ROLE OF VIRUSES IN ETIOLOGY AND PATHOGENESIS OF MULTIPLE SCLEROSIS

Samantha S. Soldan*,† and Steven Jacobson*

* Viral Immunology Section
NIH/NINDS
Bethesda, Maryland 20892
† Institute for Biomedical Sciences
Department of Genetics
George Washington University
Washington, D.C. 20052

I. INTRODUCTION

Multiple sclerosis (MS) is the most prevalent demyelinating disease of young adults, affecting an estimated 300,000 individuals in the United States alone. The natural history of the disease is unpredictable. The majority of affected individuals have a relapsing–remitting course, while a smaller subset have a more chronic–progressive presentation. Women are affected more often than men, a phenomenon associated with a number of autoimmune diseases. Although the etiology of MS is unknown, it is generally believed that genetic, immunologic, and environmental factors are involved. This chapter will highlight these issues as they suggest that exogenous factors are associated with the pathogenesis of this disorder. It has been suggested that infectious agents may comprise an environmental component of the induction and progression of this often debilitating neurological disorder. While many viruses have been investigated as potential "triggers" of MS, no virus to

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date has been definitively associated with this disease. Recently, the human herpes virus 6 (HHV-6) has received considerable attention as an infectious agent candidate that might be associated with the pathogenesis of MS. We will focus on this agent and the data that support the role of this virus in MS disease pathogenesis. We will compare and contrast these observations with the long list of viruses that have been suggested to play a role in this disease. Additionally, we propose a model whereby in genetically susceptible individuals, multiple viruses may trigger either a virus-specific or a cross-reactive autoimmune response that results in clinical MS. Importantly, we take an open but cautious view on the role that viruses may play in the pathogenesis of a chronic, progressive neurologic disorder such as MS.

II. ETIOLOGY OF MULTIPLE SCLEROSIS

A. Genetic Influences

Epidemiological, familial, and molecular studies of MS have supported a strong influence of genetic background on disease susceptibility. The worldwide distribution of MS is uneven, with areas of high prevalence being found in North America, Europe, New Zealand, and Australia, and areas of lower prevalence in Asia, Africa, and South America (Kurtzke, 1995). In general, the prevalence and incidence of MS follow a north–south gradient in both hemispheres (Sadovnick and Ebers 1993; Compston, 1994; Ebers and Sadovnick, 1994). It has been suggested that the north–south gradient observed in the New World may be reflective of the propensity of individuals from regions of Europe with a high incidence of MS to migrate to the northern regions of the United States and Canada, and of individuals from regions of Europe with a lower incidence of MS to migrate to southern regions of the United States and South America (Sadovnick and Ebers, 1993; Ebers and Sadovnick, 1994). The importance of a genetic background in influencing susceptibility to MS is further supported by epidemiologic studies demonstrating different prevalences of MS among genetically disparate populations living in the same geographic area. For example, the prevalence of MS is different in Hungarians of Caucasian descent (37/100,000) as compared to Hungarian gypsies (2/100,000) (Kalman *et al.*, 1991). A similar example has been described in the United States, where the prevalence of MS among people of Japanese descent living on the Pacific Coast (6.7/100,000) is considerably lower than that of Caucasians living in California (30/100,000) (Detels *et al.*, 1977). However, it is interesting to note that people of Japanese descent living on

the West Coast of the United States show a slightly higher prevalence of MS than those living in Japan (2/100,000), suggesting that environmental factors also have a significant impact on disease susceptibility (Detels *et al.,* 1977).

Family and twin studies have played an important role in establishing a genetic influence in the development of MS. It has been demonstrated that biological relatives of patients with MS have a greater likelihood of developing MS than have adoptees and that, conversely, family members of adopted individuals with MS do not have an increased risk of developing MS (Ebers *et al.,* 1995). Additionally, among biological relatives of individuals with MS, the lifetime risk of developing MS increases with closer biological relationships. The risk is greatest for siblings of affected individuals, especially sisters, and decreases in second- and third-degree relatives (Sadovnick *et al.,* 1988; Sadovnick and Ebers, 1993b). The rate of MS concordance is eight times greater in monozygotic than in dizygotic twins. However, the concordance among monozygotic twins is only 20%, which suggests that a susceptible genetic background alone is not sufficient to cause the disease (Bobowick *et al.,* 1978). Sadovnick and colleagues effectively illustrate the combined influence of genetic background and environment in the development of MS through a liability threshold model (Sadovnick *et al.,* 1999). As demonstrated in Fig. 1, the risk of acquiring MS is dependent on both genetic and environmental risk factors (load) that collectively represent an individual's "total liability." The degree of genetic and environmental loads vary on an individual basis. Once an individual's total disease liability crosses a particular threshold, clinical MS is observed (Sadovnick *et al.,* 1999).

Considerable effort has been made to ascribe MS susceptibility to various models of inheritance. However, MS has not been demonstrated to fit any of these models. In part, the inability to attribute a particular inheritance pattern to MS may arise from the difficulty in diagnosing this unpredictable disease (Lynch *et al.,* 1990; Tienari *et al.,* 1992). Additionally, because the age of high risk ranges from the late teens to the late 50s, an individual cannot be considered unaffected with certainty until they are past the age of high risk. Over the years, several genes, many of which are associated with immune function, have been tentatively associated with an increased risk of MS (McFarland *et al.,* 1997). Many of these associations have not been demonstrated consistently in different studies. However, a strong association between MS and the major histocompatibility complex (MHC) class II alleles DR2 and DQw1 has been demonstrated (Cook, 1997).

G + e = genetic load greater
g + E = non-genetic (environmental) loading greater
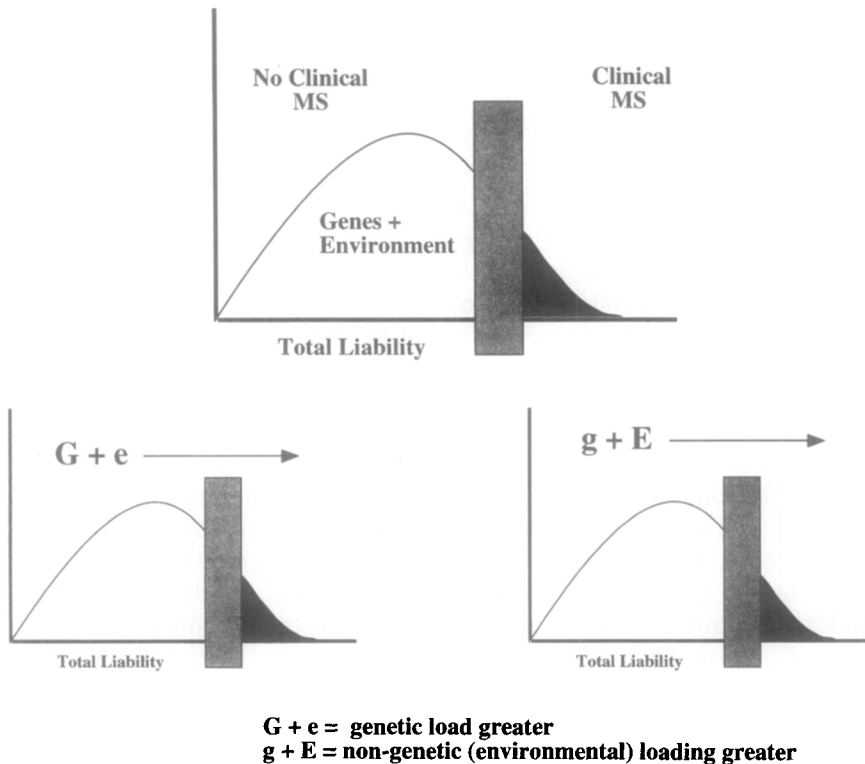
FIG. 1. Role of genetic and environmental influences in the lifetime risk of developing MS. Genes plus environmental factors comprise an individual's total disease liability. Below a particular threshold (gray bar), no clinical disease is evident. Above this threshold, expression of disease (black area) is present. Total disease liability in some individuals reflect a higher genetic burden (G) than environmental factors (e), relative to other patients in which environmental factors (E) are greater than genetic influences (g). Adapted from Sadovnick *et al.*, 1999.

B. Immunologic Influences

1. Immunologic Charactersitics of MS

In addition to genetic influences, it is widely accepted that T cell-mediated responses are involved in the etiology of MS. This is based on the association of MS with genes involved with the immune response, the immunopathology of the disease, the clinical response of MS patients to immunomodulatory and immunosuppressive treatments, and similarities with experimental immune-mediated demyelinating

diseases in animals. As described above, the MHC class II background of an individual is an important factor in disease susceptibility. Many studies have concentrated on MHC–peptide interactions in order to determine how MHC class II alleles may confer disease susceptibility. It has been determined that the binding affinity of an antigenic peptide to an MHC allele determines T cell immunogenicity and encephalitogenicity (Greer et al., 1996). The vast majority of studies concerning MHC–peptide interactions relevant to MS have focused on myelin basic protein (MBP) as the antigenic peptide. MBP-specific T cells may be demonstrated in the peripheral blood of both MS patients and normal individuals (Burns, 1983). The frequencies of MBP-specific T cells tend to be higher in MS patients than in controls (Ota et al., 1990, Olson et al., 1990). However, similar frequencies of MBP-specific T cells have been demonstrated in affected and unaffected family members, thereby suggesting that the frequency of MBP-specific T cells may be linked to the immunogenetic background of an individual and be a prerequisite of disease development (Joshi et al., 1993). It has been suggested that molecular mimicry, a phenomenon by which environmental antigens cross-react with normal host cell components, may induce an immune response against host proteins such as MBP. Therefore, individuals with higher frequencies of MBP reactive T cells may be more likely to develop autoreactive T cell responses as a result of environmental, non-self-epitopes mimicking MBP.

A number of immune abnormalities are frequently observed in MS patients and lend support to an immunologic component of the MS disease process. As represented in Fig. 2 (see color insert) a complex series of immunological mechanisms associated with events in both the peripheral blood system and the central nervous system (CNS) has been proposed (Martin et al., 1997; Brosnan et al., 1997). One of the hallmarks of MS is the intrathecal secretion of oligoclonal antibodies (Kabat et al., 1995; Tourtellotte, 1985). Oligoclonal bands (OCBs) are found in the CNS tissue and cerebrospinal fluid (CSF) of greater than 90% of MS patients and are used in the diagnosis of the disease. OCBs are not specific for MS as they are also found in several other chronic inflammatory CNS conditions of either infectious origin (such as CNS lyme or chronic viral and bacterial meningitis) or autoimmune origin (such as CNS lupus erythematosus). Although OCBs are not directed against a single antigen, antibody bands specific for viral and bacterial antigens, and self-antigens, have been described (Sindic et al., 1994; Sriram et al., 1999). Therefore, it is unclear whether the intrathecal synthesis of immunoglobulins observed in MS results from the presence of cells that are passively recruited into the CNS after the pathogenetically relevant
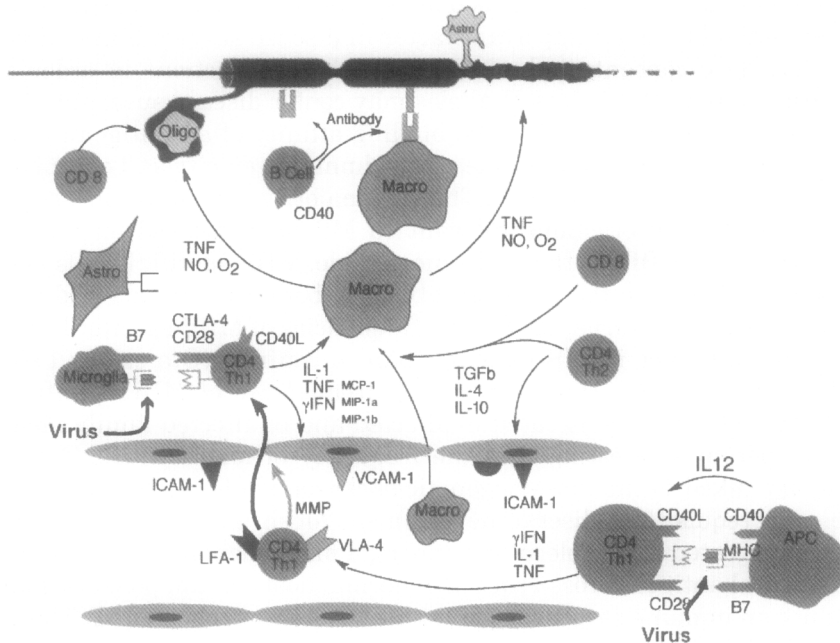
FIG. 2. [For color reproduction, see color section.] Elements of the MS lesion. A diagrammatic representation of the complex immunological mechanisms that are proposed to be involved in the initiation and maintenance of the developing MS lesion. Viruses may play a role in this model by initiating a virus-specific immune response in the periphery (lower right corner) that crosses the blood–brain barrier and encounters antigen in the CNS. Antigen may be presented on virus-infected glial cells or by resident CNS antigen-presenting cells (microglia). This interaction may result in a cascade of cytokines and chemokines that are associated with lesion development. Alternatively, autoreactive cells either in the periphery or the CNS may cross-react with viral antigens (molecular mimicry) that lead to activation of these T cells and subsequent CNS damage. (We thank H. McFarland for the use of this figure.)

cells have crossed the blood–brain barrier (BBB) or from disease-related lymphocytes. In addition to the presence of OCBs, other immunological markers of disease activity have been described in MS. The overexpression of several proinflammatory cytokines including tumor necrosis factor (TNF)-α and interferon (INF)-γ have been demonstrated in MS. Treatment of MS patients with IFN-γ resulted in a marked increase in exacerbations, which supports the model of MS as an autoimmune disease mediated by TH-1 like T cells (Panitch *et al.*, 1987). Furthermore, an increase in TNF-α expression has been found to precede relapses and inflammatory activity as measured by MRI, while the mRNA levels of

inhibitory cytokines, such as interleukin (IL)-10 and transforming growth factor (TGF)-β, declined at the same time (Riekmann *et al.*, 1995). The overexpression of these cytokines may be involved in disease pathogenesis by causing the upregulation of MHC and adhesion molecule expression on endothelial and glial cells, activation of macrophages, and recruitment of TH1 cells, or by damaging oligodendroglial cells and myelin sheaths directly (Selmaj and Raine, 1988). The soluble adhesion molecules ICAM-1 and E-selectin are elevated in MS sera while soluble VCAM-1 and E-selectin are increased in the CSF of MS patients (Dore-Duffy *et al.*, 1995).

Additional support for the concept of MS as a disease with an autoimmune component is provided by the clinical improvement obtained with immunosuppressive and antiinflammatory therapies. Although corticosteroid treatment does not alter the long-term course of MS, it is used effectively in the treatment of MS exacerbations. It has been demonstrated that the administration of high-dose steroids immediately stops BBB leakage as visualized by gadolinium-enhanced MRI (Burnham *et al.*, 1991). A number of immunosuppressive and chemotherapeutic drugs including cyclophosphamide and methotrexate have been used in the treatment of MS with variable success. Currently, two immunomodulatory therapies, namely IFN-β and copolymer-1 (Cop-1), are widely used in the treatment of MS. IFN-β counters many of the effects of IFN-γ, such as the recruitment of inflammatory cells and the upregulation of MHC and adhesion molecules. Additionally, IFN-β has been shown to lower the exacerbation rate in MS patients with a relapsing–remitting course and inflammatory activity as demonstrated by MRI (Paty *et al.*, 1993; IFN-β MS Study Group, 1993; Stone *et al.*, 1995). COP-1 is a synthetic polypeptide consisting of a random sequence of four amino acids; it blocks antigen presentation by competing with antigenic peptides for the MHC binding groove. COP-1 has been demonstrated to be approximately as effective as IFN-β in early relapsing–remitting MS (Johnson, 1995). The effectiveness of these immunomodulatory therapies lends support to the presence of an autoimmune component in the pathogenesis of MS.

2. Animal Models for MS

Experimental autoimmune encephalomyelitis (EAE) models in various animals have rendered great insight into the immunopathogenesis of MS and have been especially useful in the development of immunomodulatory therapies for the disease. EAE is an acute or chronic relapsing inflammatory demyelinating disease of the CNS that is characterized by demyelinating white matter lesions and inflammation. EAE may be induced in a number of susceptible inbred animal

strains by the injection of whole white matter or individual myelin proteins such as proteolipid protein (PLP), or MBP in Freund's complete adjuvant (Fritz and McFarlin, 1989). The ability to transfer EAE from an affected animal to a naive animal with cellular or humoral components demonstrates that EAE is a T cell-mediated autoimmune disease. EAE-resistant and- susceptible strains of mice, rats, and guinea pigs have been bred and, as in MS, are associated with particular MHC-class II backgrounds (Fritz and McFarlin, 1989). The clinical course and pathology of EAE varies among rodent species and strains (Stepaniak et al., 1994; Lorentzen, 1995). Animals sacrificed at various stages of the disease display lesions of various stages of inflammation, demyelination, and glial scarring reminiscent of MS plaques (Raine, 1983). EAE in primates is also useful as the inflammatory and demyelinating foci found in marmosets with EAE follow the same distribution pattern as those in MS (Massacesi et al., 1992). EAE has been used to develop treatment modalities that are broad, such as those which target the migration of encephalitogenic T cells into the CNS, and specific, such as interventions which target the trimolecular complex. The various EAE models have contributed greatly to our understanding of the immunopathogenesis and immunopathology of MS and will continue to be a great asset in the development of immunomodulatory therapies for this disease.

C. Environmental Influences

1. Epidemiological Evidence for Viruses in Multiple Sclerosis

For many years, an infectious etiology of MS has been suspected, as it fits with a number of epidemiological observations as well as the pathological characteristics of this disease. It has been widely speculated that the infectious component in the development of MS may be a virus. Data implicating a virus in the pathogenesis of MS include (1) epidemiological evidence of childhood exposure to infectious agents and an increase in disease exacerbations with viral infection (Johnson, 1994; Weinshenker, 1996); (2) geographic association of disease susceptibility with evidence of MS clustering (Haahr et al., 1997; Kurtzke, 1995); (3) evidence that migration to and from high-risk areas influences the likelihood of developing MS (Weinshenker, Alter et al., 1966); (4) abnormal immune responses to a variety of viruses (Neighbour et al., 1981; Jacobson et al., 1985); and (5) an analogy with animal models and other human diseases in which viruses can cause diseases with long incubation periods, a relapsing-remitting course, and demyelination.

As mentioned previously, the distribution of MS follows a geographic distribution, with an increased prevalence occurring in northern latitudes, which may be the result of both genetic and environmental influences. Migration studies, based chiefly on Europeans who immigrated to South Africa, Israel, and Hawaii, have also supported an infectious etiology of MS (Dean and Kurtzke, 1971; Kurtzke et al., 1970; Alter et al., 1966; Alter, 1971; Alter et al., 1978). In general, individuals who migrate from high-risk to low-risk areas after the age of 15 tend to take their risk of MS with them. However, individuals who migrate from high-risk to low-risk areas before the age of 15 acquire a lower risk. These data suggest that an environmental factor, perhaps a virus, must be presented before the age of 15 in order to influence MS susceptibility.

Reports of clusters or epidemics of MS also support a role for an infectious agent in MS. In the Faroe Islands, off the coast of Denmark, no cases of MS were reported from 1929 to 1943. After the occupation of the Faroe Islands by British troops in 1940, 20 islanders developed MS between 1940 and the end of the war. The areas of the Faroe Islands MS epidemic were found to correlate with the locations of British troop encampments after 1940. The sudden appearance of MS among the Faroese after the occupation by British troops suggests an interhuman transmission of the disease and the influence of an infectious agent (Kurtzke, 1995; Rohowsky-Kochan et al., 1995). This primary outbreak of MS was followed by three additional clusters of the disease, each being separated by 13 years. It has been proposed that the original cluster of MS was initiated by a virus that affected only the MS susceptibility of individuals between the ages of 11 and 12. The susceptible individuals harbored this virus in a latent state through adolescence, thus accounting for the 13-year interval between MS clusters on the Faroe Islands (Kurtzke, 1995). Other examples of MS epidemics have been described in the Shetland and Orkney Islands, Scotland; Key West, Florida; Mossyrock, Washington; and Mansfield, Massachusetts (Kurtzke, 1997).

2. Viruses in Demyelinating Diseases of Animals

Viruses have been implicated in a number of demyelinating diseases of the central nervous system in both animal and human subjects. The association of viruses in other demyelinating diseases further suggests a viral influence on the development of MS, by indicating that viruses are capable of inducing demyelination, and that they can persist for years in the CNS, presenting chronic diseases long after acute infection. Several of these viruses involved in demyelinating diseases of nonhumans include canine distemper virus (CDV); murine coron-

avirus (JHM strain); Theiler's mouse encephalomyelitis virus (TMEV); and visna virus (Appel, 1969; Kyuma and Stohlman 1990; Rodriguez *et al.*, 1987; Zink, 1992).

CDV is a member of the morbilliviruses and is related to measles and rinderpest viruses. CDV is a common infection of dogs and other members of the canine family. Acute infection with CDV is sometimes followed by a demyelinating encephalomyelitis called subacute diffuse sclerosing encephalitis. This encephalitis is characterized by tremor, paralysis, and convulsions and may not appear until weeks or months after acute infection. Lesions observed show demyelination with a sparing of axons and perivascular cuffs of lymphocytes and macrophages (Wisniewsky *et al.*, 1972). Antibodies to CDV and CNS myeline are detected in the serum and CSF of affected animals. Canine distemper demyelinating encephalomyelitis strongly resembles subacute sclerosing panencephalitis (SSPE) pathologically, virologically, and immunologically (Appel, 1969).

TMEV belongs to the family of Picornaviridae. These mouse enteroviruses are typically found in the gut. However, TMEVs are occasionally able to penetrate the CNS and cause an acute inflammation of the anterior horn cells that resembles poliomyelitis. Pathologically, this disease resembles MS in that it is characterized by demyelination with the preservation of axons. TMEV is often used as a model for MS because the pathological anomalies are limited to the CNS; infection is latent and persistent; demyelination is mediated by the immune system and occurs after a long incubation period; antibodies to myelin and proteolipid protein can be detected in diseased animals; and there are recurrences of demyelination and remyelination reminiscent of relapsing-remitting MS (Rodriguez *et al.*, 1987). Virulent and avirulent strains of TMEV have been identified. Interestingly, it is the persistent avirlulent strain that causes chronic CNS disease (Pevaar *et al.*, 1988). Susceptibility of mice to TMEV-mediated demyelinating disease is associated with MHC class I genes (Borrow *et al.*, 1992). It has been demonstrated that CD8+ T cells are critical in prevention of TMEV demyelinating disease, and that CD4+ T cells are important as helpers in the synthesis of neutralizing antiviral antibodies (Borrow *et al.*, 1992).

The murine coronavirus JHM strain is a neurotropic variant that infects small rodents. The virus readily infects oligodendrocytes and neurons and kills most animals. However, those animals that survive acute infection develop a chronic–progressive neurologic disease, while the virus establishes a persistent infection of astrocytes (Kyuwa and Stohlman 1990). These mice typically develop scattered demyelinating

lesions and areas infiltrated by macrophages and lymphocytes (Kyuwa and Stohlman, 1990). As the disease progresses, lymphocytic infiltration diminishes while demyelination and astrogliosis increase. These lesions resemble the chronic plaques of MS. JHM-resistant strains have been observed in mice and rats. In JHM-infected mice, no autoimmune reaction against brain antigens has been described.

Visna virus is a member of the lentiviruses, which include human immunodeficiency viruses I and II. Infection of sheep with visna virus results in gait abnormalities followed by paraplegia and total paralysis. The disease course is variable and ranges from slowly to rapidly progressive. Neurological signs correlate with elevations in CSF protein and pleocytosis. Visna virus has a primary tropism for monocytes and macrophages and is thought to be transported to the CNS by infected monocytes that release viral particles when they differentiate into macrophages (Zink, 1989). Once released into the CNS, the virus infects microglia and leads to the recruitment and proliferation of cytotoxic T lymphocytes (CTLs). It is believed that the demyelination lesions observed in this disease may be the result of damage by CTLs specific for viral antigen or by autoantibodies that are common in chronic lentiviral diseases (Striker et al., 1987).

3. Viruses in Demyelinating Diseases of Man

Examples of viral-induced demyelinating diseases of humans include progressive multifocal leukoencephalopathy (PML), subacute sclerosing panencephalitis (SSPE), and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). PML is a rare subacute demyelinating disease associated with JC virus, a papovavirus that is widespread in human populations worldwide. Approximately 70–50% of adult humans are seropositive for JC virus, 65% of whom are infected by the age of 14. It has been suggested that the kidneys are the site of persistent JC virus infection (Dörries and ter Meulen, 1983). Typically, PML occurs in individuals who are immunocompromised or who have defective cellular immunity. It has been reported that 3–5% of AIDS patients develop PML (Berger et al., 1987). Patients with PML present with variable symptoms, depending on the location of CNS lesions (Johnson et al., 1977). Ataxia, dementia, paralysis, and sensory abnormalities are common in PML. Pathologically, PML is typified by noninflammatory multifocal lesions scattered throughout white matter. The pathogenesis of PML is not fully understood and it is unclear why PML is such a rare disorder.

SSPE is a CNS disease of children and young adults that develops as a rare consequence of measles virus infection. The clinical course of

SSPE typically begins with subtle mental deterioration, followed by lack of coordination and other motor abnormalities (ter Meulen et al., 1983). The clinical course of SSPE may last either months or years and ultimately results in coma and death. SSPE patients have high serum and CSF antibodies to all measles virus structural proteins with the exception of the membrane (M) protein. CNS lesions in SSPE are characterized by perivascular cuffing with infiltrates of lymphocytes and plasma cells in both gray and white matter. Extensive demyelination and an increase in hypertrophic astrocytes are also observed in this disease. Cowdry type A and type B inclusion bodies containing measles virus-specific antigens are found in both neurons and glia. However, no intact measles virus particles have been observed in brain material of SSPE patients and the mechanism by which measles virus enters the CNS is unknown. The development of SSPE has not been associated with particular strains of measles virus and, therefore, it is likely that other host factors are necessary for this rare disorder to occur in measles-infected individuals.

The human T-lymphotropic virus type I (HTLV-I) is associated with a chronic, progressive neurologic disease known as HTLV-I-associated myelopathy/tropical spastic paraparesis (cited above). The clinical hallmark of HAM/TSP is a gradual onset of lower-extremity weakness, bowel and bladder dysfunction, fecal incontinence, Babinski sign, and variable sensory loss (Osame et al., 1987, 1990a, 1990b; McFarlin and Blattner, 1991). The onset of HAM/TSP is indeed gradual in most patients and the disease is clinically indistinguishable from the chronic–progressive form of MS. CSF analysis in HAM/TSP is remarkable for a mild lymphocytic pleocytosis, mild protein elevation, increased neopterin elevated IgG synthesis and IgG index, and oligoclonal bands, some of which are directed against HTLV-I (Höllsberg and Hafler, 1993; Jacobson et al., 1990). Magnetic resonance imaging has demonstrated demyelinating lesions in both the white matter and the paraventricular regions of HAM/TSP brains and swelling or atrophy in the spinal cord (Nakagawa et al., 1995). New lesions comprise mainly lymphocytes of the CD4+ lineage and monocytes, while older lesions display diffuse gliosis with macrophages and CD8+ lymphocytes in the parenchyma and perivascular spaces. Distinct plaques characteristic of MS are not observed in HAM/TSP. However, loss of myelin, with some preservation of axons, has been described. The incubation period between infection with HTLV-I and the development of HAM/TSP is typically long and, as will be described subsequently, only occurs in a minority of those infected.

III. VIRUSES IN MULTIPLE SCLEROSIS

A. *Viruses Associated with Mutiple Sclerosis*

Infectious agents have been suspected in the etiology of MS for over a century (Johnson, 1994). Over the years, several viruses have been thought to be associated with MS, and these associations are based primarily on elevated antibody titers or the isolation of a particular virus from MS material (Table I). However, none of these viruses have been definitively associated with the disease. Elevated antibody titers to several viruses, including influenza C, herpes simplex, measles, varicella-zoster, rubeola, vaccinia, Epstein-Barr, mumps, SV5, and human herpes virus 6 (HHV-6), have been reported in patients with MS, in comparison with healthy controls (Henson *et al.,* 1970, Alperovich *et al.,* 1991, Whitaker *et al.,* 1976, Ito *et al.,* 1975, Soldan *et al.,* 1997, Sumaya *et al.,* 1980). Although most of these reported agents have been discounted from consideration in the pathogenesis of MS, a few remain candidate viruses. Several bacteria have also been identified as potential etiologic agents in MS, based on the observation of increased antibody titers in MS patients as compared to controls (Salmi *et al.,* 1981, 1983; Sriram *et al.,* 1999, Vartdal *et al.,* 1980). It is not known whether elevated antibodies to infectious agents found in the CNS of MS patients represent local production of antibody in the CNS as a result of resident lymphocytes, or if they are a consequence of "spillover" of circulating serum antibodies that result from a damaged blood–brain barrier.

Of the many viruses, from a wide variety of families (Table I), that have been associated with MS, no one virus has received more consideration throughout the years than measles virus. As mentioned above, measles virus can establish persistence, both in tissue culture and *in vivo,* as is the case in SSPE, a chronic, progressive demyelinating disease of the CNS. Therefore, it has been repeatedly sought out as an etiologic agent for MS. Both humoral and cellular immune responses to measles virus differ in MS patients as compared to healthy controls. Intrathecal synthesis of measles-specific antibodies has been demonstrated in the CSF of MS patients (Norby, 1978) and, paradoxically, decreased measles-specific CTLs are found in MS patients as compared to healthy individuals (Jacobson *et al.,* 1985). Cytoplasmic tubular structures resembling measles nucleocapsids have been found in the astrocytes of one MS patient. An explant of this patient's brain tissue developed a cytopathic effect that was preventable by pretreatment with an antimeasles serum (Field, 1972). Additionally, in one study, measles virus-specific RNA has been detected by *in situ* hybridization in brain material of patients (Haase *et al.,* 1981). How-

TABLE I

VIRUSES ASSOCIATED WITH MS[a]

Virus	Evidence for Association	Ref.
Coronavirus		
Coronavirus	Isolation from mice inoculated with MS brain	Burks, 1980
Herpesviruses		
HSV	Isolated in T cells from MS patients brains	Gudnadottir, 1964
	Increased CSF antibody titers in MS	Norby, 1978
	Isolated from CSF of MS patients	Bergstrom, 1989
HCMV	Isolated from chimpanzee inoculated with MS brain	Wrobleska, 1979
EBV	Higher prevalence of EBV infection in MS	Bray, 1983
HHV-6	Detection of DNA and viral protein in MS brain	Challoner, 1995
	Increased IgM and detection of serum DNA	Soldan, 1997
	Increased lymphoproliferative response to HHV-6A variant	Soldan, 2000
Flaviviruses		
Rubella	Increased antibody titers in MS	Forghani, 1978
Tick-borne encephalitis	Isolated from mice inoculated with MS blood	Vagabov, 1982
Parainfluenza viruses		
Parainfluenza virus I	Isolation in tissue culture after cell fusion of brain cells from MS patients	ter Meulen, 1972
Simian virus 5	Development of SV5 CPE in T cells after inoculation with bone marrow from an MS patient	Mitchell, 1978

Paramyxoviruses		
Measles virus	Measles RNA detected in MS brain tissue	Haase, 1991
	Impaired CTL response in MS	Jacobson, 1985
	Increased intrathecal antibody synthesis in MS	Norby, 1978
Mumps	Increased antibody titers in MS	Alpertovich, 1991
Retroviruses		
HTLV-I	Detection of retrovirus from T cells of MS	Koprowski, 1985
MSRV	Detection of retrovirus RNA in MS CSF	Peron, 1997
Retrovirus/EBV	EBV activation of retroviral-like particles in MS CSF	Munch, 1997
Rhabdovirus		
Rabies virus	Isolation from blood and CSF of two MS patients	Margulis, 1946
Undefined viral agents		
Scrapie agent	Development of scrapie in sheep after inoculation with MS brain	Mitchell, 1978
Bone marrow agent	Development of CPE in tissue culture after CSF inoculation	Mitchell, 1978

[a] Adapted from Johnson (1995).

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ever, several other studies did not confirm these results (Stevens *et al.,* 1980, Hall, 1982).

Retroviruses, including HTLV-I, have been repeatedly targeted as potential agents in the pathogenesis of MS, in part due to the clinical and pathological similarities between HAM/TSP and MS. Kaprowski *et al.,* (1985) demonstrated the presence of antibodies that react with the HTLV-I gag (p24) protein in samples of serum and cerebrospinal fluid of patients with MS in Sweden and Florida. Additionally, HTLV-I sequences were reported in one-third of lymphocytes and CSF cell cultures from MS patients by *in situ* hybridization (Kaprowski *et al.,* 1985). Subsequently, it was demonstrated that there were both HTLV-I seronegative and seropositive MS patients from whom HTLV-I sequences could be amplified from the peripheral blood lymphocytes (PBLs) and CSF T cells (DeFreitas, 1995; Koprowski and DeFreitas, 1988; Redy, 1989). However, other studies have failed to confirm these results (Bangham, 1989; Richardson, 1989; Chen, 1990).

Although an association between HTLV-I and MS has not been supported, the possibility of a retroviral etiology of MS has not been excluded. In the absence of evidence for an exogenous retrovirus associated with MS, it has been suggested that human endogenous retroviruses (HERVs) could be involved (Rudge, 1991; Rassmussen *et al.,* 1993). HERVs comprise up to 1% of human DNA and have recently been suggested as "triggers" in a variety of autoimmune disorders (Krieg, 1992; Urnovitz, 1996; Nakagawa, 1997). The proposed pathogenic role for HERVs is based on the correlation of superantigen expression from the endogenous retrovirus termed $IDDMK_{1,2}22$ and insulin-dependent diabetes mellitus and the presence of autoantibodies that cross-react with HERV proteins in patients with systemic lupus erythematosus and Sjögren's syndrome (Benoist, 1997; Conrad, 1997; Bloomber, 1994; Garry, 1994).

A putative retrovirus, known as the multiple sclerosis retrovirus (MSRV), has recently joined the long list of viruses tentatively associated with MS (Peron *et al.,* 1997). MSRV *pol* (polymerase gene-encoding retroviral reverse transcriptase) sequences were isolated from retroviral particles released by leptomeningeal cells (LM7) cultured from the CSF of an MS patient (Peron *et al.,* 1997). Additionally, *pol* sequences were isolated from the serum of a significantly higher percentage of MS patients than of controls (Garson *et al.,* 1998). Interestingly, proteins from HSV-I have been demonstrated to transactivate MSRV *in vitro* (Peron *et al.,* 1997). This observation may be consistent with the correlation between MS exacerbations and viral infections (Sibley *et al.,* 1985). Sequence analysis of the MSRV *pol* gene indicates

that it is virtually identical to the *pol* gene of the endogenous retro-
virus –9 family that is expressed in MS and control human tissues
(Brahic and Bureau, 1997). However, an extensive characterization of
a new family of human endogenous retroviruses, which has been des-
ignated as human endogenous retrovirus-W (HERV-W), suggests that
MSRV may be a member of the HERV-W family (Blond *et al.*, 1999;
Fujinami and Libbey, 1999). Mutations in the *gag* and *pol* genes of the
HERV-W family indicated that functional proteins cannot be trans-
lated. Furthermore, preliminary studies have shown that the HERV-W
family is expressed solely in the placenta and fetal liver. These find-
ings are inconsistent with the suggestion that MSRV is derived from a
replication-competent endogenous retrovirus and do not provide a
viable explanation for the involvement of MSRV in the pathogenesis of
MS. The association of MSRV and MS is ambiguous but warrants fur-
ther investigation.

B. Potential Mechanisms of Virus-Induced Demyelination in Multiple Sclerosis

There are a number of models of virus-induced demyelination in MS.
All of these models attempt to explain the complex series of events
(Fig. 2) that ultimately result in the MS lesion. The molecular mimicry
model suggests that an immune response against viral antigens that
cross-reacts with normal host cell components may contribute to the
pathogenesis of MS. It has been demonstrated that several viral
sequences contain part of the human MBP sequence (Jahnke *et al.*,
1985). Evidence of molecular mimicry is provided by the cross-reactiv-
ity of antibodies against proteins of herpes simplex and measles virus
with human intermediate filaments (Fujinami *et al.*, 1983). Addition-
ally, rabbits immunized with a synthetic peptide containing sequences
of the hepatitis B virus polymerase developed EAE lesions. The rab-
bits that developed EAE as a consequence of immunization with this
synthetic peptide then generated a humoral and cell-mediated
immune response to both myelin and hepatitis B polymerase (Fuji-
nami and Oldstone, 1985). Additionally, cross-reactivity between a
monoclonal antibody to the VP1 protein of Theiler's murine virus and
oligodendrocytes has been demonstrated (Yamada *et al.*, 1990).
Demyelinating disease was observed in mice who were administered
the VPI monoclonal antibody. In a recent study, amino acid homologies
between immunogenic epitopes of Semliki Forest virus (SFV) and
myelin autoantigens, myelin basic protein (MBP), myelin proteolipid
protein, and myelin oligodendrocyte glycoprotein (MOG) were identi-

fied (Mokhtarian *et al.*, 1999). Immunization of B6 mice with SFV proteins induced significant lymphocyte proliferation to the SFV E2 peptide as well as the MOG peptide, 18–32, but not to MBP or PLP peptides. Immunization with both MOG 18–32 and E2 115–129 induced a later-onset, chronic EAE-like disease (Mokhtarian *et al.*, 1999). These examples of molecular mimicry support the possibility that immunological recognition of viral peptides of sufficient structural similarity to the immunodominant MBP peptide may lead to clonal expansion of MBP-reactive T cells in MS. Therefore, viruses which are known to cause latent or persistent infections, such as herpes viruses, may lead to a chronic antigenic stimulation of autoreactive T cell clones (Allegretta *et al.*, 1990). The immunodominant region of MBP (84–102) is predominantly recognized by MS patients who are carriers of the HLA-DR2 allele. This region has been found to have sequence homology with several viruses from diverse families, thereby suggesting that multiple viral agents may "trigger" autoimmune sequelae in MS (Wucherpfennig, 1995).

A second possible mechanism of virus-induced demyelination is that of a "nonspecific bystander" effect resulting from the reaction of lymphocytes or macrophages to diverse antigens (Wisniewsky *et al.*, 1975). In this case, oligodendrocytes or myelin sheaths could be damaged by lymphokines or proteases released by activated macrophages and immune cells in response to viral infection. The induction of inflammatory cytokines alone, such as TNF-α, has been shown to induce demyelination (Brosnan *et al.*, 1988). This mechanism of viral stimulation of immunocompetent cells that then nonspecifically attack the myelin sheath could explain demyelination in the number of infections with diverse viruses. This mechanism for virus-induced demyelination is also proposed in the pathogenesis of HAM/TSP. It has been suggested that the recognition of HTLV-I gene products in the CNS results in the lysis of glial cells and cytokine release (Ijichi *et al.*, 1993). This model is based on the observation that HTLV-I specific CTL restricted to immunodominant epitopes of HTLV-I gene products can be demonstrated in the PBLs and the CSF of HAM/TSP patients, and that the frequency of HTLV-I specific CTL is lower or absent in HTLV-I asymptomatic carriers. The target of the HTLV-I specific CTLs in the CNS could be either a resident glial cell (an oligodendrocyte, an astrocyte, or resident microglia), infected with HTLV-I, or an infiltrating CD4+ cell. HLA class I and II are not normally expressed in the CNS, which would prevent the antigen presentation necessary for CTL activity. However, class I and class II expression are upregulated by several cytokines including IFN-γ and TNF-α, which can be induced by HTLV-

I and are known to be upregulated in HAM/TSP patients. The release of cytokine and chemokine production by HTLV-I is potentially destructive to cells of the CNS. A similar mechanism could explain the virus induction of demyelinating disease in MS.

Demyelination may also develop as a consequence of virus-induced autoimmune reaction against brain antigens. Indirect evidence in support of this theory comes from EAE in animals, and from parainfectious encephalomyelitis in humans where virus-specific CD4$^+$ lymphocytes proliferate in response to MBP (Johnson, 1985; Leibert et al., 1988). It is not known how viruses break immune tolerance and force the host to mount a strong, cell-mediated immune response to brain antigens. It may be that as the virus replicates, it incorporates host antigens into its envelope and inserts, modifies, or coats itself, cellular antigens on the cell surface. It is biologically possible that these newly exposed antigens may be recognized and treated by the host as foreign (Hirsch, 1975). Söderberg-Naucler and colleagues (1996) have demonstrated the presence of CD13 in the envelope of human cytomegalovirus (HCMV). CD13 becomes associated with HCMV on budding in the Golgi-derived vacuoles during early egress (Söderberg-Nauclér et al., 1996). CD13-specific antibodies were detected in the majority of patients with HCMV viremia or disease after bone marrow transplantation (Söderberg-Naucler et al., 1996). These antibodies were found to cross-react with structures in normal skin biopsies (Söderberg-Nauclér et al., 1996). Alternatively, lymphotropic viruses might interact with the immune regulatory system by destroying some populations of lymphocytes or stimulating the generation of autoreactive lymphocyte clones. Many lymphotropic viruses are capable of transforming infected cells and rendering them immortal (ter Meulen, 1997). It has been demonstrated in vitro that cells immortalized by viruses, such as EBV, are capable of secreting autoantibodies (Rosen et al., 1977).

C. Ubiquity and Disease

Considerable focus has been placed on the identification of a unique virus exclusively associated with MS. However, the search for an "MS virus" (i.e., a viral infection that invariably results in MS and is not present in disease-free individuals) has been unsuccessful (Jacobson, 1998; Johnson, 1994). The inability to identify an "MS virus" could indicate that no single virus causes MS, that the putative "MS virus" has yet to be identified, or that viruses are not associated with this disease (Jacobson, 1998). Alternatively, a new paradigm of the MS dis-

ease process, which suggests that a common or ubiquitous virus may act as a trigger for MS in individuals with a genetic or an immunologic predisposition, has emerged (Jacobson, 1998). There are several examples of virus infections that lead to disease in only a subset of infected individuals. Some examples of viruses that are associated with multiple disease outcomes in different subsets of individuals include: EBV (Burkitt's lymphoma, nasopharyngeal carcinoma, mononucleosis); measles virus (SSPE); JC virus (PML); and hepatitis B and C viruses (hepatoma) (Miller, 1990; ter Meuler et al., 1983; Grinnell et al., 1983; Szmuness et al., 1978; Colombo, 1999). Perhaps the most relevant example of a virus that is common in certain populations, but results in disease only in a minority of those infected, is that of HTLV-I.

Originally identified from a T-lymphoblastoid cell line (HUT 102) of a patient diagnosed with a cutaneous T cell lymphoma, HTLV-I was the first described human retrovirus (Poiesz, 1980). In 1981, HTLV-I was established as the etiologic agent for adult T cell leukemia (ATL) (Hinuma et al., 1981), a hematological malignancy first characterized in Japan (Uchiyama et al., 1981). Since the initial description of ATL and the discovery of HTLV-I, the virus has been associated with an inflammatory, chronic, progressive neurologic disease known as HTLV-I-associated myelopathy/tropical spastic paraparesis (cited previously) and with several other inflammatory diseases (Gessain et al., 1985; Osame et al., 1986; Mochizuki et al., 1992; Nishioka et al., 1989; Morgan et al., 1989; Terada et al., 1994). While between 15 million and 25 million individuals are infected worldwide and seroprevalence rates in endemic areas can exceed 30%, the majority of individuals infected with HTLV-I are clinically asymptomatic (Gessain, 1996).

The propensity for certain individuals to develop either HAM/TSP, ATL, or other HTLV-I-associated inflammatory diseases, while at the same time, others remain clinically asymptomatic, is not fully understood. It has been suggested that host genetics and immune abnormalities influence an individual's predisposition to HAM/TSP, as they are believed to influence the likelihood of developing MS (Jacobson, 1995). Therefore, the use of HAM/TSP as a "model" of a chronic, progressive neurologic disease that occurs in only a small percentage of infected individuals is particularly germane in examining the possible involvement of a ubiquitous virus in the etiology of MS. The risk of an HTLV-I-infected individual acquiring HAM/TSP over a lifetime is estimated to be 0.25% (Osame et al., 1990c). In Japan, associations have been made between the likelihood of developing either HAM/TSP or ATL and particular HLA haplotypes (Usuku, 1988, Sonoda, 1992, Sonoda, 1996). HAM/TSP patients of Japanese descent have an increased fre-

quency of certain HLA-Cw7,B7, and DR1 alleles represented by the A26CwB16DR9DQ3 and A24Cw7B DR1DQ1 haplotypes. In contrast, Japanese ATL patients have an increased frequency of HLA-A26, B16, and DR19 and a decreased frequency of HLA-A24 and Cw1, as compared to controls. The HLA types DRB1*0901, DQB1*0303, and DRB1*1501 in ATL patients, and the HLA types DRB1*0101, DRB1*0803, DRB1*1403 and DRB1*in HAM/TSP patients, were found to be mutually exclusive (Sonoda et al., 1996).

The neuropathology of HAM/TSP indicates that immune-mediated mechanisms are involved in the progression of this disease. Furthermore, several lines of evidence indicate that the cellular and humoral immune responses of HAM/TSP patients are altered from those of HTLV-I asymptomatic carriers and uninfected controls. The immunologic hallmarks of HAM/TSP include an increase in ex vivo spontaneous lymphoproliferation in the absence of antigenic stimulation or IL-2 (Kramer et al., 1989); the presence of HTLV-I-specific, CD8+ CTLs in the PBL (Jacobson et al., 1992), and an increase in antibodies to HTLV-I in sera and CSF (Gessain et al., 1985). Natural killer cells tend to be diminished in both number and activity in HAM/TSP (Kitajima et al., 1998). Although the suggestion of disease-specific HTLV-I strains has been dismissed as a factor in the determination of disease susceptibility, increased viral load has been implicated in the pathogenesis of HAM/TSP (Nagai et al., 1998). It has been suggested that increased proviral loads may be a predictor of the progression from the asymptomatic-carrier state to HAM/TSP (Jeffery et al., 1999). It has been suggested that the HLA class I allele A2*01 may confer a protective effect on the development of HAM/TSP by influencing the proviral load in infected individuals (Jeffery et al., 1999). Interestingly, the HLA A2*01 haplotype has also been shown to decrease the overall risk of MS in an HLA allele comparison study from a cohort of Swedish and Norwegian MS patients and healthy controls using polymerase chain reaction- single-strand conformation polymorphism (PCR-SSCP) (Fogdell-Hahn et al., 2000).

Several models for the immunopathogenesis of HAM/TSP have been proposed. All of these models are based on an HTLV-I-induced, immune-mediated response in the CNS to either specific viral antigens or cross-reactive self-peptides, none of which are mutually exclusive (reviewed in Kubota et al., 2000). The proposed models for the immunopathogenesis of HAM/TSP are similar to those suggested for MS. Therefore, it is hoped that insights into the pathogenesis of HAM/TSP will lead to a better understanding of MS and other neurologic disorders, such as neuro-AIDS, in which virus-mediated immunopathogenesis may occur in a subset of infected individuals.

D. Human Herpes Virus-6 and Multiple Sclerosis

One of the most recent viral candidates as an etiological agent in MS is the human herpes virus-6 (HHV-6). HHV-6 is a beta herpesvirus for which seroprevalence rates vary from 72% to 100% in healthy adults worldwide (Yamanishi, 1992; Asano and Grose, 1994; Hall et al., 1994). Although HHV-6 replicates primarily in T lymphocytes, it is a pleiotropic virus that can either productively or nonproductively infect cells from several lineages including B cells, microglia, oligodendrocytes, and astrocytes (Lusso, 1988; Takahashi et al., 1989; He et al., 1996; Albright et al., 1998). Two variants of HHV-6 (HHV-6A and HHV-6B) have been described, and are based on genomic, antigenic, and biological differences (Ablashi, 1991). The HHV-6B variant has been identified as the causative agent of exanthem subitum and accounts for the majority of symptomatic HHV-6 infections in infants. However, the HHV-6A variant has yet to be clearly associated with a particular disease (Braun et al., 1997; Yaminishi et al., 1988). An increased neurovirulence of the HHV-6A variant as compared to the B variant has been suggested, based on a greater detection of the HHV-6A variant than of the HHV-6 B variant in the CSF of children and adults (Hall et al., 1998) Additionally, the HHV-6A variant has been isolated from the CNS of AIDS patients with areas of demyelination (Knox and Carrigan, 1995).

HHV-6 is considered to be a viable candidate as a possible etiologic agent in MS for several reasons. First, primary infection with HHV-6 usually occurs during the first few years of life, and the involvement of HHV-6 with MS is consistent with epidemiological evidence in MS suggesting exposure to an etiologic agent before puberty (Yaminishi et al., 1988; Kurtzke, 1995). Second, HHV-6, particularly the HHV-6A variant, is highly neurotropic (Hall et al., 1998). Primary infection with HHV-6 occasionally results in neurologic complications including meningitis and meningoencephalitis and febrile seizures (Ishiguro, 1990; Asano et al., 1992). HHV-6 has been demonstrated to cause fatal encephalitis in AIDS patients and in individuals immunosuppressed as a consequence of bone marrow transplantation (Knox and Carrigan, 1994, 1995; Drobyski et al., 1994). Furthermore, a neuropathogenic role for HHV-6 has been suggested, based on the development of a variety of disorders associated with active HHV-6 infection, including fulminant demyelinating encephalomyelitis, subacute leukoencephalitis, necrotizing encephalitis, progressive multifocal leukoencephalopathy, and chronic myelopathy (Kamei et al., 1997; Novoa et al., 1997; Carrigan et al., 1996; Wagner et al., 1997; Mackenzie et al., 1995). Third, one of the fundamental properties of herpesviruses is their

tendency to reactivate. The same factors that often lead to herpesvirus reactivation, such as stress and infection with another agent, have also been associated with MS exacerbations (Paniton, 1994). Unfortunately, the mechanisms by which HHV-6 achieves latency and reactivation are poorly understood (Yasukawa et al., 1999; Kondo, 1998). Fourth, herpesviruses are typically latent in nervous tissue and cannot be structurally identified in a latent state. Therefore, herpesviruses are not likely to be found by electron microscopy. Fifth, HHV-6 is pleiotropic and infects cells of both lymphoid and nonlymphoid origin. The pleiotropic nature of HHV-6 could be involved in the abnormalities observed in both the immune and nervous systems of patients with MS.

In 1995, Challoner and colleagues suggested a potential role for HHV-6 in MS that is based on an unbiased search for non-human DNA by representational-difference analysis (RDA). This technique is based on successive rounds of subtractive hybridization and PCR amplification, which are enriched for DNA sequences present in DNA preparations from MS disease material and control PBMCs. In this study, greater than 70 DNA fragments were analyzed. One of these fragments was found to be homologous to the MDBP gene of the HHV-6B variant Z29. HHV-6 DNA was found in 78% of MS brains and 74% of control brains. However, monoclonal antibodies against the HHV-6 101 K protein and the DNA binding protein p41 were detected in the brain tissues of MS patients, and not in controls. In MS brains, nuclear staining was found in oligodendrocytes surrounding MS plaques more frequently than in uninvolved white matter (Challoner, 1995). Additionally, a prominent cytoplasmic staining appeared in neurons in gray matter adjacent to plaques. While this study did not establish a causal link between HHV-6 and MS, it made a significant impact by suggesting the first association of a virus with MS using an unbiased technology.

The association of HHV-6 with MS has also been supported by several immunological and molecular studies. Significantly higher antibody titers against HHV-6 whole-virus preparations in MS patients as compared to normal controls, and an increase in HHV-6 DNA in the PBMCs of MS patients, determined by a polymerase chain reaction (PCR) assay, have been reported (Sola et al., 1993; Wilborn, 1994). While these preliminary studies were intriguing, they were based on methodologies that do not discriminate between latent and active infection of HHV-6. In order to distinguish between these stages of HHV-6 infection, early antibody responses to the HHV-6 p41/38 early antigen, and the presence of HHV-6 serum DNA, by a nested PCR

assay, were examined (Soldan, 1997). It has been demonstrated that HHV-6 serum DNA correlates with active HHV-6 infection and is not found in healthy individuals (Secchiero et al., 1995). In this original study, a significant increase in IgM response to the p41/38 early antigen was demonstrated in patients with the relapsing–remitting form of MS, in comparison to healthy controls and individuals with other neurologic disease. An increased IgM response to the p41/38 early antigen was also observed in a group of patients with other inflammatory diseases. It is notable that of the individuals in the inflammatory disease group of increased IgM titers to HHV-6 were patients with systemic lupus erythematosus, which has been tentatively associated with active HHV-6 infection (Hoffmann, 1991; Dostal, 1997). Two additional studies have confirmed the presence of increased IgM responses to HHV-6 in patients with MS (Ablashi, 1998; Friedman, 1999), while no correlation was demonstrated in another study (Enbom, 1999).

Additionally, HHV-6 serum DNA was detected in 30% of MS patients (15 of 50) and in 0% of 47 controls consisting of healthy individuals, patients with other inflammatory diseases, and patients with other neurologic diseases (Soldan et al., 1997). This NIH cohort has been expanded to include a total of 103 MS patients and 70 controls (Fig. 3). We have continued to demonstrate the presence of HHV-6 DNA in the serum of 24% of MS patients and 0% of controls. Subsequent studies

	+ HHV6 DNA	- HHV6 DNA
MS patients n=103	25	78
non MS patients NL n=36 OND n=19 OID n=15	0	70

p < .0001

FIG. 3. An extension of a study describing the presence of HHV-6 DNA in MS patients and controls, as determined by a nested PCR assay (Soldan et al., 1997). To date, serum from 103 MS patients and 70 non-MS patients, including 36 normal donors, 19 patients with other neurological disease (OND), and 15 patients with other inflammatory diseases (OIDs) were examined for HHV-6 DNA sequences as a marker of active virus. Consistent with previous results, 24% of MS patients from the National Institutes of Health (NIH) cohort were positive, while no HHV-6 DNA was found in the serum of 70 controls. These data serve to support an association of HHV-6 in a subset of MS patients.

by a number of other groups have reexamined the presence of HHV-6 serum DNA in MS patients. Overall, the results from these studies have been equivocal. It has been suggested that discrepancies in these studies may be attributable to differences in patient selection, techniques, and reagents used (Fillet, 1998; Jacobson, 1998b). In a recent study, Locatelli and colleagues (2000) validated this theory by comparing a nested PCR assay for the HHV-6 major capsid protein region of HHV-6 (Secchiero *et al.*, 1995) to a TaqMan quantitative PCR assay specific for the HHV-6 U67 open reading frame. The HHV-6 TaqMan assay had a greater sensitivity than the nested PCR assay had, in part due to the more efficient DNA-extraction method, which used glycogen as a carrier molecule prior to DNA precipitation (Locatelli *et al.*, 2000). In this study, HHV-6 cell-free DNA was detected in 18% of sera and, importantly, in 34% of CSF from 29 MS patients tested. In contrast, none of the same sera and CSF were HHV-6 DNA positive by a standard nested PCR assay. This example underscores the importance of using assays of comparable sensitivity when detecting DNA of extremely low copy number. This study confirms the presence of active HHV-6 infection, as measured by serum and CSF DNA, and suggests that HHV-6 may replicate in the central nervous system of a subset of MS patients (Locatelli *et al.*, 2000).

Lymphoproliferative responses to both the HHV-6 A and B variants have been reported in healthy adults (Wang *et al.*, 1999; Yakushijin *et al.*, 1991). A higher percentage of healthy adults have T cell responses to the HHV-6B variant than to the HHV-6A variant (Wang *et al.*, 1999). The increased frequency of healthy individuals who have lymphoproliferative responses to the HHV-6B variant may reflect a higher frequency of infection with the HHV-6 B variant in the general population (Wang *et al.*, 1999). A recent study examined the T cell lymphoproliferative responses of healthy controls and patients with MS to both variants of HHV-6 as well as to HHV-7 (Soldan *et al.*, 2000). This study demonstrated that there was no difference in either the frequency or magnitude of proliferative responses, between healthy controls and patients with MS, to either the HHV-6B variant or HHV-7. However, a significantly higher percentage of patients with MS had proliferative responses to the HHV-6A variant (66%) than did healthy controls (33%). It is, at present, not known whether the increased frequency of lymphoproliferative responses to the HHV-6A lysate in patients with MS is the result of a higher seroprevalence of the HHV-6A variant in MS patients or of an altered host immune response (Soldan *et al.*, 2000).

The description of an increased lymphoproliferative response to the HHV-6A variant further supports the association of HHV-6 with MS and suggests that the highly neurotropic A variant, rather than the B

variant, may play a role in this disease (Soldan *et al.,* 2000). Additionally, this work emphasizes that future studies concerning the putative association of HHV-6 with MS must consider variant specific tropisms and immunology. Unfortunately, the serological methods currently available do not discriminate between the HHV-6A and HHV-6B variants, thus making it difficult to assess the seroprevalence of either variant. Presently, there is indirect evidence for the involvement of both variants in MS. HHV-6B sequences have been amplified from brain material of MS patients and controls (Challoner *et al.,* 1995; Ablashi *et al.,* 1999), and one study has demonstrated an increase in the IgM response to the HHV-6B variant in the CSF of MS patients (Ongradi *et al.,* 1999). Evidence for the involvement of the HHV-6A variant, rather than the B variant, in MS is supported by the lymphoproliferative study described and by the detection of the HHV-6 A variant in the PBMC of MS patients but not in controls (Kim *et al.,* 1999). The relationship between HHV-6 and MS remains controversial and has yet to be clearly defined. Additional serological, cellular/immune response, molecular, and clinical studies are necessary to elucidate the role, if any, of HHV-6 in the pathogenesis of MS.

E. Multiple Infectious "Triggers" in Multiple Sclerosis

It is possible that multiple viruses may be involved in the etiology of MS and that particular viruses trigger disease in different subsets of individuals. Could different viruses be associated with a disease, such as MS, through a common mechanism? A possible mechanism by which HHV-6 and, potentially, other viruses could result in MS has recently been suggested by the exciting discovery of the HHV-6 cellular receptor (Santoro *et al.,* 1999). Santoro and colleagues have clearly demonstrated that CD46, also known as the membrane cofactor protein (MCP), is the cellular receptor for HHV-6. CD46 is a member of a family of glycoproteins that are regulators of complement activation (RAC), and that prevent spontaneous activation of complement on autologous cells. CD46 is expressed on all human nucleated cells, and soluble forms can be found in plasma tears and in seminal fluid of normal individuals (Hara *et al.,* 1992). The use of a virtually ubiquitous human molecule as a surface receptor helps to explain the pleiotropism of HHV-6. Of particular interest is the fact that CD46 is also the primate-specific receptor for measles (Dorig *et al.,* 1993; Oldstone *et al.,* 1999). Significantly, HHV-6 and measles virus, which are from disparate virus families, have been associated with MS and, interestingly, use the same receptor.

Could viruses that share a receptor in common, such as HHV-6, and measles virus cause MS by a similar mechanism? It is theoretically

possible that the engagement of CD46 by one or both of these viruses may result in increased activation of the complement cascade on autologous cells through downregulation of the receptor. This abnormal increase in complement could lead to widespread tissue damage through cytokine disregulation, cytolysis, and nitric oxide production (Karp *et al.*, 1996, Ghali and Schneider-Schaulies, 1998). Furthermore, an increase in soluble CD46 has been described in several autoimmune disorders, including systemic lupus erythematosus and Sjogren's syndrome and may be implicated in the pathogenesis of other autoimmune diseases (Cuida *et al.*, 1997; Kawano *et al.*, 1999). Additionally, other viruses use various members of the RAC family as cellular receptors. Epstein-Barr virus, which has also been implicated in MS, uses CD21, while CD55 is used by several echoviruses and coxsackie viruses (Yerfenol *et al.*, 1976; Bergelson *et al.*, 1994, 1995). Further studies are needed to determine whether these members of the RAC family that serve as virus receptors play a role in the pathogenesis of MS.

The potential influence of viruses that utilize members of the RAC family, such as HHV-6 and measles virus, on the pathogenesis of MS may, in part, be elucidated by animal studies. Recently, a CD46 transgenic mouse that can be infected by measles virus was described (Oldstone *et al.*, 1999). Measles virus infection in these transgenic mice was associated with immunosuppression and virus replication in the CNS. Measles virus infection was also associated with CNS disease in infected mice (Oldstone *et al.*, 1999). The generation of a CD46 transgenic mouse provides an excellent model for studying the role of measles virus infection in CNS disease. Additionally, the CD46 transgenic mouse may provide a model for studying the neuropathogenesis of HHV-6 infection in the CNS if in fact the virus, which has an extremely limited host range, may productively infect these mice that now express the HHV-6 receptor. Furthermore, a recent study has demonstrated that EAE may be inhibited by the use of a complement inhibitor, which indicates an important role for complement in EAE as well as in MS (Davoust *et al.*, 1999). Future studies investigating the interactions of measles virus and HHV-6 with CD46 may elucidate the role of both viruses and complement in the pathogenesis of MS.

IV. Conclusions

The pathogenesis and etiology of MS have yet to be well defined. Epidemiologic evidence suggests that it is a multifactorial disease that develops as a result of host genetics, immune response, and environment. Several lines of evidence, including the documentation of viruses

that induce a variety of demyelinating diseases in both humans and animals, suggest that a virus may comprise the environmental component in the etiology of MS. While many viruses have been proposed as etiologic agents in MS, none of these viruses have been firmly associated with disease pathogenesis. Additionally, mechanisms by which virus–host interactions may lead to demyelination are not fully understood. Currently, HHV-6 and MSRV are receiving much attention as potential MS "triggers." However, the role of these viruses in the pathogenesis of MS is unclear. We suggest that multiple viral agents may induce a virus-specific and/or a cross-reactive autoimmune process resulting in clinical disease in a subset of genetically susceptible individuals. The involvement of multiple infectious agents in MS may explain the difficulty in identifying a single viral agent responsible for this highly variable and chronic disease. Moreover, we encourage extreme caution in attempts to readily associate viruses in a chronic, progressive neurologic disorder such as MS. As outlined in this review, it is difficult to determine cause from effect, particularly when a ubiquitous viral agent is suggested to play a role in disease pathogenesis. Uniformity in assay design, viral-isolation techniques, molecular probes, etc., must be employed by different research groups on a large number of MS cohorts to confirm these virus associations. Perhaps only through well-controlled, clinical, antiviral therapeutic trials, with defined clinical, virological, and radiographic outcome measures, can we ever determine the role that viruses, if any, may play in the pathogenesis of MS.

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