkey glioblastoma cells in culture: biological properties associated with the viral early gene product. Virology **136**:359–367.
126. **Major, E. O., and R. Traub.** 1986. JC virus T protein during productive infection in human fetal brain and kidney cells. Virology **148**:221–225.
127. **Major, E. O., and D. A. Vacante.** 1989. Human fetal astrocytes in culture support the growth of the neurotropic human polyomavirus, JCV. J. Neuropathol. Exp. Neurol. **48**:425–436.
128. **Major, E. O., D. A. Vacante, R. G. Traub, W. T. London, and J. L. Sever.** 1987. Owl monkey astrocytoma cells in culture spontaneously produce infectious JC virus which demonstrates altered biological properties. J. Virol. **61**:1435–1441.
129. **Mantyjdurvi, R. A., O. H. Meurman, L. Vihma, and B. Berglund.** 1973. A human papovavirus (BK): biological properties and seroepidemiology. Ann. Clin. Res. **5**:282–287.
130. **Mandl, C. W., and R. J. Frisque.** 1986. Characterization of cells transformed by the human polyomavirus JC virus. J. Gen. Virol. **67**:1733–1739.
131. **Mandl, C., D. L. Walker, and R. J. Frisque.** 1987. Derivation and characterization of POJ cells, transformed human fetal glial cells that retain their permissivity for JC virus. J. Virol. **61**:755–763.
132. **Mark, A. S., and S. W. Atlas.** 1989. Progressive multifocal leukoencephalopathy in patients with AIDS: appearance on MR images. Radiology **173**:517–520.
133. **Marriott, P. J., M. D. O'Brien, I. C. Mackenzie, and I. Janota.** 1975. Progressive multifocal leukoencephalopathy: remission with cytarabine. J. Neurol. Neurosurg. Psychiatry **38**:205–209.
134. **Martin, J. D., K. H. Brackmann, B. Grinnell, and R. J. Frisque.** 1982. Recombinant JC virus DNA: verification and physical map of prototype. Biochem. Biophys. Res. Commun. **109**:70–77.
135. **Martin, J. D., R. J. Frisque, B. L. Padgett, and D. L. Walker.** 1979. Restrictive endonuclease cleavage map of the DNA of JC virus. J. Virol. **29**:846–855.
136. **Martin, J. D., D. M. King, J. M. Slauch, and R. J. Frisque.** 1985. Differences in regulatory sequences of naturally occurring JC virus variants. J. Virol. **53**:306–311.
137. **Martin, J. D., B. L. Padgett, and D. L. Walker.** 1983. Characterization of tissue culture-induced heterogenicity in DNAs of independent isolates of JC virus. J. Gen. Virol. **64**:2271–2280.
138. **Matsuda, M., K. Yasui, K. Nagashima, and W. Mori.** 1987. Genetic characterization of JC Virus Tokyo-1 strain, a variant oncogenic in rodents. Virus Res. **7**:159–168.
130. **Mazló, M., and I. Tariska.** 1980. Morphological demonstration of the first phase of polyomavirus replication in oligodendroglia cells of human brain in progressive multifocal leukoencephalopathy (PML). Neuropathology **49**:133–143.
140. **Mazló, M., and I. Tariska.** 1982. Are astrocytes infected in progressive multifocal leukoencephalopathy (PML)? Acta Neuropathol. **56**:45–51.
141. **McKeever, P., B. Chronwall, S. Houff, J. Sever, P. Kornblith, B. Padgett, D. Walker, and W. T. London.** 1983. Glial and

divergent cells in primate central nervous system tumors induced by JC Virus isolated from human progressive multifocal leukoencephalopathy (PML), p. 239–252. *In* J. L. Sever and D. Madden (ed.), Polyomaviruses and human neurological disease. Alan R. Liss, Inc., New York.

142. **Miller, N. R., W. T. London, B. L. Padgett, D. L. Walker, and W. C. Wallen.** 1984. Brain tumors of owl monkeys inoculated with JC virus contain the JC virus genome. J. Virol. **49**:848–856.
143. **Miyamura, T., A. Furuno, and K. Yoshiike.** 1985. DNA rearrangement in the control region of early transcription in a human polyomavirus JC host range mutant capable of growing in human embryonic kidney cells. J. Virol. **54**:750–756.
144. **Miyamura, T., H. Jikuya, E. Soeda, and K. Yoshiike.** 1983. Genomic structure of human polyoma virus JC: nucleotide sequence of the region containing replication origin and small-T-antigen gene. J. Virol. **45**:73–79.
145. **Miyamura, T., K. Yoshiike, and K. K. Takemoto.** 1980. Characterization of JC papovavirus adapted to growth in human embryonic kidney cells. J. Virol. **35**:498–504.
146. **Monier, R.** 1986. Transformation by SV40 and polyoma viruses, p. 247–294. *In* N. P. Salzman (ed.), The papovaviridae, vol. 1. The polyomaviruses. Plenum Press, New York.
147. **Myers, C., R. J. Frisque, and R. R. Arthur.** 1989. Direct isolation and characterization of JC virus from urine samples of renal and bone marrow transplant patients. J. Virol. **63**:4445–4449.
148. **Myers, R. M., D. C. Rio, A. K. Robbins, and R. Tjian.** 1981. SV40 gene expression is modulated by the cooperative binding of t antigen to DNA. Cell **25**:373–384.
149. **Nagashima, K., K. Yasui, J. Kimura, M. Washizu, K. Yamaguchi, and W. Mori.** 1984. Induction of brain tumors by a newly isolated JC virus (Tokyo-1 strain). Am. J. Pathol. **116**:455–463.
150. **Nandi, A., G. Dos, and N. P. Salzman.** 1985. Characterization of a surrogate TATA box promoter that regulates in vitro transcription of the simian virus 40 major late gene. Mol. Cell. Biol. **5**:591–594.
151. **New England Journal of Medicine. Case records of the Massachusetts General Hospital.** 1987. Weekly clinicopathological exercises. Case 1-1987. A 58-year-old-man with a malignant thymoma and confusion. N. Engl. J. Med. **316**:35–42.
152. **New England Journal of Medicine. Case records of the Massachusetts General Hospital.** 1988. Weekly clinicopathological exercises. Case 45-1988. A 67-year-old woman with chronic lymphocytic leukemia, homonymous hemianopia, confusion, and poor balance. N. Engl. J. Med. **319**:1268–1280.
153. **Ohsumi, S., M. Motoi, and K. Ogawa.** 1986. Induction of undifferentiated tumors by JC virus in the cerebrum of rats. Acta Pathol. **36**:815–825.
154. **O'Riordan, T., P. A. Daly, M. Hutchinson, A. G. Shattock, and S. D. Gardner.** 1990. Progressive multifocal leukoencephalopathy—remission with cytarabine. J. Infect. **20**:51–54.
155. **Osborn, J. E., S. M. Robertson, B. L. Padgett, G. M. ZuRhein, D. L. Walker, and B. Weisblum.** 1974. Comparison of JC and BK viruses with simian virus 40: restriction endonuclease digestion and gel electrophoresis of resultant fragments. J. Virol. **13**:614–622.
156. **Padgett, B. L., C. M. Rogers, and D. L. Walker.** 1977a. JC virus, a human polyomavirus associated with progressive multifocal leukoencephalopathy: additional biological characteristics and antigenic relationships. Infect. Immun. **15**:656–662.
157. **Padgett, B. L., and D. L. Walker.** 1973. Prevalence of antibodies in human sera against JC virus, an isolate from a case of progressive multifocal leukoencephalopathy. J. Infect. Dis. **127**:467–470.
158. **Padgett, B. L., and D. L. Walker.** 1983. Virologic and serologic studies of progressive multifocal leukoencephalopathy. Prog. Clin. Biol. Res. **105**:107–117.
159. **Padgett, B. L., D. L. Walker, G. M. ZuRhein, A. E. Hodach, and S. M. Chou.** 1976. JC papovavirus in progressive multifocal leukoencephalopathy. J. Infect. Dis. **133**:686–690.
160. **Padgett, B. L., D. L. Walker, G. M. ZuRhein, and J. N. Varakis.** 1977. Differential neurooncogenicity of strains of JC virus, a human polyoma virus, in newborn Syrian hamsters. Cancer Res. **37**:718–720.
161. **Padgett, B. L., G. ZuRhein, D. Walker, R. Echroade, and B. Dessel.** 1971. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. Lancet **i**:1257–1260.
162. **Parr, J., D. S. Horoupian, and C. Winkelman.** 1979. Cerebellar form of progressive multifocal leukoencephalopathy (PML). Can. J. Neurol. Sci. **6**:123–128.
163. **Peters, A. C. B., J. Versteeg, G. T. A. Bots, W. Boogerd, and G. J. Vielvoye.** 1980. Progressive multifocal-leukoencephalopathy: immunofluorescent demonstration of SV40 antigen in CSF cells and response to cytarabine therapy. Arch. Neurol. **37**:497–501.
164. **Portegies, P., P. R. Algra, C. E. M. Hollar, J. M. Prins, P. Reiss, J. Valk, and J. Lange.** 1991. Response to cytarabine in progressive multifocal leukoencephalopathy in AIDS. Lancet **337**:680–681.
165. **Post, M. J., J. J. Sheldon, G. T. Hensley, K. Soila, J. Tobias, J. Curan, R. Querar, and L. Moskowitz.** 1986. Central nervous system disease in acquired immunodeficiency syndrome: prospective correlation using CT, MR imaging and pathologic studies. Radiology **158**:141–148.
166. **Preskorn, S. H., and I. Watanabe.** 1979. Progressive multifocal leukoencephalopathy: cerebral mass lesions. Surg. Neurol. **12**:231–234.
167. **Price, R. W., S. Nielsen, B. Horten, M. Rubino, B. Padgett, and D. Walker.** 1983. Progressive multifocal leukoencephalopathy: a burnt-out case. Ann. Neurol. **13**:485–490.
168. **Rand, K. H., K. P. Johnson, L. J. Rubenstein, J. Wolinsky, J. Penney, D. Walker, B. Padgett, and T. Merigan.** 1977. Adenine arabinoside in the treatment of progressive multifocal leukoencephalopathy: use of virus containing cells in the urine to assess response to therapy. Ann. Neurol. **1**:458–462.
169. **Raymondjean, M., S. Cereghini, and M. Yaniv.** 1988. Several distinct "CCAAT" box binding proteins coexist in eukaryotic cells. Proc. Natl. Acad. Sci. USA **85**:757–761.
170. **Rentier-Delrue, F., A. Lubiniecki, and P. M. Howley.** 1981. Analysis of JC virus DNA purified directly from human progressive multifocal leukoencephalopathy brains. J. Virol. **38**:761–769.
171. **Rhodes, R. H., J. M. Ward, D. L. Walker, and A. A. Ross.** 1988. Progressive multifocal leukoencephalopathy and retroviral encephalitis in acquired immunodeficiency syndrome. Arch. Pathol. Lab. Med. **112**:1207–1213.
172. **Richardson, E. P., Jr.** 1961. Progressive multifocal leukoencephalopathy. N. Engl. J. Med. **265**:815–823.
173. **Richardson, E. P., Jr.** 19674. Our evolving understanding of progressive multifocal leukoencephalopathy. Ann. N.Y. Acad. Sci. **230**:358–364.
174. **Richardson, E. P., Jr., and P. C. Johnson.** 1975. Atypical progressive multifocal leukoencephalopathy with plasma-cell infiltrates. Acta Neuropathol. Suppl. **6**:247–250.
175. **Richardson, E. P., and H. deF. Webster.** 1983. Progressive multifocal leukoencephalopathy: its pathological features, p. 191–203. *In* J. L. Sever and D. Madden (ed.), Polyomaviruses and human neurological disease. Alan R. Liss, Inc., New York.
176. **Rockwell, D., F. L. Ruben, A. Winkelstein, and H. Mendelow.** 1976. Absence of immune deficiencies in a case of progressive multifocal leukoencephalopathy. Am. J. Med. **61**:433–436.
177. **Rosenthal, N., M. Kress, P. Gruss, and G. Khoury.** 1983. BK viral enhancer element and a human cellular homology. Science **233**:749–755.
178. **Rziha, H. J., G. Bornkamm, and H. ZurHausen.** 1978. Seroepidemiological studies and serologic response to viral infection. Med. Microbiol. Immunol. **165**:73–92.
179. **Salzman, N., V. Natarajan, and G. Selzer.** 1986. Transcription of SV40 and polyoma virus and its regulation, p. 27–98. *In* N. P. Salzman (ed.), The papovaviridae, vol. 1. The polyomaviruses. Plenum Press, New York.
180. **Sangalang, V. E., and J. A. Embil.** 1984. Emergence of

papovavirus in long-term cultures of astrocytes from progressive multifocal leukoencephalopathy patients. J. Neuropathol. Exp. Neurol. **43**:553–567.

181. **Saxton, C. R., P. Gailiunas, J. Helderman, R. Farkas, R. McKay, A. Diehl, A. Sagalowsky, K. Murphy, E. Ross, F. Silva, and D. Walker.** 1984. Progressive multifocal leukoencephalopathy in a renal transplant recipient: increased diagnostic sensitivity of computed tomography scanning by double dose contrast with delayed films. Am. J. Med. **77**:333–337.
182. **Schlitt, M., R. Morawetz, J. Bonnin, B. Chandra-Sekar, J. Curtiss, A. Diethelm, J. Whelchel, and R. Whitley.** 1986. Progressive multifocal leukoencephalopathy: three patients diagnosed by brain biopsy with prolonged survival in two. Neurosurgery **18**:407–414.
183. **Schmidbauer, M., H. Budka, and K. V. Shah.** 1990. Progressive multifocal leukoencephalopathy (PML) in AIDS and in the pre-AIDS era. A neuropathological comparison using immunocytochemistry and in situ DNA hybridization for virus detection. Acta Neuropathol. **80**:375–380.
184. **Schneider, A., U. Papendick, L. Gissmann, and E. M. DeVilliers.** 1987. Interferon treatment of human genital papillomavirus infection: importance of viral type. Int. J. Cancer **40**:610–614.
185. **Selhorst, J. B., K. F. Ducy, J. M. Thomas, and W. Reggelson.** 1978. Remission and immunologic reversals. Neurology **28**: 337.
186. **Shafran, B., M. E. Roke, R. M. Barr, and J. G. Cairncross.** 1987. Contrast enhancing lesions in progressive multifocal leukoencephalopathy: a clinicopathological correlation. Can. J. Neurol. Sci. **14**:600–602.
187. **Shapshak, P., W. W. Tourtellotte, M. Wolman, N. Verity, M. Verity, P. Schmid, K. Syndulka, E. Bedows, R. Boonsanfar, M. Darvish, S. Nakasuma, U. Tomyasa, R. Steiner, S. Hawkins, D. Hoffman, F. Adhami, and S. Martinez.** 1986. Search for virus nucleic acid sequences in postmortem human brain tissue using in situ hybridization technology with cloned probes: some solutions and results on progressive multifocal leukoencephalopathy and subacute sclerosing panencephalitis tissue. J. Neurosci. Res. **16**:281–301.
188. **Shein, H. M.** 1965. Propagation of human fetal spongioblasts and astrocytes in dispersed cell cultures. Exp. Cell Res. **40**:554–569.
189. **Sidman, R. L., M. M. Dickie, and S. H. Appel.** 1984. Mutant mice (*quaking* and *jimpy*) with deficient myelination in the central nervous system. Science **144**:309–311.
190. **Sima, A. A. F., S. D. Finkelstein, and D. R. McLachlan.** 1983. Multiple malignant astrocytomas in a patient with spontaneous progressive multifocal leukoencephalopathy. Ann. Neurol. **14**: 183–188.
191. **Small, J., G. A. Scangos, L. Cork, G. Jay, and G. Khoury.** 1986. The early region of human papovavirus JC induces dysmyelination in transgenic mice. Cell **46**:13–18.
192. **Small, J. A., G. Khoury, G. Jay, P. M. Howley, and G. A. Scangos.** 1986. Early regions of JC virus and BK virus induce distinct and tissue-specific tumors in transgenic mice. Proc. Natl. Acad. Sci. USA **83**:8288–8292.
193. **Smith, C. R., A. A. F. Sima, I. E. Salit, and F. Gentili.** 1982. Progressive multifocal leukoencephalopathy: failure of cytarabine therapy. Neurology **32**:200–203.
194. **Sogos, V., F. Bussolino, E. Pilia, S. Torelli, and F. Gremo.** 1990. Acetylcholine-induced production of platelet-activating factor by human fetal brain cells in culture. J. Neurosci. Res. **27**:706–711.
195. **Stoner, G., and C. Ryschkewitsch.** 1991. Evidence of JC Virus in two progressive multifocal leukoencephalopathy (PML) brains previously reported to be infected with SV40. J. Neuropathol. Exp. Neurol. **50**:342.
196. **Stoner, G., C. Ryschkewitsch, D. Walker, D. Stoffer, and H. deF. Webster.** 1988. A monoclonal antibody to SV40 large T-antigen labels a nuclear antigen in JC virus-transformed cells and in progressive multifocal leukoencephalopathy (PML) brain infected with JC virus. J. Neuroimmunol. **17**:331–345.
197. **Stoner, G. L., C. F. Ryschkewitsch, D. L. Walker, and H. deF. Webster.** 1986. JC papovavirus large tumor (T)-antigen expression in brain tissue of acquired immune deficiency syndrome (AIDS) and non-AIDS patients with progressive multifocal leukoencephalopathy. Proc. Natl. Acad. Sci. USA **83**:2271–2275.
198. **Stoner, G. L., D. Soffer, C. F. Ryschkewitsch, D. L. Walker, and H. DeF. Webster.** 1988. A double-label method detects both early (T-antigen) and late (capsid) proteins of JC virus in progressive multifocal leukoencephalopathy brain tissue from AIDS and non-AIDS patients. J. Neuroimmunol. **19**:223–236.
199. **Stoner, G. L., D. L. Walker, and H. D. Webster.** 1988. Age distribution of progressive multifocal leukoencephalopathy. Acta Neurol. Scand. **78**:307–312.
200. **Tada, H., M. Lashgari, J. Rappaport, and K. Khalili.** 1989. Cell type-specific expression of JC virus early promoter is determined by positive and negative regulation. J. Virol. **63**:463–466.
201. **Tada, H., J. Rappaport, M. Lashgari, S. Amini, F. Wong-Staal, and K. Khalili.** 1990. Trans-activation of the JC virus late promoter by the tat protein of type 1 human immunodeficiency virus in glial cells. Proc. Natl. Acad. Sci. USA **87**:3479–3483.
202. **Takemoto, K., P. Howley, and T. Miyamura.** 1979. JC human papovavirus replication in human amnion cells. J. Virol. **30**: 384–389.
203. **Tamura, T., T. Inoue, K. Nagata, and K. Mikoshiba.** 1988. Enhancer of human polyoma JC virus contains nuclear factor I-binding sequences; analysis using mouse brain nuclear extracts. Biochem. Biophys. Res. Commun. **157**:419–425.
204. **Tarsy, D., E. M. Holden, J. M. Segarra, and R. G. Feldman.** 1973. 5-Iodo-2′-deoxyuridine (IUDR): (NSC-39661) given intraventricularly in the treatment of progressive multifocal leukoencephalopathy. Cancer Chemother. Rep. **57**:73–78.
205. **Tashiro, K., S. Doi, F. Moriwaka, Y. Maruo, and M. Nomura.** 1987. Progressive multifocal leukoencephalopathy with magnetic resonance imaging verification and therapeutic trials with interferon. J. Neurol. **234**:427–429.
206. **Telenti, A., A. J. Aksamit, J. Proper, and T. F. Smith.** 1990. Detection of JC virus DNA by polymerase chain reaction in patients with progressive multifocal leukoencephalopathy. J. Infect. Dis. **162**:858–861.
207. **Tooze, J.** 1980. Human papovaviruses, p. 205–296. *In* J. Tooze (ed.), Molecular biology of tumor viruses, vol. 2. DNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
208. **Tornatore, C. S., S. Houff, B. Curfman, and E. O. Major.** 1991. Detection of JCV genome in a prolonged survivor of progressive multifocal leukoencephalopathy. Neurology **41**(Suppl.): 400.
209. **Trapp, B. D., J. A. Small, B. A. Pulley, G. Khoury, and G. A. Scangos.** 1988. Dysmyelination in transgenic mice containing JC virus early region. Ann. Neurol. **23**:38–48.
210. **Tyring, S. K., R. Cauda, V. Ghanta, and R. Hiramoto.** 1988. Activation of natural killer cell function during interferon-alpha treatment of patients with condyloma acuminatum is predictive of clinical response. J. Biol. Regul. Homeo Agents **2**:63–66.
211. **Vacante, D. A., R. Traub, and E. O. Major.** 1989. Extension of JC virus host range to monkey cells by insertion of a simian virus 40 enhancer into the JC virus regulatory region. Virology **170**:353–361.
212. **Van Horn, G., F. O. Bastien, and J. L. Moake.** 1978. Progressive multifocal leukoencephalopathy: failure of response to transfer factor and cytarabine. Neurology **28**:794–797.
213. **Varakis, J., G. M. ZuRhein, B. L. Padgett, and D. L. Walker.** 1978. Induction of peripheral neuroblastomas in Syrian hamsters after injection as neonates with JC virus, a human polyoma virus. Cancer Res. **38**:1718–1722.
214. **Vazeux, R., M. Cumont, R. M. Girard, X. Nassif, P. Trotot, C. Marche, L. Matthiessen, C. Vedrenne, J. Mikol, D. Henin, C. Katama, F. Bolgert, and L. Montagnier.** 1990. Severe encephalitis resulting from coinfections with HIV and JC virus. Neurology **40**:944–948.
215. **Walker, D. L.** 1978. Progressive multifocal leukoencephalopathy: an opportunistic viral infection of the central nervous system, p. 307–329. *In* P. J. Vinken and G. W. Bruyn (ed.),

Handbook of clinical neurology, vol. 34. Elsevier/North-Holland Publishing Co., New York.
216. **Walker, D. L.** 1985. Progressive multifocal leukoencephalopathy. Demyelinating diseases, p. 503–524. *In* P. J. Vinken, G. W. Bruyn, H. L. Klawans, and J. C. Koetsier (ed.), Handbook of clinical neurology, vol. 47. Elsevier Biomedical Press, Amsterdam.
217. **Walker, D. L., and B. L. Padgett.** 1983. The epidemiology of human polyomaviruses, p. 99–106. *In* J. L. Sever and D. Madden (ed.), Polyomaviruses and human neurological disease. Alan R. Liss, Inc., New York.
218. **Walker, D. L., B. L. Padgett, G. M. ZuRhein, A. E. Albert, and R. F. Marsh.** 1973. Human papovavirus (JC): induction of brain tumors in hamsters. Science **181**:674–676.
219. **Wallen, W., W. T. London, R. G. Traub, K. Petersen, and N. Witzel.** 1983. Antibody response to JC Virus associated antigens by tumor bearing owl monkeys, p. 261–270. *In* J. L. Sever and D. Madden (ed.), Polyomaviruses and human neurological disease. Alan R. Liss, Inc., New York.
220. **Weber, T., R. W. Turner, B. Ruf, W. Luer, W. Luke, J. Haas, E. Stark, S. Poser, H. Pohle, G. Hunsman, and K. Felgenhauer.** 1990. JC virus detected by polymerase chain reaction in cerebrospinal fluid of AIDS patients with progressive multifocal leukoencephalopathy, p. 100. *In* J. R. Berger and R. L. Levy (ed.), Neurological and neuropsychological complications of HIV infection. Proceedings from the Satellite Meeting of the International Conference on AIDS.
221. **Weck, P. K., D. A. Buddin, and J. K. Whisnant.** 1988. Interferons in the treatment of genital human papillomavirus infections. Am. J. Med. **85**:159–164.
222. **Weiner, L., R. M. Herndon, and R. T. Johnson.** 1972. Further studies of a simian virus 40 like virus isolated from human brain. J. Virol. **10**:147–149.
223. **Weiner, L. P., R. M. Herndon, O. Navayan, R. T. Johnson, K. Shah, L. J. Rubenstein, T. J. Prefiosi, and F. K. Conley.** 1972. Isolation of virus related to SV40 from patients with progressive multifocal leucoencephalopathy. N. Engl. M. Med. **288**: 1103–1110.
224. **Weiner, L. P., O. Narayan, J. B. Penney, Jr., D. L. Walker, and B. Dupont.** 1973. Papovavirus of JC type in progressive multifocal leukoencephalopathy. Arch. Neurol. **29**:1–3.
225. **Wekerle, H., H. Linington, H. Lassman, and R. Meyerman.** 1986. Cellular immune reactivity within the central nervous system. Trends Neurosci. **9**:271–278.
226. **Wiley, C. A., M. Grafe, C. Kennedy, and J. A. Nelson.** 1988. Human immunodeficiency virus (HIV) and JC virus in acquired immune deficiency syndrome (AIDS) patients with progressive multifocal leukoencephalopathy. Acta Neuropathol. **76**:338–346.
227. **Willoughby, E., R. W. Price, B. L. Padgett, D. L. Walker, and B. Dupont.** 1980. Progressive multifocal leukoencephalopathy (PML): in vitro cell-mediated immune responses to mitogens and JC virus. Neurology **30**:256–262.
228. **Winkleman, N. W., and M. T. Moore.** 1941. Lymphogranulomatosis (Hodgkin's disease) of the nervous system. Arch. Neurol. Psychiatry **45**:304–318.
229. **Wolinsky, J. S., K. P. Johnson, K. Rand, and T. C. Merigan.** 1976. Progressive multifocal leukoencephalopathy: clinical pathological correlates and failure of a drug trial in two patients. Trans. Am. Neurol. Assoc. **101**:81–82.
230. **Wroblewska, Z., M. Wellish, and D. Gilden.** 1980. Growth of JC virus in adult human brain cell cultures. Arch. Virol. **65**:141–148.
231. **Yogo, Y., T. Kitamura, C. Sugimoto, T. Ueki, Y. Aso, K. Harra, and F. Taguchi.** 1990. Isolation of a possible archetypal JC virus DNA sequence from nonimmunocompromised individuals. J. Virol. **64**:3139–3143.
232. **Yoshiike, E., T. Miyamura, H. W. Chan, and K. K. Takemoto.** 1982. Two defective DNAs of human polyomavirus JC adapted to growth in human embryonic kidney cells. J. Virol. **42**:395–401.
233. **Zochodne, D. W., and J. C. Kaufmann.** 1987. Prolonged progressive multifocal leukoencephalopathy without immunosuppression. Can. J. Neurol. Sci. **14**:603–607.
234. **ZuRhein, G. M.** 1967. Polyoma-like virions in a human demyelinating disease. Acta Neuropathol. **8**:57–68.
235. **ZuRhein, G. M.** 1969. Association of papovavirions with a human demyelinating disease (progressive multifocal leukoencephalopathy). Prog. Med. Virol. **11**:185–247.
236. **ZuRhein, G. M.** 1983. Studies of JC virus-induced nervous system tumors in the Syrian hamster: a review, p. 205–221. *In* J. L. Sever and D. Madden (ed.), Polyomaviruses and human neurological disease. Alan R. Liss, Inc., New York.
237. **ZuRhein, G. M., and S. M. Chou.** 1965. Particles resembling papovavirions in human cerebral demyelinating disease. Science **148**:1477–1479.
238. **ZuRhein, G. M., and J. N. Varakis.** 1979. Perinatal induction of medulloblastomas in Syrian golden hamsters by a human polyoma virus (JC). Natl. Cancer Inst. Monogr. **51**:205–208.