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VIRUSES AND DEMYELINATION

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INTRODUCTION

Multiple sclerosis (MS) has been recognized as a distinct clinical entity for over 100 years, but its etiology remains elusive. In all likelihood, a viral infection during childhood or adolescence triggers an autoimmune response to oligodendrocytes and/or myelin in susceptible individuals (1). Patients with MS are now being treated with cyclophosphamide (2,3), cyclosporine (4), azathioprine (5), whole body radiation (6,7), or plasmapheresis (8), on the assumption that MS is an autoimmune disease. These therapies have potentially serious hematologic, gastrointestinal, infectious, or neoplastic side effects (9). Immunosuppressive therapy, even if effective in stabilizing multiple sclerosis, is less than ideal because of the above mentioned side effects. Establishment of the etiology of MS may allow for more earlier, more specific, and less toxic treatment. In this paper we will review the epidemiologic evidence for a viral etiology of MS, the current state of candidate viruses, viral associated human demyelinating diseases other than MS, and the animal models of viral-induced demyelination.

EPIDEMIOLOGY OF MULTIPLE SCLEROSIS

The epidemiology of MS has been extensively studied in the hope of establishing its etiology. MS begins primarily, although not exclusively, in young adulthood. Risk rises steeply from early adolescence, reaches a peak about age 30, and declines to near zero by age 60 (10). This same unimodal age-specific onset curve is present in various ethnic groups (11) and in areas of high and low prevalence (12). Women are 1.4 times more likely to develop MS than men (13). Ethnic groups demonstrate a range of susceptibilities from the Anglo-Saxons, who are susceptible, to the Japanese, who are resistant. Multiple sclerosis is more common in temperate rather than tropical regions (30 to 80 per 100,000 versus 4 to 6 per 100,000) (12). This observation holds for both the Old and New Worlds as well as the northern and southern hemispheres (14). The risk of developing MS within one ethnic group varies by a factor of 10 to 20 based on latitude (12). There is an age-specific component to the effect of factors encountered at various latitudes. Migration from a high to low incidence region prior to age 15 reduces an individual's risk of contracting MS (15,16). Migration from a low to

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high incidence region prior to age 15 increases an individual's risk of contracting MS (12).

The risk of developing MS is in part due to the inheritance of specific histocompatibility (HLA) antigens. In Caucasians, the presence of the HLA A3 or B7 antigens increases an individual's risk of developing MS by two to three fold (17,18), while the presence of DR2 increases risk by four to five fold (19,20). An examination of the HLA of family members of patients with MS did not detect a single HLA haplotype which differed among affected and non-affected individuals (21). HLA provides a marker of susceptibility in Caucasians. There is, however, no consistent relationship between MS and HLA in other races (12).

A current hypothesis is that MS arises as a consequence of an abnormal immune response to a virus which occurs at a critical age in a susceptible individual. Rubella, measles and typhoid vaccinations occurred at a later age in MS patients compared to controls (21). Individuals who are at higher risk to develop MS because of their MHC haplotype were more likely to have measles or mumps at a later age than controls (24). MS may arise as a result of a susceptible individual contracting a common childhood infection at a point when there is a regulatory abnormality of immune system, which permits the development of autoimmunity against myelin and/or oligodendrocytes.

The increased incidence of MS in family members compared to the general population provides further evidence for the etiologic role of an environmental factor. MS is 6 to 8 times more frequent in siblings and 2 to 4 times more common in parents than unrelated controls (12). Monozygotic twins demonstrate a 50 percent concordance. The clinical signs of MS frequently develop in the same year, rather than at the same age, in siblings (23). This suggests MS may develop following a common triggering agent in susceptible individuals. Siblings discordant for MS have been shown to have fewer and less severe viral illnesses as children (24). Taken together, the increased rate of MS in family members suggest a common exposure to an environmental pathogen (25).

Further evidence for an environmental factor in the etiology of MS is provided by Kurtzke's studies of the epidemiology of Ms in the Faroe Islands. Prior to World War II, Faroe Islanders were not afflicted with MS. In contrast, MS is common in the British, who are of the same genetic stock and live at the same latitude (26). During World War II, the Faroe Islands were occupied by British soldiers. Subsequently, MS was diagnosed in native Faroe Islanders. A detailed examination of individuals who contracted MS revealed they had closer contact to the British forces than those who did not. Since World War II, MS has become endemic in native Faroe Islanders. MS appears to occur in a small percentage of individuals six to twelve years after the exposure to a presumably infectious agent (27).

VIRAL-INDUCED HUMAN DEMYELINATING DISEASES

A. Multiple Sclerosis

Intensive efforts have been made to isolate a virus from the brains of patients with MS. While a variety of viruses have been isolated, including rabies, herpes simplex, scrapie, parainfluenza I, measles, chimpanzee cytomegalovirus, simian viruses I and V, coronaviruses, MS-associated (Carp) agent, and the bone marrow (Mitchell) agent, all probably represent contaminants or adventitious, rather than causal agents (28). A variety of viruses have been identified in the brain of

MS patients by other techniques. Both measles (29) and herpes simplex type 1 (30) were found to in situ hybridization. Coronavirus-like particles were detected by electron microscopy (31). It remains unclear if the presence of these viruses is causal or coincidental in the etiology of MS. Attempts to produce MS in primates by intracerebral injection of brain tissue from patients with MS have proven unsuccessful (32).

Antibodies to a variety of viruses have been found in the serum and cerebrospinal fluid (CSF) of patients with MS. Adams and Imagawa (32) found elevated levels of measles antibodies in the serum of MS patients compared to controls. Most subsequent studies have confirmed this observation (33). Increased levels of measles antibodies, however, are not found in every patient, and th absolute titer of measles antibodies is low (12). Antibodies against a variety of other viruses have been found in the cerebrospinal fluid (CSF) of patients with MS, but no virus has been detected universally (34,35). There is no consistent relationship between viral antibodies and the presence of oligoclonal bands in the CSF (36). The significance of viral antibodies in the serum or CSF of MS patients has recently bee reinterpreted. Elevated antibody titers to measles virus envelope, hemolysin, and hemagglutinin, antigens, Epstein-Barr virus capsid and nuclear antigen, and rubella hemagglutinin antigen were found in serum samples of patients with MS and rheumatoid arthritis compared to age and sex matched controls (37). The presence of elevated viral antibody titers may be a marker of abnormal immune regulation rather than being indicative of a specific etiologic agent.

The human lymphotropic virus type I (HTLV-I) was recently implicated as the etiologic agent of MS after antibodies to HTLV-I were identified in the CSF of MS patients from Sweden, and Key West, Florida (38). HTLV-I nucleotide sequences were also found in cells from the CSF by in situ hybridization under low stringency conditions from these patients (38). A second group reported detecting antibodies to HTLV-I proteins in one quarter of Japanese patients with MS (39). In spite of these promising early reports, HTLV-I does not appear to play an etiologic role in MS. In subsequent studies, HTLV-I was not detected by enzyme-linked immunosorbent assay (ELISA) or in situ hybridization techniques nor directly isolated from cultured lymphocytes, peripheral blood monocytes or brain tissues of patients with MS (40-43). Antibodies to HTLV-I, II, or III (human immuno-deficiency virus or HIV) do not occur more commonly in patients with MS compared to those with optic neuritis or other neurologic diseases (45). Finally, while HTLV-I is found in Japanese patients with MS, it was not statistically more common among patients with MS compared to those with other neurologic diseases and normal controls (44). At present, the weight of evidence is against HTLV-I being the "MS agent".

B. Tropical Spastic Paraparesis

HTLV-I was recently identified as the etiologic agent of tropical spastic paraparesis (TSP). HTLV-I associated TSP produces a slowly progressive, symmetrical, predominantly upper motor neuron disorder, characterized clinically by spastic paraparesis. It is associated with minimal sensory or autonomic dysfunction (49). Japanese (46) and Caribbean (50) patients with TSP have elevated serum antibodies to HTLV-I compared to controls. High levels of antibodies to HTLV-I are present in the CSF of patients with TSP (47) and these antibodies are synthesized intrathecally (48). Pathologic examination of the spinal cord reveals intense chronic meningomyelitis with demyelination; patchy collections of lymphocytes, plasma cells and macrophages are distributed in both grey and white matter. Demyelination is present predominantly in the

pyramidal and dorsal medial columns. In chronic cases, spongiform changes develop in the white matter (51,52). TSP is diagnosed in the appropriate clinical setting by presence of antibodies against HTLV-I. Computerized tomography, magnetic resonance imaging, and/or myelogram, are normal. It has not been determined if demyelination is due to the direct effect of HTLV-I on oligodendrocytes and/or myelin, or if it is immune-mediated. Tentative evidence for the later mechanism is provided by the observation that some patients with TSP improved during immunosuppressive treatment with prednisone and subsequently deteriorated when prednisone was withdrawn (46). The identification of HTLV-I as the agent of TSP represents a major breakthrough and significantly enlarges the domain of human viral-induced demyelinating diseases.

C. Acquired Immunodeficiency Syndrome (AIDS)

While HIV (HTLV-III) has not been shown to be MS agent, it has been found to produce a variety of neurologic conditions, including vacuolar myelopathy, subacute encephalopathy, aseptic meningitis, sensory polyneuropathy and dysimmune motor polyneuropathy. CNS demyelination is a major feature of the first two syndromes. Vacuolar myelopathy is characterized clinically by paraparesis, ataxia and incontinence (53,54). Pathologic examination reveals demyelination, predominantly in the lateral and posterior columns of the thoracic spinal cord. Vacuolar myelopathy is found in up to 20 percent of patients with the acquired immunodeficiency syndrome (AIDS). Demyelination appears to result from interfering with the normal metabolism of oligodendrocytes (55). The subacute encephalopathy of AIDS is characterized clinically by impaired memory and concentration with psychomotor slowing (55a). The course is progressive and may be accompanied by motor or behavioral changes. On pathologic examination abnormalities are present in the white matter and in subcortical structures. They consist of white matter pallor, microglial nodules, and infiltrations of lymphocytes, macrophages and multinucleated giant cells (56,56a). The earliest pathologic feature of the subacute encephalopathy of AIDS is the white matter pallor and vacuolation (56). HIV has demonstrated in monocytes and multinucleated cells in the regions of demyelination, but not, so far, in oligodendrocytes (56). The mechanism of demyelination in subacute encephalopathy is, as yet, unknown. Subacute encephalopathy is a significant source of morbidity and mortality in AIDS. Establishment of the mechanism of demyelination is an important goal in the effort to design more effective therapies of AIDS.

D. Progressive Multifocal Leukoencephalopathy

Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease due to human papovaviruses, the JC virus (JCV) (56,57) and the SV-40-like agent (58). PML occurs primarily in individuals with diseases which impair the immune system, such as leukemia, lymphoma, or AIDS (54,59-61). PML is seen in approximately 2% of patients with AIDS (62). If the present exponential increase in AIDS cases continues, PML will become as prevalent as Huntington's disease or myasthenia gravis by 1991 (62).

The clinical sign and symptoms of PML relate to the multifocal nature of the disease. PML is usually progressive and unrelenting, leading to death within six months of onset in immuno-incompetent individuals. PML may run a more protracted course in immuno-competent patients and, on rare occasion, may spontaneously resolve (63). Infection precedes without producing a fever or a pleocytosis in the CSF. PML can be diagnosed in the proper clinical setting with a characteristic appearance

on CT scan: Multiple hypodense, nonenhancing lesions present in the white matter. These lesions do not respect vascular borders nor been demonstrated to be effective in the treatment of PML.

Pathologic examination of the brain of patients with PML reveals multifocal regions of demyelination, which become confluent as the disease progresses. Basophilic, enlarged oligodendrocytes, and bizarre, enlarged astrocytes with irregularly lobulated, hyperchromatic nuclei, are seen by light microscopy in conjunction with the demyelination (65). Large numbers of papovavirus particles, single or in crystalline arrays, can be visualized in the nuclei of oligodendrocytes by electron microscopy (66). Virus particles are not present in astrocytes or neurons. JCV nucleotide sequences are found in oligodendrocytes, occasionally in astrocytes, but not in vascular endothelial cells as detected by in situ hybridization techniques (67). PML probably arises as the result of reactivation of JCV in immunologically compromised patients (68). JCV virus is acquired subclinically during childhood (65). JCV virus can be recovered from spleen and bone marrow cells as well as mononuclear cells in the CSF. PML may occur as a result of JCV entering the perivascular space of the brain from tissues in which it has been dormant. Clinical signs develop when oligodendrocytes are infected (69).

The molecular basis of JCV-induced demyelination has recently been elucidated through application of the powerful techniques of modern molecular biology. Early attempts at identifying thee mechanism of demyelination were hampered by the restricted host range of JCV infection of oligodendrocytes (70). This barrier was overcome by creating transgenic mice containing the JCV early region (71). Transgenic mice which inherit the JCV early region develop "shaking", one phenotype seen in mice with defects in myelin synthesis. The JCV early region codes for the T-antigen. Expression of the T-antigen correlates with the severity of "shaking". The presence of the T-antigen in oligodendrocytes results in a decrease in the transcription, compared to the translation, of the major structural proteins of CNS myelin (72). The T-antigen shares a C-terminal subsequence with myelin basic protein (MBP). This sequence functions as a phosphate acceptor site in the latter. The T-antigen sequence appears to competitively inhibit the protein kinase phosphorylation of the Pro-Arg-Thr-Pro-Pro sequence of MBP (74). This blocks the production of myelin and arrests the maturation of oligodendrocytes. The T-antigen has been detected in the nuclei of oligodendrocytes of patients with PML by use of the immunoperoxidase staining technique (73). The T-antigen has not been demonstrated in oligodendrocytes of patients with MS (75). In conjunction with the rise in the number of cases of AIDS, PML promises to become an increasingly important clinical disease.

ANIMAL MODELS OF VIRAL-INDUCED DEMYELINATION

A variety of viruses which cause demyelination in animals have been studied as models of MS. These models have provided many insights into mechanisms of viral-induced demyelination. Martin and Nathanson (76) observed that these systems share the following characteristics: One, the diseases are biphasic with a stage of acute encephalitis followed by a stage of chronic demyelination. Two, virus persists in the white matter. Three, the lesions are multifocal, and are located primarily in the spinal cord. Recently, some of the models have been modified so that they more closely resemble MS.

A. JHM Strain of Mouse Hepatitis Virus

JHM virus (JHMV), the neurotropic strain of mouse hepatitis virus (MHV), is a coronavirus which produces an acute, diffuse encephalomyelitis with patchy demyelination in mice and rats (77,78,88). Lesions develop in the white matter five to seven days after intracerebral inoculation. Inflammation and necrotic lesions are present in gray and white matter. The degree of demyelination is dependent on the age and strain of the animal dose of virus and route of infection (79-82). Demyelination is due to the lytic effect of JHMV on oligodendrocytes (79). Demyelination occurs in conjunction with the presence of JHMV as demonstrated by fluorescent microscopy or immunoperoxidase techniques. JHMV can be visualized in oligodendrocytes by electron microscopy. There is no temporal or anatomic association with the occurrence of demyelination and thee presence of inflammatory cells; demyelination occurs even in the absence of perivascular inflammation aft treatment with cyclophosphamide (79). Animals which survive the acute encephalitis remain persistently infected and develop subclinical chronic recurrent demyelination (83,84). The study of viral-induced demyelination has been facilitated by the identification of temperature sensitive and antibody selected mutants of JHMV which cause chronic demyelination with minimal encephalitis (85-87) and a clinically relapsing disease in association with the recurrence of demyelination (89).

The immune system may play a role in the development of demyelination following JHMV infection of rats. Demyelination can be transferred from infected to naive rats by adoptive transfer of the lymphocytes, following in vitro stimulation with myelin basic protein (90). JHMV may cause demyelination by altering the regulation of cell mediated immunity in the brain. JHMV induces class II proteins on astrocytes (91), cells which do not ordinarily express class II (92). This may result in oligodendrocytes and/or myelin proteins becoming targets of the immune system, resulting in demyelination. JHMV remains a useful model for studying mechanisms of virus-induced demyelination.

B. Canine Distemper Virus

Canine distemper virus (CDV) is a paamyxovirus related to measles which produces either acute or chronic demyelinating disease in dogs, based on the strain of the virus (93-95). Demyelination is a result of a lytic infection of oligodendrocytes; myelin breakdown occurs in the absence of inflammatory cells. Demyelination occurs anatomically and temporally separate from inflammatory infiltrates. Mononuclear cells are present in the brain but occur around blood vessels, and represent a secondary response to demyelination. CDV is a useful model of PML because in both diseases demyelination is due to the oligodendrocidal effects of the virus.

C. Semliki Forest Virus

Semliki Forest Virus (SFC) is a non-human pathogenic alphavirus which was discovered in mosquitoes of the Semliki Forest of Uganda. SFV produces multifocal demyelination in the CNS when inoculated intracerebrally in mice (96). SFV-infected mice provide a very useful model to study the physiology of demyelination (97,98). SFV-induced demyelination is immune-mediated (99). Demyelination occurs in conjunction with inflammatory infiltrates. It does not occur in immuno-incompetent (100) or immunosuppressed mice, in spite of higher titer of virus in the brain tissue compared to control mice (101). Reconstitution of SFV-infected immuno-incompetent mice with normal spleen cells leads to

demyelination (101). SFV is an excellent model of immune-mediated demyelination.

D. Herpes Simplex Virus

Herpes simplex virus type 1 (HSV-1) is a DNA virus which produces, on occasion, meningitis and encephalitis in man. HSV-1 has recently been found to produce demyelination in mice. Following oral-facial inoculation, HSV-1 induces lesions characterized by demyelination in association with an inflammatory mononuclear cell infiltrate in the brainstem adjacent to the trigeminal nerve root entry zone (102,103). Demyelination is immune-mediated; demyelination is prevented by immunosuppression with cyclophosphamide prior to infection (104). The extent of demyelination following infection with HSV-1 is under genetic control (105); certain strains of mice develop multifocal demyelination throughout the brain independent of the presence of virus. Demyelination in these latter strains is probably on an autoimmune basis. HSV-1 induced demyelination in mice is an important new model of MS.

E. Visna

Visna is a retrovirus which produces pneumonia and/or a chronic progressive, although occasionally relapsing-remitting, myelopathy in sheep (106). Visna persists at low levels for years, in part by evolving into antigenically distinct forms over time (107). Pathologically, demyelination occurs in two phases (108). During the initial phase, demyelination occurs in regions of inflammatory infiltrates with relatively little tissue necrosis. During the latter phase, demyelination occurs in conjunction with necrosis, of both gray and white matter. Immunosuppression inhibits early but not late demyelination (109). Visna may provide an excellent model for TSP and may be very useful as a means to test new therapies.

F. Theiler's Murine Encephalomyelitis Virus

Wild-type Theiler's murine encephalomyelitis virus (TMEV) usually produces an asymptomatic enteric infection in mice, and only rarely encephalomyelitis. One strain of TMEV, DA, has been isolated which reliably produces a biphasic neurologic disease in Swiss mice (110). Mice strains vary in their degree of susceptibility to TMEV (118,119). Nine to 20 days following intracerebral inoculation with the DA strain of TMEV, 80 percent of mice develop encephalomyelitis. Between one and five months post-infection, survivors develop a mild gait disturbance in conjunction with the occurrence of demyelination in areas of intense mononuclear inflammation in the spinal cord leptomeninges and white matter. During the acute phase, TMEV can be found in neurons and glial cells. During the late phase, TMEV is present only in glial cells (111,112). Immunosuppression prevents demyelination, although results in increased neuronal necrosis with a concomitant increase in mortality (113). Timing of the initiation of immunosuppression is critical in preventing demyelination (114). Immunosuppression initiated at the time of infection prevents early demyelination. Immunosuppression begun later is ineffective. MBP appears in the CSF and serum during chronic TMEV infection. The level of MBP parallels the clinical severity of demyelination (115). MBP appears to be a marker of demyelination rather than a target of attack by the immune system. Treatment with myelin components cannot prevent demyelination in TMEV (116) as is observed in experimental allergic encephalomyelitis, or even perhaps, in MS (117).

Demyelination occurs during TMEV infection as a result of a delayed type hypersensitivity (DTH) response against persistently infected

Table 1 Animal Models of Viral-Induced Demyelination

Virus	Host	Possible Mechanism
Theiler's	Mouse	Immunopathological in a persistent infection
Semliki Forest	Mouse	Immunopathological
JHM	Mouse	Oligodendrocidal in a persistent infection
	Rat	Immunopathological
Herpes Simplex	Mouse	Immunopathological
Canine Distemper	Dog	Oligodendrocidal
Visna	Sheep	Oligodendrocidal +/or immunopathologic

Table 2 Possible Mechanisms of Virus-Induced Demyelination (124)

Direct viral effects

- Viral infection of oligodendrocytes or Schwann cells causing demyelination through cell lysis or an alteration of cell metabolism
- Myelin membrane destruction by the virus or viral products

Virus-induced immune-mediated reactions

- Antibody and/or cell-mediated reactions to viral antigens on cell membrane
- Sensitization of host to myelin antigens
  - Breakdown or myelin by infection with introduction into the circulation
  - Incorporation of myelin antigens into the virus envelope
  - Modification of antigenicity of myelin membranes
- Cross-reacting antigens between virus and myelin proteins
- Bystander demyelination

Viral disruption of regulatory mechanisms of the immune system

oligodendrocytes (120). The development of demyelination correlates with the establishment of high levels of DTH against TMEV antigens (121). It does not appear to be due to an autoimmune response against CNS antigens. Examination of the fine specificity of class II restricted T cell responses reveals that the DTH is against viral antigens. Mice chronically infested with TMEV do not mount a DTH response against mouse spinal cord homogenate, myelin basic protein, or proteolipid protein (122). While demyelination during TMEV is not due to autoimmunity, procedures which increase inflammation, such as opening the blood brain barrier, lead to increased demyelination (123). TMEV provides an excellent system for studying viral-induced immune-mediated demyelination.

MECHANISMS OF VIRAL-INDUCED DEMYELINATION

Viral infections can induce demyelination through a variety of mechanisms (124). We have previously discussed new demyelination may result from lysis (JHMV), or interference with the normal metabolism (PML) of oligodendrocytes and immune-mediated destruction of virus infected oligodendrocytes (TMEV, SFV). Demyelination has recently been shown to arise as a consequence of molecular mimicry, where antibodies-synthesized against a viral protein inadvertently cross-react with a host protein. Experimental allergic encephalomyelitis (EAE) can be elicited by inoculation of MBP in Freund's adjuvant. MBP and the polymerase protein of hepatitis B are homologous for six amino acids. Inoculation of those six amino acids in Freund's adjuvant results in pathologic lesions which resemble EAE (125). Finally, demyelination may result as a consequence of a virus infection disrupting the normal regulatory mechanisms of the immune system which prevents autoimmunity. Chronic JHM infection in rats induces Class II antigens on glial cells, which may allow astrocytes to function as antigen-presenting cells and process an oligodendrocyte and/or myelin protein, such as MBP into an auto-antigen. Demyelination could result if a normal host protein becomes a target of the immune system. MS does not appear to arise as a consequence of the direct effect of a viral infection on oligodendrocytes and/or myelin. Instead, MS probably occurs as a result of either a virus-induced immune-mediated reaction or through alteration of the regulatory mechanisms of the immune system. Further studies into the pathogenesis of MS will be greatly aided by the availability of animal models of both mechanisms.

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