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REVIEW

Polyomavirus Models of Brain Infection and the Pathogenesis of Multiple Sclerosis

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Multiple sclerosis (MS) is generally considered to be an autoimmune disorder with myelin as the target and with several unidentified viruses playing ancillary roles, possibly through molecular mimicry. Although this paradigm has led to important progress on potential mechanisms of myelin loss, neither a target antigen in myelin nor a triggering mechanism has yet been identified, leaving the etiology of MS still unknown. Animal models of viral demyelination and studies showing that JC virus (JCV), the polyomavirus which causes progressive multifocal leukoencephalopathy (PML), may be latent in some normal human brains suggest another possibility. A host immune response targeting proteins expressed at low levels from viral DNA latent in the central nervous system (CNS) might underlie a focal demyelinating disease such as MS. A shift from autoimmunity to a latent-virus model is not a trivial substitution of target antigens. This shift would expand the search for a definitive laboratory test for MS and could lead to improved therapeutic and preventive approaches.

Introduction

For more than 25 years the autoimmune theory of multiple sclerosis (MS) etiology has dominated the field. The origins of the experimental allergic encephalomyelitis (EAE) / autoimmunity paradigm go back nearly 60 years to the work of Rivers and colleagues on demyelination following repeated injections of normal rabbit brain homogenate into monkeys (1). Despite occasional dissent by some neuropathologists (2-4), EAE has generally been considered to have sufficient clinical and pathological similarities to MS to provide a valid model. Development of the chronic-relapsing variant of experimental allergic encephalomyelitis (CR-EAE) added to the utility of this model (5,6). Over the years, increasingly sophisticated immunological approaches have dissected all aspects of the EAE model, as well as the immune dysregulation evident in MS (7-15). However, while T cells specific for myelin basic protein (MBP) can be found in the circulation of MS patients, similar cells can be demonstrated in healthy individuals as well (9,16-21). Thus, questions persist about the significance of these immunological abnormalities and anti-myelin reactivities, some of which might be a reflection of natural immunity or epiphenomena resulting from myelin breakdown, rather than its cause (7,22-25). Other immunological abnormalities might be secondary to virus recrudescence (26). A recent report of the development of white matter lesions in severe combined immunodeficiency (SCID) mice following intracisternal injection of CSF mononuclear cells from MS patients in exacerbation (27) supports a pathogenetic role for these cells, but their antigen specificity, if any, remains undefined.

Experimentally, viral concepts of MS etiopathogenesis owe much to the elegant studies of demyelination mediated by the immune response to viral gene products delineated in the Theiler's murine encephalomyelitis virus (TMEV) model (28). However, reports of a variety of candidate MS viruses isolated from the human brain have remained largely unconfirmed in other laboratories (29), and an appropriate viral model involving latency and reactivation of a human virus in glial cells has been lacking. More recently, the molecular mimicry hypothesis of viral induction of autoimmunity by chance cross-reactivity with brain antigens (30) has led to the "multiple virus hypothesis" (31) which further weakens the case for a single specific etiologic viral agent in the brain. Under these circumstances any single-virus etiology proposed for MS tends to face a skeptical reception. It is not the purpose of this presentation to review the entire field of virus persistence and demyelination, nor to make a case for a particular virus as a major cause of MS, but rather to use the example of a ubiquitous virus with tropism for glial cells to illustrate the value of a modified paradigm for investi-

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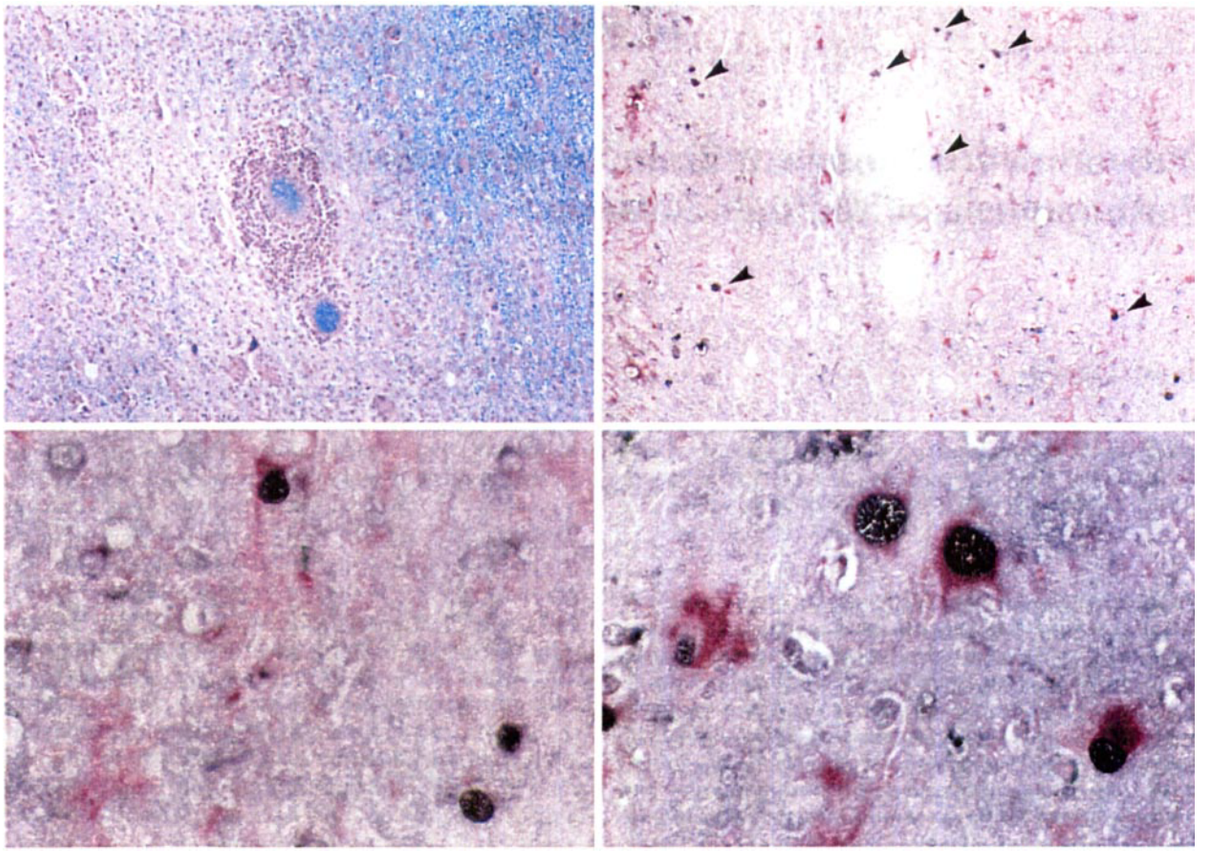

Figure 1 Perivascular cellular infiltrates in a case of macaque progressive multifocal leukoencephalopathy (PML) due to SV40 occurring on a background of simian AIDS; **a** Perivascular mononuclear cell infiltrates characteristic of cell-mediated immune response to antigen in brain tissue. Hematoxylin & Eosin and Luxol fast blue staining. x 140; **b** Double-label immunostaining with antibody to simian virus 40 (SV40) virus capsid proteins (black peroxidase label) and glial fibrillary acidic protein (GFAP) (red alkaline phosphatase label) shows SV40-infected glial cells in the surrounding brain parenchyma (arrowheads). No counterstain. Method modified from *J Neuroimmunol* 19: 223-236, reference 80. x 140; **c** SV40-infected oligodendrocytes, one near a reactive astrocyte, enlarged from (b). x 600; **d** SV40-infected reactive astrocytes in another region of the same macaque brain, immunostained as in (b). Note large nuclei with mottled staining for JCV capsid antigens in cells immunostained for GFAP in the cytoplasm. x 900

gating and explaining MS. For previous discussions of virus persistence in relation to demyelinating disease see references (32-39).

The Nature of a Multilple Sclerosis (MS) Virus

It is thought that an MS virus could be one of two basic types: 1) A rare agent which infects relatively few individuals but is frequently pathogenic, or 2) A common agent which infects a majority of the population and perhaps a significant number of normal brains, but, in which the virus expression and the host response to the infection (for genetic and environmental reasons) is pathogenetic in only a few individuals. Into the former group would fall certain retroviruses when they invade non-endemic areas (40), the unconventional agents which cause spongiform encephalopathies, e.g., Jakob Creutzfeldt disease and canine distemper virus. The latter group would include such agents as Epstein-Barr virus (41), coronavirus (42,43), herpes simplex virus (44) and measles virus (45) (prior to the introduction of the measles vaccine). Other examples of common agents would be the closely related human polyomaviruses known as JC virus (JCV) (46) and BK virus (BKV) (47). Serological evidence suggests that JCV and BKV each infect more than 70% of the population, mainly during childhood (48). JCV causes a fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML), in rare immunocompromised adults (49-53). This review addresses the implications of the recent findings of JCV DNA in human brain tissue without known neurological disease (54-58). Curiously, although JCV has been known for 20 years (46) and its lytic infection of oligodendrocytes in PML (59) make it arguably the best example of a human viral demyelinating agent (53,60), its possible significance for MS has not been widely noted. For example, several compendiums of recent basic and clinical MS research either fail to mention JCV and PML (61-63), or they refer to PML only in passing (64).

However, all of these books discuss measles virus and subacute sclerosing panencephalitis, and three of them also consider canine distemper virus and canine distemper encephalomyelitis (61,63,64). This oversight is in part a consequence of virus nomenclature. The two human polyomaviruses, along with the highly homologous simian virus 40 (SV40) and the more distantly related mouse polyoma virus, are all considered to be small DNA tumor viruses which are oncogenic in small animal hosts (65-69). This categorization as tumor viruses has focused attention on their possible role in human tumor induction (70-73) but, unfortunately, has tended to obscure the significance of these viruses for chronic demyelinating disease in their natural hosts.

An Animal Model for Progressive Multifocal Leukoencephalopathy (PML) and Antiviral Immunity in the Central Nervous System

PML in the macaque provides an animal model for the study of the pathogenesis of neurotropic polyomaviruses (74-76). Simian PML is caused by SV40 (77,78) which shares 69% genomic homology with JCV (79). In analogy to human PML, simian PML occurs spontaneously on a background of natural or experimental simian autoimmune deficiency syndrome (SAIDS) (75,76). Although the existence of brain latency in this model has not yet been established, the occurrence of PML in a macaque known to be seropositive for SV40 several years before onset of neurological disease suggests that reactivation of SV40 does occur (76).

Immunocytochemical studies in our laboratory indicate that perivascular cellular infiltration, apparently due to immunological reactivity to SV40 antigens, occurs in this model (Fig. 1). These results suggest that SV40 in brain glial cells around vessels can trigger lymphocytic infiltration. Although human PML is thought to characteristically lack perivascular mononuclear cell infiltration because of the underlying immunocompromising condition, in fact perivascular cuffing does occur (e.g., see description of cases 4, 6 and 7 of Richardson (81)) and the simian model may not be different in this regard.

While its relevance as a PML model is apparent, the SV40-infected macaque might also provide a model for MS-like disease induced by a latent virus. If some of these animals carry a latent SV40 infection in the brain, and the balance between viral expression and anti-SV40 immunity tips to the other side so that SV40 reactivation is abortive, then the pathology might be limited to immune-mediated destruction of glial cells with primary demyelination. Thus, simian immunodeficiency virus (SIV)-infected macaques should be observed regularly for the possible occurrence of MS-like signs with demyelination attributable to mononuclear cell infiltration in response to abortively infected glial cells, rather than to productive SV40 infection or to SIV-induced pathology.

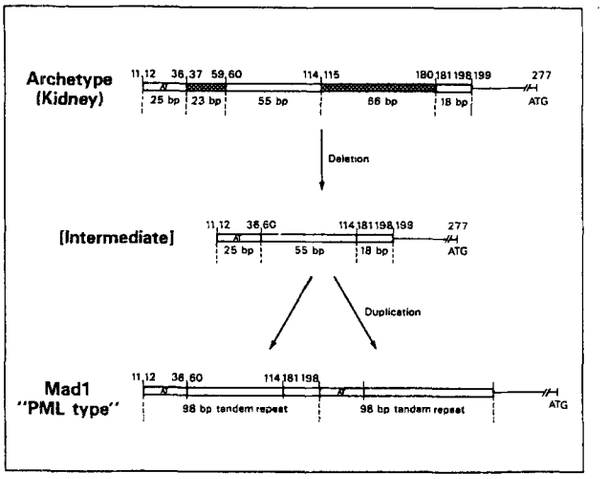

Figure 2 JC virus (JCV) regulatory region DNA undergoes a two-stage process of deletion and duplication known as "brain adaptation" to generate the progressive multifocal leukoencephalopathy (PML)-type viral genome capable of replicating in glial cells of the human brain. It is this rearranged promoter/enhancer, not the archetypal form circulating in the population, which becomes neurotropic in its host. The JCV isolates obtained from 11 PML brains were each uniquely rearranged; none was identical to the prototype Mad-1 strain. The host enzymes mediating these rearrangements are unknown. Diagram modified from *J Virol* 64: 3139-3143, reference 83.

Presence of JC Virus DNA at Low Levels in Some Human Brains without Neurological Disease

Our studies with the polymerase chain reaction (PCR) on frozen sections of brain tissue without known neurological disease have shown that as many as half of the brains may contain very low levels of JCV DNA (54). Sequences of amplified DNA fragments from these brains have a rearranged regulatory region showing deletion followed by duplication of promoter/enhancer elements (54), rather than the stable "archetypal" form which is found in the kidney and shed into the urine (82,83) (Fig. 2).

There is additional data in support of these findings. PCR analysis of DNA extracted from fixed brain tissue, obtained from aged individuals without PML, was able to detect JCV DNA in one-third of the brains (55). In another study in which PCR was used to detect JCV in the brains of human immunodeficiency virus (HIV)-positive individuals without AIDS or other CNS diseases such as PML, 4 of 13 brains were found to contain JCV DNA (56). In that same study, JCV DNA was found in 1 of the 12 normal brains. In a third study, 19 of 67 brains without PML (28%) were found to harbor JCV DNA by both PCR and Southern blot hybridization analysis (57). Two of the positive cases were confirmed by direct cloning of free full length JCV DNA from the infected

tissue. In the same study, 2 of 8 MS brains showed evidence of JCV DNA (57). In addition, 18 of the 19 JCV-positive brains were also positive for BKV DNA, but at much lower levels (57). However, in a fifth study PCR failed to detect JCV in DNA preparations from either MS or normal brains (84). Additional studies are required to ascertain the proportion of non-PML brains with polyomavirus infection and the state of the viral DNA.

In these individuals there was no reported evidence for virus particles, nor for JCV expression in the brain or neuropathological changes attributable to the presence of viral DNA. However, in another study evidence for JCV DNA was found by *in situ* hybridization in brains of 3 of 10 elderly patients without clinical PML, but with very small foci of demyelination (58). It should be noted that these findings could not be reproduced in a study of Alzheimer's disease brain tissue (85). Thus, it is currently not clear whether latent JCV infections may sometimes reactivate minimally in elderly individuals with little, if any, pathological change and without overt neurological symptoms (86).

What do these findings suggest about the pathogenesis of PML or about a role for a latent DNA virus in neurological diseases of unknown etiology?

Implications for the Pathogenesis of Progressive Multifocal Leukoencephalopathy (PML)

PML was once an extremely rare complication of chronic lymphocytic leukemia and lymphomas, but now occurs in approximately 2-5% (or more) of AIDS patients (87,88). The finding of JCV DNA in normal brains suggests that some cases of PML, which is almost exclusively an adult disease, may represent the reactivation of latent viral DNA harbored in the brain following a childhood infection. When PML occurs in AIDS, it seems likely that two main factors combine to promote JCV reactivation. First, there is the immunosuppressive effect of HIV-1 infection. In addition, since the HIV-1 Tat protein can transactivate JCV late transcription *in vitro* (89-91), it seems likely that Tat produced by HIV-1 infection of the CNS transactivates latent JCV in the brain. Brain tissue doubly infected with HIV and JCV has been observed in several studies (92-94). However, since Tat is a regulatory protein expressed prior to viral replication, HIV particles need not be present for Tat to be expressed. Furthermore, there is no apparent requirement for superinfection of JCV-containing glial cells by HIV since a single HIV-infected cell can transactivate as many as 1,000 uninfected bystander cells with which it is in contact (95,96).

Implications for the Etiology of MS

As postulated from the TMEV model (97), and in analogy to the prediction derived above from the macaque model, a latent polyomavirus infecting the human brain could provide a target for immune-mediated demyelination. Thus, while PML represents one type of host response to a latent virus infection following immunosuppression and transactivation, quite another host response to the same latent virus might involve strictly limited viral antigen expression, which when combined with a hyperactive immune response to CNS viral antigen, could lead to demyelinating disease (98). In the latent-virus model a viral protein, or possibly a cellular protein aberrantly expressed, provides the antigenic target. In addition, degenerative changes in latently infected cells leading to cell death with antigen release cannot be excluded.

Variables Influencing Viral Pathogenesis in the Central Nervous System

There is likely to be a continuum between latent infections which remain entirely quiescent on the one hand, and those which progress to clinically definite demyelinating disease on the other. For example, primary demyelination without clinical MS has been discovered during routine pathological study at autopsy (99,100). Abnormalities on neuroimaging suggestive of subclinical MS have been found in healthy identical twins (101) and unaffected siblings of MS patients (102), as well as 20% of older adults (103). What would determine whether a latent virus infection remains entirely benign, or erupts as a chronic demyelinating disease such as MS? The precise mechanisms which trigger JCV and BKV expression in kidney tissues leading to virus excretion under certain conditions, e.g., late in pregnancy (104) and during immunosuppression (105,106) are not yet understood. Nevertheless, it is possible to suggest a variety of factors capable of influencing virus expression in human glial cells. The following likely factors would include intrinsic (i.e., genetics of host and virus) and extrinsic (e.g., environmental) influences on the host's immune response.

Genetics of the host. Animal models have documented the important influence of host genetics in determining the outcome of virus infections (107,108). These genetic influences most likely include determinants of the specificity, type and strength of the immune response. They may in turn control the severity and extent of the primary infection and thus the extent of latency in tissues, e.g., kidney, spleen, bone marrow and brain. The immunogenetic aspects of MS (109) can be understood in this context, as well as in the context of autoimmunity to MBP.

Genetics of the virus. At least two distinct genotypes of JCV circulate in the human population (110,111). Two types of JCV in the United States, termed type 1 and type 2, have been identified by DNA coding region sequence data from 11 PML isolates (111). While both are also present in Europe, to date only

one of these has been found in Asian populations (110). Most of the DNA sequence differences between JCV types 1 and 2 are silent, i.e., do not cause type-specific amino acid substitutions (111). Thus, the capsid proteins of the two types of JCV are antigenically similar, and types 1 and 2 viruses are not serologically distinguishable. However, a few type-specific amino acid changes are predicted in the C-terminus of T-antigen which might have functional significance (111). By analogy to SV40 T-antigen (112), the C-terminus of JCV T-antigen may contain a host-range function. The possible existence of distinct types of JCV characteristic of Northern Europe and Asia suggests that differing types of a virus circulating in European and Japanese populations might contribute to the contrasting clinical presentations of MS (113), in addition to the immunogenetic differences in Asian populations which have been previously invoked (114).

Site of integration into the host genome. Small DNA tumor viruses such as the polyomaviruses have a propensity to be integrated into host cell DNA during transformation, with oncogenic effects mediated by subsequent viral oncogene (T-antigen) expression. If the viral agents were integrated into the DNA of scattered glial host cells early in life (perhaps during early brain development) and thus had been passed on to both daughter cells during each host cell mitosis, a ready explanation for multifocal infection (and multifocal lesions) would be provided. (However, free viral genomes could also be lineally passed to daughter cells if viral replication is negatively regulated by a cis-acting mechanism and restricted to once per cell generation (115)). Each focus of infection would represent the progeny of a single infected precursor cell. The cellular genes flanking the insertion site could influence viral expression, and, in turn, viral promoters / enhancers could alter the expression of nearby cellular genes.
The site of cleavage of the circular viral genome (5,130 bp for JCV (Mad-1) (116)) would be important, as interruption of either the regulatory region or viral early coding region would preclude production of an intact T-antigen. A truncated polypeptide might, however, be sufficient to provide an antigenic target in glial cells.

Timing of primary infection. It can be assumed that virus latent in the adult brain may be acquired during primary infection early in life, or possibly transplacentally from a mother who reactivates the virus during pregnancy (104,105). Unfortunately, primary infection in the child is difficult to pinpoint as it lacks known disease symptoms and can only be identified by seroconversion. Maternal reactivation with shedding of virus in the urine is asymptomatic as well. The timing, duration, and site of the primary infection, as well as the magnitude of the resulting viremia, will be important determinants of whether and to what extent the virus penetrates the developing brain and establishes latency, and will influence the eventual outcome of viral latency. In general, for a given virus load, the earlier in brain development this seeding occurs, the greater the number of daughter cells to which the viral genome could be lineally passed. This is true whether the viral DNA integrates into host cell DNA or free virus replicates synchronously with cell DNA during mitosis.

Cell types targeted by brain infection. Although JCV grows best in human fetal glial cells, glial cells are not the exclusive target of JCV infection *in vivo*. Infection of the kidney (117), spleen (118), and bone marrow (119) is well documented. Within the brain other cell types may be involved. Abortive granule cell infection has been observed in cerebellar PML (120). Other cell types infected include microglial cells (121) and brain endothelial cells (122). JCV infection has been observed in umbilical vein endothelial cells in culture (123) and in vascular endothelial cells of the neonatally infected hamster brain (124). Since astrocytic endfeet contact up to 95% of the endothelial cells of the brain microvasculature (125), infection of brain endothelial cells following early viremia would provide an avenue for an agent coming from the circulation to reach astrocytes, and after replication, to infect surrounding oligodendrocytes. Macaque PML brain showed several isolated clusters of SV40-infected oligodendrocytes around an infected astrocyte (Fig. 3). Although other interpretations are possible, this unique array is best explained by direct passage of virus from the centrally located astrocyte to the surrounding oligodendrocytes which it contacts.
In human PML brain clusters of a few enlarged oligodendrocytes have been described as the earliest recognizable microscopic lesion (128,129). They created demyelinated foci of pinhead size (130), usually 1 to 2 millimeters (mm) in diameter (128,129), but sometimes as small as 0.1 to 0.5 mm (131,132). In these lesions small blood vessels were often seen (129,131,132), although no consistent perivascular location was proved. Giant astrocytes in PML lesions have been observed with a cellular process attached to the wall of an adjacent blood vessel (133). In one study intracytoplasmic clusters of JCV particles were observed by electron microscopy in infected astrocytes, sometimes near foot processes (134). These might be passed to susceptible oligodendrocytes, although in late stages of disease such intracytoplasmic particles can also be interpreted as an early step in astrocyte infection by virions taken up from surrounding cell debris (135).
The presence of small clusters of infected oligodendrocytes in the human PML brain can be interpreted in one of the following ways. Firstly, the characteristic pinpoint foci of demyelination in

a b

Figure 3 Clusters of simian virus 40 (SV40)-infected oligodendrocytes surround an infected astrocyte in the macaque brain. **a** Note the immunostained astrocyte process extending to an infected oligodendrocyte (arrowhead). Smaller groups of infected oligodendrocytes were seen at this location in both adjacent sections indicating that the infected cells surround the astrocyte on all sides. x 350; **b** A similar cluster in another section. Immunostaining by monoclonal antibodies BH3 and CE5 to SV40 capsid proteins (126) with biotinylated goat antibody to mouse IgG, followed by peroxidase conjugated streptavidin as described (127). No counterstain. x 175

early PML could result from cytolysis of a cluster of oligodendrocytes infected by contact with the same infected astrocyte. The length of the processes of this central astrocyte would determine the diameter of this early focus of infection, and thus the extent of the lesion (0.1 to 2.0 mm). The infected astrocyte could have received its virus from an infected endothelial cell. Although endothelial cell infection is well recognized as a possible route for virus penetration into the brain (136), it must be emphasized that direct evidence for polyomavirus transfer along this pathway is lacking and at present this scheme is a working hypothesis. Secondly, clusters of infected glial cells could arise when virus is passed to daughter cells from a common infected glial precursor during cell proliferation. This could occur with free or integrated virus. The size of such clusters would be determined by the stage of development at which infection initially occurs and the efficiency with which the viral minichromosome persists and replicates. Thirdly, a cluster of infected cells might arise by infection with extracellular virus released from a lysed cell and diffused locally. This latter possibility seems less likely, as it should be a recurring feature of spread beyond coalescing lesions within the brain, not a feature limited to very early lesions and very early disease.

Immune response: environmental determinants. Environmental factors will modulate the viral and host genetic determinants. These could include coinfection with closely related viruses which may influence the immune response to the pathogenic virus. In the case of JCV, these would include various serotypes of BKV (137,138). The existence of distinct serotypes of BK virus has been confirmed by DNA sequence comparison of wildtype BKV(Dun) and the variant known as BKV(AS) (139). BKV infection will probably modulate the immune response to the potentially cross-reactive JCV (≈ 80% amino acid sequence identity (79)). The BKV(AS) strains, which comprised 15% of BKV strains in one series of urinary isolates (140), lack the primary antigenic epitope on the wild type BKV capsid (139), as do the BKV(DB) strains (138). Which one of these potentially cross-reactive BKV serotypes is associated with a JCV infection, and the order in which the two infections were acquired, might significantly influence JCV immunoregulation. In general, antibodies to BKV are acquired by the population at a younger age than are those to JCV (48). Reversal of the usual order of infection, i.e., acquisition of JCV infection before BKV, might influence the strength and specificity of the immune response to JCV (and BKV). The cellular immune responses to JCV and BKV virion proteins or T-antigens have been studied very little in any human populations (141,142) due to lack of antigen.

The Role of Retroviruses: Late Region Transactivation. The ability of HIV-1 Tat to transactivate the JCV late region promoter (90) suggests that HIV-1 may play a more direct role in viral reactivation leading to the induction of PML in AIDS than by immunosuppression alone. In fact, HIV-1 Tat protein is a stronger activator of JCV late region transcription than is T-antigen itself, and the two act synergistically (89). The potential role of HIV-1 Tat in modulating JCV antigen expression during latency, i.e., prior to DNA replication and in the absence of capsid protein synthesis, remains to be explored.

MS-like Disease in AIDS Patients

It is noteworthy that HIV-1 infection can promote MS-like disease prior to or during onset of clinical AIDS (143-147). This phenomenon might be a direct

Table 1 Models of Polyomavirus-induced Disease in the CNS

Animal	*Virus*	*Comments*	*References*
Rhesus monkey	SV40	Natural SV40 infection followed by natural or experimental SIV infection	75,76
Hamster	JCV (Mad-1)	Latent glial/endothelial infection following intracerebral inoculation of newborns[1]	124,127
Transgenic mouse	JCV[2]	Dysmyelination in second and subsequent generation mice	149,150
Transgenic mouse	Polyoma[3]	Expression primarily in astrocytes with dysmyelination	151

[1] Tumors observed at four to six months, but demyelination not evident at the light microscope level (H.G. Ressetar, personal communication)

[2] Early region genes, large and small T antigens.

[3] Early region gene, large T antigen.

Abbreviations: SV40, simian virus 40; SIV, simian immunodeficiency virus; JCV, JC virus

result of HIV-1 brain infection, or an indirect effect mediated by activation of another latent virus. If the latter, these patients might represent a group in which abortive viral reactivation is sufficient to cause neurological disease, but stops short of active viral replication and fatal productive CNS infection. To date only one AIDS patient presenting with MS-like symptoms has been reported to have developed PML (143). In another of these patients whose brain was biopsied, JCV replication was not detected by *in situ* hybridization, although PCR revealed that bone marrow and peripheral blood lymphocytes were infected by JCV (147). Note, however, that if it is postulated that MS patients show a hyperactive immune response to a latent viral brain antigen, then these patients may be less likely to progress to a productive viral infection of the brain than those who lack an antiviral CNS immune response. Thus, in any cases of MS-like demyelinating disease in which JCV provides the antigenic target, progression to PML under the influence of HIV-1 infection may actually be less likely than it would in ordinary JCV latency.

Animal Models

A useful and instructive animal model for immune-mediated demyelination triggered by viral antigen in the CNS has been provided by the Theiler's murine encephalomyelitis virus infection (34,148). Another well-developed model is provided by coronavirus-induced demyelinating encephalomyelitis in rats (35-37). For study of the pathogenesis of polyomaviruses in the CNS and the potential role of these small DNA viruses in demyelinating disease, four different animal models are available (Table 1).

These models of polyomavirus CNS infection include simian PML due to SV40 in the context of simian AIDS (74-77), and the neonatal hamster brain infection (67,124,127) with persistence of JCV in brain cells (Ressetar, unpublished data). Note that the ham-

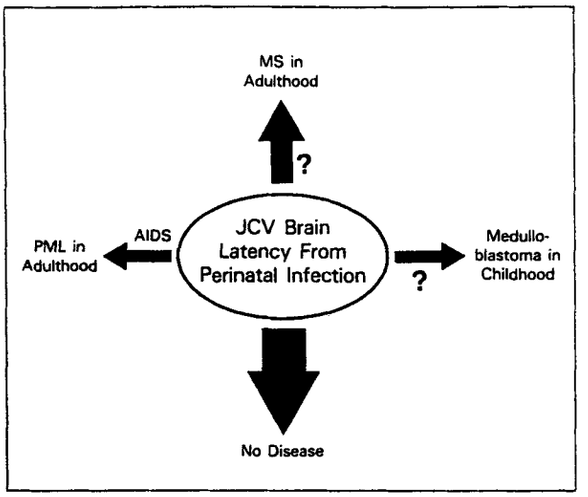

Figure 4 Four possible outcomes of latent JC virus (JCV) (or other small DNA tumor virus) infection of the brain following primary infection in the perinatal period or in childhood. **a** JCV remains entirely latent and undetected by the immune response in most individuals; **b** Following immunosuppressive disease, immunosuppressive therapy, and/or retroviral transactivation, a few may develop a productive infection, e.g., progressive multifocal leukoencephalopathy (PML); **c** The immune response to the multifocal infection might trigger chronic demyelinating disease such as multiple sclerosis (MS); **d** Expression of a viral oncogene (e.g., T-antigen) could lead to transformation of susceptible cells and tumorigenesis.

ster brain infection currently provides a model of brain tumor induction and brain endothelial cell infection by JCV (124,127). Additional experimental manipulation will likely be required to create a model of immune-mediated demyelination triggered by polyomavirus antigen in hamster glial cells. Transgenic mice carrying the JCV early region expressed in oligodendrocytes showed dysmyelination in the CNS in the second generation (149). In another transgenic mouse model, severe dysmyelination occurred in animals carrying mouse polyoma virus T-antigen DNA which was expressed primarily in astrocytes (151). This finding points toward a key role for the astrocyte in oligodendrocyte support and myelin formation and maintenance.

Discussion

There are more data available in all medical disciplines for MS than for any other neurological disease, yet its etiopathogenesis remains mysterious. Could brain infection with a latent virus capable of limited reactivation in glial cells explain this wealth of data? The example of the polyomaviruses suggests that it could. Unfortunately, our knowledge of the natural history of polyomavirus infections of humans is still fragmentary, and there is very little data available on these infections in relation to MS. Although JCV was first cultured and characterized in 1971 (46), primary JCV infections in humans have never been identified and have therefore been impossible to study. Serological tests are presently the only available indicator of acquisition of this (apparently) silent initial infection. The most widely used antibody assay, hemagglutination inhibition, requires large amounts of the virus which is in limited supply due to the difficulty with which JCV is cultured in primary human fetal glial cells (152). A single report on BKV serology in MS (153) did not reveal an association. There is no published information on JCV serology in relation to MS.

With respect to pathology, a systematic comparison of lesion development and distribution in PML and MS brains has never been reported. However, comparison of the recognized pathological features of each disease suggests some interesting parallels (98). It is noteworthy that the very small early foci described for PML (128-132) are in some ways reminiscent of the earliest MS lesions as described by Lumsden (2). (For discussion of early MS pathology see also reference 154). The relationship of many small MS plaques to the course of a vessel (2,155) is consistent with the proposed route of polyomavirus entry into the CNS. At onset the topology of MS lesions may be defined in large part by the distribution of viral antigen along the pathway taken by the virus on leaving the circulation and entering the brain. This path could be within dividing cells or between cells in contact, or both.

Recent neuroimaging data suggest that disturbance of the blood-brain barrier with or without clinical symptoms is the initial event in the development of new demyelinated lesions in relapsing/remitting MS (103,156,157). These findings emphasize a role for the endothelium in MS lesion pathogenesis, and have generally been interpreted in terms of the EAE model (158). Can they also be accommodated by a latent-virus model? In fact, as described above, JCV can infect brain endothelial cells. Productive infection of endothelial cells by JCV is unusual in PML (122), but an abortive infection of hamster endothelial cells in which T-antigen is expressed at 30 days of age is particularly pronounced following immunosuppressive treatment with cyclophosphamide (124). Thus, initiating events at the blood-brain barrier in MS pathogenesis can be accommodated by a viral hypothesis in which immune responses targeting cells in the parenchyma follow viral reactivation in infected endothelial cells nearby, as originally postulated by Wisniewski et al. (3).

Possible Outcomes of Central Nervous System Polyomavirus Infection

The polyomaviruses are involved not only in demyelinating disease, but are best known as DNA tumor viruses in animal models. This has encouraged a continuing search for these viruses in human tumors (73). Thus, three possible outcomes of JCV latency in the human brain can be envisioned (see

Fig. 4). The etiological role of JCV in PML is well established, but the other outcomes (Figs. 4c,4d) remain speculative. With respect to tumorigenesis by JCV, interest has centered around medulloblastomas (67,127,159,160), but there is currently no direct evidence for involvement of JCV in human tumors. However, from this perspective the unexplained reports of MS lesions and gliomas occupying the same anatomical locations (161-166) may not be chance occurrences. Reports that PML and multiple gliomas (167) or astrocytomas (168) can coincide are also of interest in this context. Finally, the coincidence of clinical MS and PML (169,170) completes the reported associations between these three different outcomes. This latter association occurred before the AIDS epidemic under the combined influence of systemic lupus erythematosus (SLE) and corticosteroid therapy (170). It is not certain that this was a case of MS as the demyelinated lesions observed at autopsy could not be distinguished from those of chronic PML (171).

Conclusion

A latent virus distributed multifocally in the CNS with its expression modulated by the immune response as well as by transactivating factors acting within or between cells, could explain the varied clinical course of MS. In view of the evidence for their presence in normal brain, the small human DNA viruses with gliotropic properties provide a compelling argument for a disease mechanism in which a common virus latent in CNS white matter provokes an uncommon immunopathological response in a few susceptible individuals.

Along with the recent evidence for coronavirus sequences in MS brains (42,43), these polyomavirus findings emphasize the relevance of the latent-virus model to demyelinating disease. Virus latency in the CNS could be the essential precondition for onset of MS pathology, i.e., the so-called "MS trait" (172). Although the number of different viruses which may eventually be implicated in CNS latency remains unclear, it is apparent that the immune response to a single virus in the CNS could provoke a disease as diverse and variable in its expression as MS. The interplay of the genetic and environmental factors listed above acting on a viral infection capable of latency and reactivation could explain the complex and unpredictable clinical course of MS as well as does the CR-EAE model. The latent-virus model of MS pathogenesis has several conceptual advantages: 1) Provides a mechanism for multifocal distribution of antigen in the CNS without requiring viral replication independent of cell division, if the infection occurs early in life; 2) Allows for regulated antigen expression, variable in time, within those foci, and 3) Has the potential for modulation of the immune response by cross-reactive viral antigen in organs outside the CNS (e.g., by antigens of BKV if the primary CNS agent were JCV). With the development of highly sensitive PCR techniques, the tools to detect MS candidate viruses latent in the human brain are in hand. Even if many latently infected cells have been destroyed by the immune response in the course of the disease, some infected cells which express little or no antigen and therefore escape destruction may continue to harbor detectable levels of viral DNA.

The choice of paradigm is important because it determines the range of questions asked, and can, in turn, limit the answers obtained. Is it time to change the paradigm? Within the framework of the EAE / autoimmunity paradigm have come significant advances in our understanding of the pathogenesis of MS (173,174), and promising new approaches to treatment (175-177), but much remains uncertain (4,178,179). After 25 years of intensive investigation, there is still no definite autoantigen, no breakthrough in laboratory diagnosis, no effective long-term treatment for MS (180,181), and there are no approaches to disease prevention. A fuller understanding of the disease could lead to new diagnostic laboratory tests, and to new possibilities for therapy and prevention. The direction of MS therapy research indicated by a latent-virus paradigm would be a combined attack on both the afferent and efferent arms of the immune response. This would involve inhibition of viral reactivation to reduce both the (peripheral) immunogenic stimulus as well as the (central) antigenic target and, if possible, selective immunosuppression directed toward a CNS antiviral immune response to reduce inflammation without provoking increased antigen expression. Clearly, a solid rationale for the use of antiviral agents would ensure new emphasis on their development and future testing.

A final resolution of the seemingly intractable MS problem, which has been aptly described (borrowing from Churchill) as "a riddle wrapped in a mystery inside an enigma" (172), will require the framework of an authentic paradigm. Although MS is now frequently described as an autoimmune disease (182), the use of a latent-virus paradigm might accelerate progress by emphasizing avenues of research which the more narrowly based EAE / autoimmunity paradigm bypasses.

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