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INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). Although the cause of MS is unknown, it is thought to be an autoimmune condition mediated by autoreactive lymphocytes. The pathogenesis of MS seems to be multifactorial in nature, and it is thought to be triggered by multiple environmental factors in genetically susceptible individuals. Viruses have long been suspected to play a role in the development of MS, and there seems to be increasing evidence of a link between MS and several different viruses. Although viruses may not be causal to the development of MS, there is speculation that they may act as environmental triggers in susceptible individuals. While multiple viruses have been linked with MS, including Epstein–Barr virus (EBV), human endogenous retroviruses (HERVs), varicella zoster virus (VZV), and measles, to name a few, some of the most compelling evidence for a viral etiology of MS has been found in studies of HHV-6. This chapter will review the current evidence linking viruses and, more specifically, HHV-6 with MS.

MULTIPLE SCLEROSIS

Multiple sclerosis is the most common inflammatory demyelinating disease of the CNS and is estimated to affect between 2 to 2.5 million people worldwide. It is a heterogeneous disease with variable clinical presentations and pathological findings. MS is a chronic degenerative disease that involves CNS inflammation and demyelination in acute presentations, as well as axonal damage in the long term. It is classically characterized by white matter lesions disseminated in space and time. MS is also characterized by gray matter atrophy, which is less well defined but may be related to neuronal degeneration caused by white matter lesions and axonal loss. In the most common form of MS,
relapsing-remitting MS (RRMS), the inflammation and demyelination occur episodically, correlating clinically with neurologic dysfunction.  

Multiple sclerosis is thought to be autoimmune in nature, although direct proof of an autoimmune cause is lacking. In this disease, it is thought that autoreactive lymphocytes cross the blood–brain barrier and infiltrate the CNS, leading to inflammation and damage to myelin and axons. Although some remyelination does occur, it is typically not durable. There is usually recovery from the dysfunction after the inflammation resolves; however, this recovery may be incomplete. Over time, MS patients accumulate gliosis and axonal degeneration, which correlates with progression of the disease and further disability. Although T cells are well recognized in the pathogenesis in MS, greater than 90% of MS patients are found to have oligoclonal bands in the cerebrospinal fluid (CSF) and an elevated immunoglobulin G (IgG) index, indicating a role for B cells in the immune response to MS, as well.

Multiple sclerosis tends to present at a relatively early age, with a peak age of onset at 30. It affects more females than males, with a ratio approaching 3:1. Although most patients have a relapsing-remitting course, 65% of these patients will eventually enter a secondary progressive phase. In about 20% of patients with MS, the course is a primary progressive course from the beginning. Presenting symptoms commonly include visual disturbances such as optic neuritis and extraocular movement abnormalities, motor and sensory symptoms, and ataxia. Other common symptoms in MS patients include spasticity, urinary dysfunction, vertigo, and fatigue. Relapsing and remitting MS patients are treated with a variety of disease-modifying agents, including interferon-γ, glatiramer acetate, natalizumab, and fingolimod; these agents reduce the frequency of relapses but do not reverse the acquired deficits, and they have unclear effects on disease progression. They have not been found to be effective in primary progressive and secondary progressive MS.

**Genetic Factors in MS**

Multiple genetic factors have been found to play a role in the development of MS. There is a higher risk of developing MS if a family member has MS, with an odds ratio of 16.8 in siblings of MS patients compared to the general population. There is also a higher concordance rate in monozygotic twins (24 to 30%) compared to dizygotic twins (3 to 5%). Certain alleles of the major histocompatibility complex (MHC) have been linked to a higher risk of developing MS, particularly the HLA-DRB1 locus. Epigenetic factors have also been implicated in the development of MS. There seems to be a maternal parent-of-origin effect in MS; this has been described in studies of extended family pedigrees, avuncular pairs, and half siblings.

**Geographical Distribution in MS**

There is also an interesting geographical distribution of MS prevalence. It has long been thought that MS prevalence increases with increasing latitude.
away from the equator, but there are many contradictions to this notion; for example, some areas with a high frequency of MS, such as Sardinia in the Mediterranean, are relatively near the equator. Another example is the Inuits, who live in a cold northern climate and have a low frequency of MS. Nevertheless, recent observations suggest that, in general, a latitudinal gradient in MS frequency does exist. Genetic factors could potentially explain this apparent geographical distribution of MS. Certain races seem to be more susceptible to developing MS, including Caucasians from Scandinavia and Scotland. Conversely, among Mongolians, Japanese, Chinese, and American Indians, MS is relatively rare. MS also occurs less frequently in African blacks, Aborigines, Norwegian Lapps, and Gypsies.

**Immigration Studies in MS**

Changes in the incidence of MS in immigration populations that cannot be explained by genetic factors have been well described. A systematic review of migrant studies of MS undertaken by Gale and Martyn revealed two trends: (1) migrants moving from an area with a higher prevalence of MS to an area with a lower prevalence of MS have a decreased rate of disease, and (2) migrants who move from an area of lower to higher prevalence retain the lower risk of developing the disease, although offspring of migrants who move from an area of lower to higher prevalence will have a risk of MS approaching the host area. Based on the immigration studies that took age at immigration into account, Gale and Martyn surmised that the risk of developing MS is established within the first two decades of life; however, other immigration studies found that this risk may be established within an even earlier time frame. Studies of migrants to Los Angeles County in California and King and Pierce counties in Washington State revealed that migrants to Los Angeles County had lower rates of MS than migrants to King and Pierce counties; these results seem to be even more pronounced in individuals who migrated to Los Angeles County at the age of 10 years or less when compared to individuals who migrated at older ages. A study in Israel found that immigrants from Afro-Asian countries, who typically have low rates of MS, developed a risk of MS similar to that of European immigrants, who have higher rates of MS; this study noted, however, that the higher rates were only noticed in immigrants who migrated at a very early age, between infancy and preadolescence. Despite these studies, other studies suggest that no particular age at immigration causes the change in risk of MS, but rather duration of the exposure to the new environment. These immigration studies, as well as the moderate discordance in monozygotic twins, suggest a strong environmental factor in the development of MS.

**Environmental Factors and MS**

Many different environmental factors have been linked to MS, including vitamin D, exposure to sunlight and ultraviolet (UV) light, smoking, and viruses.
The observation of an association between MS and latitude has led to studies on the nature of vitamin D and UV exposure in relation to MS. Vitamin D levels have been found to be low in MS patients at time of diagnosis, and there also seems to be an association between low vitamin D levels and an increased risk of relapse. Similarly, studies showed that an increase in vitamin D levels in MS patients correlated with a lower risk of relapse. UV exposure has also been studied; higher UV exposure at a young age seems to be associated with a decreased risk of MS. Several different studies indicate a higher risk of MS in smokers; it has also been observed that parental smoking increases the risk of MS in children.

VIRUSES AND MULTIPLE SCLEROSIS

Another environmental factor associated with MS is viruses. The role of viruses in MS is a somewhat controversial issue that has been of interest in one form or another for over a century. In the late 19th century, when Jean-Martin Charcot and his pupil Pierre Marie were describing MS, they postulated an infectious etiology for this disease. Marie in particular asserted his hypothesis for an infectious etiology of MS when in 1884 he noted the following: “I was struck by the coincidental occurrence of sclérose en plaques with infectious illnesses, and by the close relationship that, from a theoretical point of view, unites these diseases. Therefore, I made an effort to renew my idea that sclérose en plaques often starts as an infectious process.” In the late 19th and early 20th century, multiple infectious agents were implicated, including bacterial, spirochetal, and viral infections; however, to date, no infectious agent has been proven to cause MS. Several clusters of MS reported in the 20th century were suspected to be linked to an infectious cause. One such cluster occurred in 1947 when four of seven scientists developed MS; this group was working on a disease in lambs called swayback. Another cluster of MS occurred in the Faroe Islands after British troops were stationed there during World War II; it was thought that British troops introduced canine distemper virus to the islands. However, these and other clusters of MS are controversial and disputed by many.

Although the infectious theory in MS has been a controversial topic, more interest is being placed on the role of viruses in MS, not necessarily as the sole cause of the disease but as a potential trigger or risk factor in the development of this complex disease in genetically susceptible individuals. Numerous viruses have been implicated in the development of MS, including measles, mumps, rubella, EBV, VZV, HERVs, and more recently HHV-6. Some of the most convincing data for an association between viruses and MS is found in EBV and HHV-6, two different herpes viruses.

EBV and MS

Epstein–Barr virus (also known as human herpesvirus 4) is the causative agent of infectious mononucleosis. EBV has been of interest as a potential cause or
trigger of MS since the 1970s, but the association with MS has been strengthened recently in light of growing research. EBV seropositivity has been found to be more common in patients with MS compared to controls, with a seroprevalence of 99% in MS patients and 89% in controls. A meta-analysis studying infectious mononucleosis and MS risk found that individuals who are not infected with EBV have a risk of MS close to zero, and MS risk increases after infection with EBV; this risk was further increased if individuals developed the infection in adolescence or adulthood.

The Hygiene Hypothesis

The observation that a delayed exposure to EBV leads to a higher risk of MS lends support to the hygiene hypothesis, which postulates that in more developed, and hence “hygienic,” areas individuals are less likely to be exposed to infections early in life and may have abnormal immunological responses later in life. These abnormal responses may be produced when encountering these infectious agents as an adolescent or young adult. This could at least in part explain the geographic distribution of MS and other autoimmune diseases, and the hypothesis could be partly explained by the idea that infections early in life may help regulate the immune system. Another potential mechanism for this hypothesis could be related to maternal exposure to virus and transfer of antiviral antibodies. A decreased exposure to viral antigens in women prior to pregnancy leads to a reduction in the degree of protection conferred to newborns via maternal antiviral antibodies; later exposure of the child to the virus could provoke an immune response, possibly leading to autoimmune disease in the child.

Animal Models of Virally Induced Demyelination

Numerous observations have led to the implication of viruses in MS. For one, there are several animal models of virally induced demyelination. One of the most well-known associations between a virus and demyelination in an animal model is seen in Theiler’s murine encephalomyelitis virus (TMEV). TMEV, a picornavirus, was first isolated in 1934. TMEV causes a biphasic illness in laboratory mice, the first phase of which is a polioencephalitis causing hindlimb paralysis that resolves in a few weeks. The second phase involves a relapse of the hindlimb paralysis, occurring within three weeks. During the first phase, the virus affects the gray matter of the brain and spinal cord and is subsequently cleared over a few weeks, corresponding to clinical improvement in the mice. However, the virus persists in the white matter, and inflammatory demyelination characterizes the second phase of the illness. Another example of viral-induced demyelination is seen in BALB/c mice infected with JHM virus, a coronavirus. This virus infects oligodendrocytes with resultant demyelination; however, the demyelination is not associated with inflammation or immune-mediated mechanisms, indicating that direct viral-induced damage of oligodendrocytes leads to demyelination.
Murine hepatitis coronavirus also causes demyelination in mice. It causes a biphasic disease, with an acute encephalitis followed by disease relapse after a few weeks. Although the virus is cleared in a matter of weeks, viral antigen and RNA persist in the brain, inducing a chronic inflammatory response. This leads to inflammatory demyelinating lesions in the spinal cord, similar to those seen in MS. Of note, these demyelinating lesions do not occur in immune-deficient mice infected with the virus. Additionally, CNS demyelination is seen in dogs with canine distemper virus, sheep with visna virus, goats with caprine arthritis-encephalitis virus, and mice with Semliki Forest virus.

Recently, Japanese macaque encephalomyelitis has been described as a spontaneous disease in the Oregon National Primate Research Center’s colony of Japanese macaques. Clinically, affected monkeys develop paralysis, ataxia, and ocular motor paresis. Initial symptoms are severe, and animals with the disease often do not recover; in those that do recover, relapses often occur. Pathologically, multifocal demyelination is found throughout the CNS, along with infiltrates of lymphocytes and macrophages and variable areas of axonal loss. In studying the diseased tissue, a previously unknown herpesvirus was identified, called JM rhadinovirus (JMRV); this virus was not identified in healthy macaques or in healthy-appearing tissue of the diseased macaques.

**Virally Induced Demyelination in Humans**

In addition to the animal models mentioned above, there are several well-known examples of viral-induced demyelination occurring in humans, as well. Measles virus, a paramyxovirus, is known to cause demyelination through two separate clinical entities: acute postinfectious measles encephalomyelitis (APME) and subacute sclerosing panencephalitis (SSPE). APME, also known as measles-induced acute disseminated encephalomyelitis (ADEM), typically occurs during the resolution phase of systemic measles, or even weeks to months afterward. It is an immune-mediated process that does not involve direct viral infection of the CNS. It occurs in 1 in 1000 cases of measles and very rarely following vaccination with a measles-containing vaccine. This disorder is characterized by demyelination, with clinical symptoms including motor and sensory deficits, ataxia, and altered mental status. With treatment, which may include intravenous corticosteroids and intravenous immunoglobulin (IVIG), some patients may make a full recovery; however, others are left with permanent neurological deficits. SSPE is related to a persistent infection with a defective measles virus. It more commonly occurs in children who contract measles before the age of two. Most patients who develop SSPE remain symptom free for 6 to 15 years after the initial infection but then later develop behavioral problems, intellectual disability, motor dysfunction, myoclonic jerks and other movement disorders, and ocular abnormalities before progressing to death within 1 to 3 years of symptom onset. Pathologically,
cellular inclusion bodies, neuronal loss, and demyelination are seen.\textsuperscript{30,32} It is known that there is a strong immune response in SSPE; despite this response in patients without immunological deficits, the virus persists in the CNS. The precise mechanism of demyelination in SSPE is unknown.\textsuperscript{32}

Another example of virally induced demyelination occurring in humans is JC virus infection leading to progressive multifocal leukoencephalopathy (PML) in immunocompromised individuals. JC virus is a polyomavirus that is acquired in childhood or young adulthood. It is a very common virus, with at least 50% of the adult population being JCV seropositive. The exact pathogenesis of CNS infection by JC virus is unclear; it is debated whether the virus is latent in the CNS, or if immunosuppression leads to dissemination of the infection to the brain.\textsuperscript{33} PML is characterized by JCV infection and destruction of oligodendrocytes, leading to focal demyelination.\textsuperscript{32} This disease entity occurs in immunocompromised patients from, for example, AIDS, lymphoreticular malignancies, or immunosuppression related to organ transplantation, MS, or rheumatologic diseases. Clinically, patients may develop focal or multifocal neurologic deficits, depending on what area of the brain is affected; potential symptoms include dementia, weakness, visual disturbances, aphasia, apraxia, or ataxia. Magnetic resonance imaging (MRI) shows characteristic abnormalities localized to the subcortical white matter at the gray–white junction, typically with little to no post-contrast enhancement. No treatments have been proven effective for PML, although improvement in immune status may improve the overall clinical picture.\textsuperscript{33}

Human T-cell leukemia virus type I (HTLV-I) as the causative agent of HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is an additional example of a virus causing demyelination in human subjects. HTLV-I is a human retrovirus that is found predominantly in Africa, South America, the Caribbean, and southeast Japan. Of those infected with HTLV-I, fewer than 5% develop HAM/TSP.\textsuperscript{34} This disease is characterized by a chronic progressive spastic paraparesis with sphincter disturbance and mild or no sensory loss. The disease typically occurs after a long incubation period, with an average age of onset in the fifth decade. Pathologically, an inflammatory demyelinating process affecting the thoracic spinal cord is found.\textsuperscript{35} The pathogenesis of HAM/TSP is likely related to both viral and immunological factors. HTLV-I infection causes a proinflammatory state; additionally, it infects and causes dysfunction of regulatory T cells, leading to impaired modulation of lymphocyte activity. As a result, high levels of proinflammatory cytokines and mediators are secreted, causing demyelination and spinal cord damage.\textsuperscript{34}

**Oligoclonal Bands and IgG Index in MS**

Oligoclonal bands and IgG elevation in the cerebrospinal fluid (CSF) provide further evidence to support a viral etiology in MS. The presence of oligoclonal
bands and elevated IgG index in the CSF have long been used to aid in the diagnosis of MS. IgG index is obtained by calculating the ratio between CSF and serum IgG after correcting for albumin concentrations in the CSF and serum. Oligoclonal bands are detected by isoelectric focusing methods that separate proteins in the CSF and serum, including IgG. Immunoblotting is then used to detect separated IgG molecules. CSF and serum samples are compared; although there are multiple patterns of CSF oligoclonal bands, a significant pattern is one in which two or more bands are present in the CSF but absent in the serum. While oligoclonal bands are detected in the majority of MS patients, the presence of oligoclonal bands is not specific to MS. Oligoclonal bands are found in a variety of inflammatory and infectious CNS disorders, including paraneoplastic disorders, CNS lupus, neurosarcoidosis, and Behçet’s disease; however, the majority of cases in which oligoclonal bands are seen are infectious. Moreover, in many infectious disorders with the presence of oligoclonal bands, including neurosyphilis, tuberculous meningitis, fungal meningitis, HAM/TSP, and SSPE, the oligoclonal bands are specific for the causative agent. Given these findings, it is possible that the oligoclonal bands identified in MS may be an antibody response directed against a viral or other infectious agent. Oligoclonal bands in MS patients have been studied, with the goal of identifying an infectious agent against which these bands are directed; oligoclonal bands directed against Chlamydia pneumoniae, EBV, and HHV-6 have all been identified in MS patients. Oligoclonal bands in MS could also be immunopathologic in nature and possibly indicative of cell-mediated immunopathology induced by viral infections.

**Viruses Leading to MS Exacerbation**

Another piece of evidence to support a viral link to MS is the fact that viral infections are known to trigger MS relapses. Several studies have found that upper respiratory infections in particular seem to trigger exacerbations. Moreover, it has been suggested that clinical exacerbations preceded by viral illness are often more prolonged than usual clinical relapses and may even contribute to the long-term decline of MS patients.

**Antiviral Effects of Interferon-β and MS**

Additional data to support a potential viral etiology is the fact that some of the therapies used in MS, primarily interferon-β (IFN-β), are also active against viruses. IFN-β is a natural cytokine in humans that is expressed in response to viral infections. Although the exact mechanism of action of IFN-β in MS is incompletely understood, its efficacy may be related to its antiviral properties. The effects of IFN-β on HHV-6 in patients with MS are detailed later in the chapter.
HHV-6 AND MS

Although numerous viruses have been implicated in MS, HHV-6 has some of the most compelling evidence associated with it. HHV-6 is a betaherpesvirus discovered in 1986 when it was isolated from immunocompromised patients with human immunodeficiency virus (HIV) and lymphoproliferative disorders.\(^{47,48}\) HHV-6 is a ubiquitous virus, with an estimated seroprevalence of greater than 95% in the adult population.\(^{47}\) HHV-6 was originally classified into subtypes A and B, or HHV-6A and HHV-6B; however, HHV-6A and HHV6-B have recently been reclassified as separate viruses.\(^{49}\) HHV-6B is acquired early in life, with infection usually occurring before the ages of 2 to 3 years. This primary infection can either be asymptomatic or manifest as exanthema subitum, also known as roseola infantum. Afterward, the virus becomes latent, generally found in the peripheral blood mononuclear cells (PBMCs). Less is known about the acquisition and seroprevalence of HHV-6A, partly due to a lack of appropriate serologic assays for detection.\(^{23}\) HHV-6A is thought to be more neurotropic, given that it is detected more commonly in the CSF than in PBMCs.\(^{50}\) HHV-6 is known to infect a variety of cells, both \textit{in vivo} and \textit{in vitro}, including brain tissue \textit{in vivo} and glial cells \textit{in vitro}. HHV-6 reactivates in immunocompromised states, such as in bone marrow transplantation, and can act as an opportunistic infection, leading in some cases to encephalitis.\(^{47}\) HHV-6 has also been implicated in a variety of other neurological disorders, including mesial temporal lobe epilepsy, encephalitis in immunocompetent patients, and chronic fatigue syndrome, as well as MS.\(^{51}\)

History of HHV-6 Link with MS

Human herpesvirus 6 was first implicated in MS in the early 1990s. In 1993, Sola et al.\(^{52}\) found significantly higher HHV-6 serum antibody titers by immunofluorescence analysis in MS patients compared to controls; however, analysis of viral DNA in PBMCs indicated that HHV-6 DNA was rarely found in the PBMCs of either MS patients or controls. It was surmised that the higher titers seen in MS patients were more likely related to immunological impairment rather than reactivation. Shortly after, Challoner et al.\(^{53}\) used representation differential analysis (RDA) in MS and control brain tissue to provide some of the first direct evidence implicating HHV-6 in the pathogenesis of MS. RDA is an unbiased search method allowing for enrichment of nonhuman DNA sequences by successive rounds of PCR amplification. Through RDA of MS and control brain tissue, the major DNA binding protein (MDBP) gene of HHV-6B was found in MS brains. When PCR analysis was performed on the MS brains and control brains, however, HHV-6 DNA was found to be comparable in both groups; this was thought to be evidence that HHV-6 is a commensal virus of the brain. Immunocytochemistry directed against HHV-6 proteins was also
performed and revealed protein expression in MS brains but not in control cases. Moreover, the expression was more precisely localized to the oligodendrocytes, further suggesting an association between MS and HHV-6.

**Tissue Evidence of HHV-6 in MS**

Since then, more studies have additionally supported a link between HHV-6 and MS. HHV-6 DNA has been found frequently in CNS tissue. In 2000, Blumberg et al.\(^5^4\) used a sensitive two-step *in situ* PCR to search for HHV-6 DNA in formalin-fixed, paraffin-embedded tissue that was archived from patients with MS. High gene expression for both HHV-6 p41 and p101 was consistently found in the white matter of MS patients, particularly in oligodendrocytes as well as neurons. Cermelli and Jacobson\(^5^5\) explored the frequency of HHV-6 DNA by PCR in MS plaques compared to normal-appearing white matter in MS patients as well as controls through the use of laser microdissection. In this study, it was found that HHV-6 DNA was significantly more frequent in MS plaques compared to normal-appearing white matter in MS patients or controls. Goodman et al.\(^5^6\) looked for the presence of HHV-6 DNA via *in situ* PCR in acute, untreated MS lesions. In this study, biopsy specimens were evaluated from patients who presented clinically as patients with cerebral tumors but were subsequently found to have MS based on pathology and clinical course. In all of the specimens, numerous oligodendrocytes, lymphocytes, and microglia were positive for HHV-6 DNA, although no clear HHV-6 antigens were identified in these cells. The fact that these immunomodulation-naïve specimens exhibited HHV-6 DNA indicated that HHV-6 may be associated with MS outside of potential reactivation due to the immunosuppressive therapies associated with MS. A later study by Opsahl and Kennedy\(^5^7\) used fluorescent *in situ* hybridization (FISH) to study early and late viral gene expression in both lesions and normal-appearing white matter from MS patients as well as normal brain tissue. It was found that both the lesions as well as the normal-appearing tissue in MS patients had significantly higher levels of HHV-6 expression when compared to the normal tissue. However, the lesions expressed the highest levels, while the normal-appearing MS tissues exhibited intermediary levels of HHV-6. In addition, active translation of HHV-6 mRNA was found in oligodendrocytes in MS brain tissue.\(^5^7\) Other studies have shown a relative lack of viral transcripts in MS brain tissues for other closely related herpesviruses (EBV, HHV-7, and HHV-8), further strengthening the association between HHV-6 and MS.\(^5^8,5^9\)

**DNA Evidence for HHV-6 in MS Outside of the CNS**

Human herpesvirus 6 DNA has also been studied in fluids outside of the CNS in MS patients and controls. Akhyani et al.\(^6^0\) investigated the presence of HHV-6 DNA in saliva, urine, sera, and PBMCs in a cohort of MS patients and
healthy controls. HHV-6 DNA was found in the saliva and PBMCs of both groups; however, it was found in the sera and urine of 23% of MS patients and in none of the controls. Subtype analysis of the PCR products further revealed a predominance of HHV-6A variant in the MS patient samples. A larger study by Alvarez-Lafuente et al.\textsuperscript{61} showed similar results: HHV-6 DNA was found in the sera of 14.6% of MS patients and none in healthy controls. Although HHV-6B was commonly found in the PBMCs in both controls and MS patients (30.4% and 53.4%, respectively), HHV-6A was seen more often in the PBMCs of MS patients (20.4% of patients) compared to controls (4.4% of controls). Furthermore, the HHV-6 DNA found in the sera of MS patients, but not controls, was predominantly HHV-6 variant A. HHV-6 is typically a cell-associated virus with viral particle shedding only occurring during active viral replication;\textsuperscript{51} hence, the fact that HHV-6 DNA was found in extracellular compartments (e.g., sera and urine) in MS patients is suggestive of active HHV-6 viral replication occurring more commonly in MS patients. Similarly, Berti et al.\textsuperscript{62} devised a longitudinal study following a cohort of 59 MS patients over 5 months; multiple serum samples were taken throughout various points of the study and were tested for HHV-6 DNA by PCR. While HHV-6 DNA was detected in the patients during both relapses and remissions, it was detected significantly more often during clinical relapses, suggesting a possible association between active HHV-6 replication and clinical MS exacerbations.

**DNA Evidence for HHV-6 in MS in CSF**

Numerous studies have also investigated the presence of HHV-6 DNA in CSF in MS patients compared to controls. The results vary, with multiple positive studies showing an increase in HHV-6 DNA detection in MS patients compared to controls,\textsuperscript{61,63–67} as well as a number of negative studies showing no difference between the two groups.\textsuperscript{68–74}

**Serological Evidence for HHV-6 in MS**

There is also serological evidence for an association between HHV-6 and MS. As mentioned previously, Sola et al.\textsuperscript{52} found higher serum antibody titers in MS patients compared to controls. In a subsequent study, Soldan et al.\textsuperscript{75} found higher IgM serum antibody response to HHV-6 early antigen (p41/38) in patients with RRMS, compared to patients with chronic progressive MS, other neurological diseases, other autoimmune diseases, and healthy controls. IgG levels were not significantly different among the different groups, given the ubiquity of HHV-6. However, elevated IgM levels in the RRMS group indicate that recent exposure or reactivation of HHV-6 may be associated with RRMS. A later study found that serum IgM and IgG antibody levels to HHV-6 were higher in patients with early MS (particularly early RRMS and clinically isolated syndromes) in comparison to SPMS patients and healthy
controls, indicating a potential role of HHV-6 as a possible trigger for MS.\textsuperscript{76} Some studies have confirmed the elevated serological titers to HHV-6 in MS,\textsuperscript{64,65,73,74,77,78} while others have found less convincing data.\textsuperscript{69,72,79} The differences in these studies may be attributed to differences in patient and/or control populations, or the different serological assays.

**Effects of IFN-β on HHV-6 in MS**

Another piece of evidence linking HHV-6 to MS is the effect of IFN-β on HHV-6 in MS patients. In a study by Hong et al.,\textsuperscript{46} serum HHV-6 IgM antibodies and HHV-6 DNA were measured in MS patients treated with IFN-β, untreated MS patients, and healthy controls. Findings from this study suggest that treatment with IFN-β significantly decreased HHV-6 replication, given that the cell-free DNA was decreased in the treated MS group. A study by Garcia-Montojo et al.\textsuperscript{45} also found a decrease in prevalence of HHV-6 serum DNA in MS patients after treatment with IFN-β; however, it was also found in this study that MS patients with continuous presence of HHV-6 DNA detected in the blood generally fared more poorly and experienced more frequent and severe relapses than MS patients with undetectable serum HHV-6 DNA.

**Evidence for HHV-6A in MS**

While the findings of Challoner et al.\textsuperscript{53} in 1995 suggested a possible role of HHV-6 B as a trigger for MS, more recent studies seem to indicate that HHV-6A plays a greater role in the association with MS. HHV-6A DNA detection in MS patient sera and increased serum antibody to HHV-6A p31/48 protein in MS patients, as mentioned previously, suggest an association between the HHV-6A variant in MS.\textsuperscript{60,75} In addition, Soldan et al.\textsuperscript{80} observed an increased lymphoproliferative response to HHV-6A in MS patients that was not seen in healthy controls. In this study, lymphoproliferative responses to HHV-6A, HHV-6B, and HHV-7 cell lysates were compared in healthy controls and MS patients. Although both groups showed lymphoproliferation in response to HHV-6B lysates, the MS group showed a significantly increased response to HHV-6A lysates compared to controls (67% of MS patients compared to 33% of controls).

**POTENTIAL MECHANISMS FOR HHV-6 INDUCED AUTOIMMUNITY IN MS**

**Molecular Mimicry**

Although it is difficult to definitively establish HHV-6 as a causative agent in MS, there is an abundance of evidence associating the virus with MS. If there is a viral etiology or trigger in MS, HHV-6 would be a very likely candidate, given its ubiquity, neurotropism, and latency; its characteristically early
period of infection would also fit with the idea that the risk of developing MS occurs early in life. Moreover, there are multiple potential mechanisms that could link HHV-6 as a trigger to autoimmunity in MS. Molecular mimicry has been suggested as one possible mechanism. Molecular mimicry arises when there is cross-reactivity between self epitopes and viral epitopes, possibly due to homologous amino acid sequences, leading to activation of autoreactive T cells. When this occurs, the immune system may then recognize the cross-reactive self epitopes as nonself; subsequently, an immune response will be directed against the cross-reactive self epitope, even if the virus is no longer present. The U24 gene of HHV-6 has been found to share a homologous sequence (residues 4–10) with myelin basic protein (MBP) (residues 96–102). In a study by Tejada-Simon et al., it was shown that a significant percentage of T cells recognizing MBP cross-reacted with a synthetic peptide corresponding to HHV-6 U24, in MS patients. It was also found that T cells with specificity for both peptides were significantly increased in MS patients compared to controls.

**Bystander Activation**

Bystander activation is another possible mechanism by which HHV-6 could lead to an autoimmune response in MS. Bystander activation can occur when a viral infection causing direct inflammation or necrosis of a target tissue leads to nonspecific activation of autoreactive T cells. Additionally, virus-specific T cells could also lead to bystander activation. In a viral infection, virus-specific T cells migrate to the area of active viral infection and encounter virally infected cells. These infected cells present viral antigens via the MHC-I molecules and are recognized by the virus-specific T cells. CD8+ T cells then release cytotoxic granules, killing the virally infected cells. In this context, the dying cells, CD8+ cells, and other inflammatory cells release inflammatory cytokines, leading to bystander damage of the uninfected surrounding cells. This tissue damage and subsequent release of sequestered antigen can lead to further lymphocyte recruitment to the damaged tissue. Lymphocytes may then become reactive to self antigens (such as MBP) in this inflammatory setting, potentially leading to autoimmunity.

**Epitope Spreading**

Epitope spreading is an additional mechanism by which a virus could lead to autoimmunity. This phenomenon occurs when an immune response is directed against several different epitopes, although initially the immune response was directed against a single epitope. This can be seen when B cells act as antigen presenting cells (APCs) in response to a viral infection. A B cell will bind a particular epitope in an antigen, which is then internalized and processed for antigen presentation. The antigen, however, may contain other epitopes in addition to
the one initially recognized by the B cell. These epitopes may fit into the binding grooves of the B cell’s MHC-II molecule, leading to presentation of these additional epitopes. In this way, self antigens that were not the initial immune targets can later become targeted antigens, leading to autoimmunity.  

**ADDITIONAL MECHANISMS BY WHICH HHV-6 MAY AFFECT MS**

**HHV-6 Leading to Apoptosis**

In addition to mechanisms leading to autoimmunity, HHV-6 may affect the pathogenesis and course of MS through a variety of other mechanisms. HHV-6 infection, particularly with HHV-6A, could lead to cell death of neurons and oligodendrocytes. Gardell et al. showed that *in vitro* exposure to HHV-6A led to apoptosis in neurons, astrocytes, and oligodendrocytes, while exposure to HHV-6B did not. Death of oligodendrocytes could lead to demyelination, while neuronal death could lead to the axonal loss seen later in the course of MS.

**HHV-6 Causing Inflammation**

An HHV-6 infection also leads to inflammation, which may be related to the pathogenesis of MS. HHV-6 has been reported to induce a type 1 (also known as Th-1), or proinflammatory, immune response in T cells. When T cells are infected with HHV-6A or HHV-6B, proinflammatory genes are increased and anti-inflammatory genes are decreased at the mRNA and protein levels. This leads to an increase in inflammatory cytokines such as IL-2, IL-18, and TNF-α and downregulation of anti-inflammatory cytokines such as IL-10 and IL-14. A type 1 or Th-1 immune response in MS is well described to be related to worsened symptoms and disease progression. Inflammatory Th-1 cytokines are typically increased during MS relapses, while anti-inflammatory cytokines are associated with MS remission. Levels of inflammatory cytokines such as TNF-α in the CSF have also been shown to correlate with levels of disability and rate of progression in MS patients.

**HHV-6 May Impair Remyelination**

Infection with HHV-6 may also interfere with remyelination in MS patients. Efficient repair of CNS demyelination depends on the ability of oligodendrocyte precursor cells to fully mature into oligodendrocytes. Dietrich et al. found that infection of glial precursor cells with HHV-6 disrupts glial cell differentiation and proliferation. In the case of MS, this observed disruption could lead to fewer precursor cells being recruited to an area of demyelination and an inability of these glial precursor cells to mature effectively into oligodendrocytes for proper remyelination.
HHV-6 and Glutamate Dysregulation

Human herpesvirus 6 could also potentially have an effect on MS pathogenesis through glutamate dysregulation. The dysregulation of glutamate has been suggested to play a role in the pathogenesis of MS, particularly through excitotoxicity. It has been demonstrated that cells with persistent HHV-6 infection exhibit dysregulated glutamate uptake. This could lead to glutamate-related excitotoxicity and subsequent neurologic disease.

HHV-6 and Impaired Phosphorylation of MBP

Impairment in phosphorylation has also been suggested as a potential mechanism for a role of HHV-6 in the pathogenesis of MS. It has been discovered that certain parts of myelin basic protein are phosphorylated less in MS patients, which may lead to impairment in the integrity of the myelin sheath and possibly decreased nerve conduction. Tait and Straus suggested that HHV-6 could lead to impaired phosphorylation of MBP. In particular, the homologous area of HHV-6 U24 may compete with MBP for phosphorylation and potentially confound signaling in which phosphorylated MBP might normally participate.

HHV-6 and HERVs

Additionally, HHV-6 may participate in the activation of human endogenous retroviruses (HERVs), which have been linked in the pathogenesis of MS. HERVs are retroviruses that entered into the human genome millions of years ago. HERVs have been found to have effects on host gene transcription and to even have effects on other viruses. HERVs were first implicated in MS in 1989 when what eventually became to be known as the multiple sclerosis-associated retrovirus (MSRV) was identified in the supernatants of cell cultures from patients with MS. MSRV has been identified as a new family of HERVs, HERV-W. HERV-W has been found to be more prevalent in MS patients compared to controls and has been associated with a poorer clinical outcome. In particular, the env gene encoded by HERV-W has been implicated in the pathogenesis of MS through inflammation and potential oligodendrocyte damage. This gene encodes the protein syncytin, which has been reported to have indirect oligodendrotoxic effects by promoting the release of cytokines and reactive oxygen viruses. Several different herpesviruses, including HSV-1, EBV, VZV, and HHV-6, have been found to cause reactivation of HERV-W, hence, HHV-6 could play a role in MS through the reactivation of HERV-W, leading to inflammation and oligodendrocyte damage.

CONCLUSION

This chapter has detailed the existing evidence for a potential viral etiology in multiple sclerosis, with a particular focus on HHV-6. Although there is
substantial evidence suggesting a viral link in MS, no direct evidence exists for a viral etiology in MS; however, there is compelling evidence for an association between MS and HHV-6. With HHV-6, particular difficulties in proving causation exist, given the fact that the virus is so ubiquitous. However, the isolation of this virus from diseased CNS tissues in MS patients, along with compelling serologic evidence, is highly suggestive of a role for this virus in MS, either as a trigger or in relation to the ongoing course of the disease. Of course, the presence of HHV-6 in these tissues in MS may be reflective of MS leading to reactivation of HHV-6; nevertheless, there is abundant evidence to suggest that HHV-6 could play a role in the inflammation, demyelination, and cell damage seen in MS. More information is needed to prove either a cause or a role for HHV-6 in the course of MS. If indeed HHV-6 is a trigger for MS, further research could examine the prevention of HHV-6 infection through vaccination. Given the high likelihood that HHV-6 has some effect on the overall course of MS, further research on the effects of antivirals active against HHV-6 in MS patients may also yield exciting new information. There is still much to be learned about this virus; it is hoped that a new nonhuman primate model of HHV-6 infection will elicit additional information with which to further establish a more causal relationship between HHV-6 and MS.\[^{100}\]

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