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Viral Infection and Multiple Sclerosis

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1 Introduction

Studies during the 1960s were the first to discover that persistent viral infections can cause chronic neurological disease (Gilden, 2005). Since that time, many viruses have been positively associated with numerous neurological and chronic diseases. The characterization of virus in affected tissues, increased risk of disease in immunosuppressed patients, and response of patients to antiviral therapy all point investigators toward an infectious etiology of the disease. Multiple viruses have been shown to produce demyelination, and a temporal association of postinfectious encephalomyelitis has been shown after smallpox vaccination and measles, varicella, or rubella infection. Viruses are capable of establishing persistent, latent infection in host organisms leading to continuous viral replication

over time without killing the host. Reactivation of latent virus can produce clinical disease, although the stimulus of reactivation has yet to be characterized.

The association of viruses with numerous neurological disorders in animals and humans has led to the theory that multiple sclerosis (MS) may also be the result of infectious or virus-triggered neuropathology (Gilden, 2005). One such example is the correlation of JC virus with progressive multifocal leukoencephalopathy (PML), the only human demyelinating disease with a proven viral cause. Another example is the temporal association of viruses with postinfectious encephalomyelitis, a demyelinating disorder that occurs as a complication of either smallpox vaccination or measles infection. Theiler's murine encephalomyelitis virus (TMEV) leading to central nervous system (CNS) demyelination is another example that supports a role for virus in MS. Although a viral association with MS may be the result of primary infection, more likely it is the reactivation of virus years after the primary infection that leads to the development of MS, similar to JC virus in PML and TMEV, both of which result from reactivation of latent virus in the CNS that may lyse oligodendrocytes, initiating an immunopathological process that leads to demyelination (Gilden, 2005).

Although the etiology of MS is still unknown, much research has been done to gain a better understanding of the factors involved in the development of disease. Epidemiological studies have supported both a genetic component and an environmental trigger in MS. These environmental triggers have been suggested to be transmissible agents such as a viral or bacterial infection. Although the immunology of MS is complex, further support for infectious triggers is indicated by the role of Th1-type CD4+ cells in disease pathogenesis. Th1 cells dominate viral infections and allow for the proliferation of CD8+ cells. In addition, studies have demonstrated increased CD8+ cytotoxic T cells in MS lesions, which suggests a role for these effector T cells in the pathogenesis of this disorder. The observation of increased immunoglobulin G (IgG) responses in MS further supports a role for infection in disease, as most diseases that show high concentrations of IgG are inflammatory and infectious in nature. The involvement of viral infections in MS coupled with the long-held view of MS as an autoimmune disease suggests that molecular mimicry may play a role in MS disease pathogenesis, as studies have found that myelin basic protein (MBP)-specific T cells can be effectively activated by MBP homologue from numerous viruses. This review highlights a number of these diverse observations that lend support to a viral etiology in MS.

2 Multiple Sclerosis

Multiple sclerosis is a demyelinating disease affecting the CNS, with its onset occurring between 20 and 40 years of age. The pathological marker of disease is white matter lesions or plaques resulting from loss of axonal myelination due to inflammation, with noticeable lymphocyte infiltration and oligodendrocyte loss (Ffrench-Constant, 1994). Lesions are characterized by demyelination, edema, and disruption of the blood-brain barrier (Hemmer et al., 2002). Symptoms of

disease begin with recurring inflammatory attacks involving significant neurological impairment, ranging from vision problems and difficulty walking to paralysis (Steinman and Zamvil, 2003). Although the cause of MS is unknown, it has typically been considered a CD4+ Th1 cell-mediated disease. Disease begins when the blood-brain barrier is breached, and activated immune cells attack components of the myelin sheath insulating axons. Epidemiological studies have suggested both a genetic and environmental component in MS, leaving open the possibility of a viral trigger in MS.

2.1 *Epidemiology and Etiology*

The prevalence of MS in specific ethnic groups residing in the same environment supports a role for genetic susceptibility (Haines et al., 1998). The familial aggregation of disease, observed in population and family studies, shows an increased risk in first-, second-, and third-degree relatives over the general population (Dyment et al., 2004). Adoption studies show an increased risk of developing MS only in biologically related individuals (Ebers et al., 1995). In twin studies, monozygotic twins have a higher concordance rate than dizygotic twins (Haines et al., 1998). Although the monozygotic twin concordance rate of 20% to 30% indicates that genetics can predispose an individual to MS, nongenetic factors such as environmental triggers and immune responses must play a role in determining the overall etiology of disease (Dyment et al., 2004). The genetic component most often observed is the HLA-DR2 allele on chromosome 6p21, and this gene could contribute 10% to 60% of the genetic risk factor (Haines et al., 1998). Allelic variants of chromosomal regions are linked to increased disease risk, and these MHC genes could predispose disease through thymic selection or presentation of antigen to T cells, or both (Ermann and Fathman, 2001).

Multiple sclerosis affects approximately 1 million people worldwide, although it is much more prevalent in Caucasians, and 350,000 of people affected reside in North America (Steinman, 2001a). A geographical gradient of north to south has been found, with the Northern Hemisphere having an increased prevalence of disease. The geographical distribution is not related to genetics alone, as the prevalence of disease in Caucasians who migrate outside of Europe or North America is one-half that of those living in many parts of the Northern Hemisphere. Migration studies found that the geographical risk is acquired by the age of 15, as migration from an area of high risk to one of low risk during adolescence confers a reduced risk of developing disease. One explanation for the geographical distribution observed is the reduced sunlight exposure at higher latitudes, as ultraviolet (UV) radiation may exert an effect on vitamin D or cause an excess of melatonin, which enhances Th1 responses (Sospedra and Martin, 2005).

Twice as many women as men develop MS, a phenomenon observed with other autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis (Steinman, 2001a). This finding suggests a role for hormones as risk factors in MS and is supported by the finding that relapse rates are decreased during pregnancy and increase afterward. Additionally, disease often worsens during

menstruation; high estradiol and low progesterone have been correlated with increased magnetic resonance imaging (MRI) disease activity; and estriol has shown a therapeutic effect in RR-MS (Sospedra and Martin, 2005). The mechanism of hormonal action is unknown, although the stimulatory effect of estrogen on proinflammatory cytokine secretion is a likely mechanism.

2.2 *Environmental Factors*

The mechanisms of action for the observed geographical distribution could be numerous, with the “hygiene hypothesis” frequently mentioned as a possible mechanism. The “hygiene hypothesis” states that less hygienic environments, which predispose children to infections, helps protect against later disease by driving the immune system toward Th1 responsiveness. In contrast, children in a more hygienic environment with low exposure to infectious disease, as is the case in developed countries, tend toward Th2 responsiveness, and these are the children who are thought to be more prone to developing atopic allergic disease. Although this hypothesis is difficult to substantiate, it elucidates a possible mechanism as to the increased prevalence of disease in the Northern Hemisphere.

Interestingly, epidemics of MS have been observed, suggesting that a transmissible agent may be associated with disease pathogenesis. The most well known epidemic is that of the Farøe Islands during World War II, where MS was unknown until 1940 when British soldiers arrived and an epidemic broke out shortly thereafter (Kurtzke, 2000). Prospective studies have also shown that MS relapses often follow viral infections, and seasonal variation in the incidence of new MS cases exists. Experimental autoimmune encephalomyelitis (EAE) studies have also suggested a transmissible agent, as almost 100% of transgenic mice expressing T cell receptors (TCRs) specific for an encephalitogenic peptide of MBP develop EAE when housed in non-pathogen-free conditions, whereas those in pathogen-free conditions do not (Goverman et al., 1993). Additionally, common viral infections are temporally associated with MS exacerbations in some patients (Sibley et al., 1985). Many groups have searched for bacteria and viruses that may be associated with disease, and to date about 20 organisms have been associated, although none has gained acceptance as the causal agent of MS (Table 11.1).

Although the etiology of MS is unknown, epidemiological studies point to a role for both genetics and environmental triggers in the development of disease. Figure 11.1 depicts a visualization of how these overlapping criteria may combine to lead to autoimmune disease.

2.3 *Immunology of Disease*

2.3.1 T Helper Lymphocytes

Like many other chronic inflammatory diseases, MS has traditionally been thought to be a CD4+ Th1-mediated disease. Naive T cells differentiate into Th1 cells in the presence of interferon- γ (IFN γ) and interleukin-12 (IL-12) and into

TABLE 11.1. Viruses Implicated in Multiple Sclerosis.

Family	Virus	Year
Bornaviridae	Bornea disease virus (BDV)	1998
Coronaviridae	Human coronavirus (HCV) 229E	1976
Herpesviridae	Herpes simplex virus 1 and 2 (HSV-1, HSV-2)	1981
	Epstein-Barr virus (EBV)	1983
	Cytomegalovirus (CMV)	1979
	Varicella-zoster virus (VZV)	1975
	Human herpesvirus-6 (HHV-6)	1994
Papovaviridae	JC virus	1998
Paramyxoviridae	Measles virus	1972
	Mumps virus	1972
	Parainfluenza virus type 1	1972
	Simian virus 5	1978
	Canine distemper virus (CDV)	1979
Poxviridae	Vaccinia virus	1975
Retroviridae	Human T cell leukemia virus (HTLV-1)	1986
	Human endogenous retrovirus (HERVs)	1989
Togaviridae	Rubella virus	1976

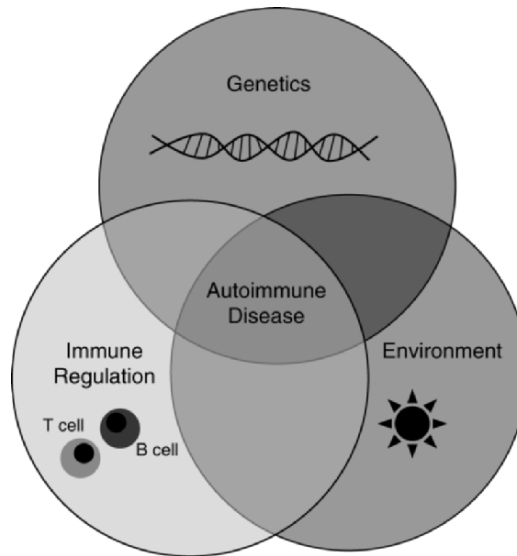


FIGURE 11.1. Development of autoimmune disease. (Adapted from Ermann and Fathman, 2001.)

Th2 cells in the presence of IL-4 and/or IL-6. The presence of Th1- or Th2-type cytokines also function to inhibit the generation of the other response. Th1 cells dominate viral and bacterial infections owing to IL-12 (produced by dendritic cells and macrophages) and IFN γ , produced by natural killer (NK) cells and CD8+ T cells. The two subsets of CD4+ cells have very different functions: Th1

cells activate macrophages and allow proliferation of CD8+ cells and upregulation of major histocompatibility complex class I (MHC I) molecules, and Th2 cells are the most effective activators of B cells, binding to antigen-specific B cells, which leads to the secretion of B cell stimulatory cytokines and drives the proliferation and differentiation of B cells into antibody-secreting plasma cells.

2.3.2 Cytotoxic T Lymphocytes

CD8+ cytotoxic T lymphocytes (CTLs) have come to the forefront of MS research after it was found that CD8+ CTL for MBP can induce severe EAE via adoptive transfer (Huseby et al., 2001; Steinman, 2001b). Previous to this, the focus of EAE research was on the role of myelin-specific CD4+ T cells because of their ability to induce EAE predominantly and lead to cytokine activation similar to that seen in MS (Steinman, 2001b). Additionally, lesions in EAE induced by CD8+ CTLs for MBP closely resemble MS lesions and exhibit extensive demyelination, indicating that CD8+ CTLs are capable of damaging MHC I-expressing oligodendrocytes (Huseby et al., 2001).

CD8+ CTLs are the primary effector T cells that lyse virus-infected cells by recognizing short peptide fragments (usually nine amino acids in length) in association with HLA class I molecules (Neumann et al., 2002; Shrestha et al., 1998; Trapani et al., 2000). They are associated with antigen-specific apoptosis in the CNS, as their specificity for virus-infected cells prevents widespread tissue damage (Barry and Bleackley, 2002; Russell and Ley, 2002). CTLs kill target cells by a number of mechanisms, including the Ca²⁺-dependent release of lytic granules and the Ca²⁺-independent binding of Fas to Fas-L on the CTLs leading to the activation of caspases (Barry et al., 2000; Green et al., 2003; Medana et al., 2000).

CD8+ CTLs accumulate around lesions in infectious and autoimmune neurological disorders including MS (Neumann et al., 2002). It has come to be appreciated that MHC I-restricted CD8+ CTLs have been found to outnumber CD4+ T cells in MS lesions almost 10:1 (Booss et al., 1983). CD8+ CTLs have been shown to be enriched at the site of actively demyelinating lesions in MS (Monteiro et al., 1995) and the clonal proliferation of CD8+ CTL cells has been observed to be much greater than that of CD4+ cells in the T cell infiltrate in inflammatory lesions (Babbe et al., 2000). CD8+ CTL responses to MHC I peptides of MBP are elevated in MS patients compared to healthy individuals, suggesting that CTLs specific for MBP may become activated in MS patients (Crawford et al., 2004). Neuronal cells do not constitutively express the MHC I molecule necessary for CD8+ activation (Neumann et al., 2002), but invading CD8+ CTLs release proinflammatory cytokines including IFN- γ , tumor necrosis factor- α (TNF α), and TNF β (Medana et al., 2000; Woodland and Dutton, 2003). IFN γ inhibits viral replication, activates macrophages, and induces the express of MHC I molecules on glial cells and neurons (Vass and Lassmann, 1990).

Recognition of the involvement of CTLs in the pathogenesis of MS is suggestive of a role for virus in lesions, as CD8+ CTLs are implicated in the direct killing of resident cells during infection. The preponderance of CTLs in inflammatory lesions

supports a role for CTLs as important effectors in MS, and further study of these cells is crucial to our understanding of their involvement in inflammation and MS.

2.3.3 Antibody in Brain and CSF

The response of IgG to autoantigens on cell surfaces leads to the rapid destruction of these cells and inflammatory injury. About 90% of MS patients have high concentrations of IgG in brain and cerebrospinal fluid (CSF), further suggesting an infectious etiology for MS. Other diseases that show high concentrations of IgG are inflammatory and most are infectious (Gilden et al., 1996). Attempts to bind IgG extracted from MS brain and CSF to that of healthy controls and MS patients has been relatively unsuccessful, and points to a possible flaw in the hypothesis of autoantigen involvement in MS (Gilden, 2005). Research on IgG oligoclonal bands from other studies have suggested that oligoclonal IgG in MS is antibody-directed against the infectious cause of MS (Swanborg et al., 2003).

2.4 *Mechanism of Immunopathology*

Viral infections could lead to autoimmune diseases, including MS, via three possible mechanisms: molecular mimicry, bystander activation, and viral persistence. Each of these mechanisms involves the interaction of the virus with the immune system, “priming” the host for subsequent immunopathology by initiating the immunoreactivity that leads to autoimmune disease (Fujinami et al., 2006). In molecular mimicry, T cells react in the periphery to foreign molecules with enough similarity to self-antigen, causing the T cells to become activated and allowing them to cross the blood-brain barrier (Wucherpfennig and Strominger, 1995). Resting autoreactive T cells cannot cross the blood-brain barrier and therefore must be activated in the periphery. Many microbial proteins share sequence homologies with components of the myelin sheath, most notably myelin oligodendroglial glycoprotein (MOG), MBP, and proteolipid protein (PLP); and this homology allows activated T cells to attack myelin (Steinman, 2001a).

Viruses have been shown to cross-react with host proteins, activating MOG- or MBP-specific T cells (Fujinami et al, 2006; Guggenmos et al., 2004). MBP-specific T cells can be effectively activated by MBP homologues from various viruses, including herpes simplex, human papillomavirus, adenovirus type 12, and Epstein-Barr virus (Wucherpfennig and Strominger, 1995). Cross-reaction between viruses and hosts, although common, must occur in a disease-related epitope for autoimmune disease to occur; and this epitope must include peptides that can be presented by MHC II molecules on antigen-presenting cells (APCs) to CD4+ cells (Fujinami et al., 2006). Viruses such as those of the herpesvirus family are good candidates for molecular mimics, as they can establish latent and persistent infection; and chronic infection may permit constant antigenic stimulation of autoreactive T cell clones (Wucherpfennig and Strominger, 1995). Persistent viral infections could damage the immune system as a result of the continual presence of viral antigen (Fujinami et al., 2006).

3 Making a Viral Association

Although many pieces of evidence point toward a role for viral infection in the development of MS, making a positive association between a virus and neurological disorders such as MS is challenging, especially when the infection may be ubiquitous in the population. A number of chronic human illnesses have been suggested to be “triggered” by microorganisms, and proving the infectious etiology of these diseases has required extensive support from multiple sources (Carbone, 2005). The traditional rules for proof of pathogenicity, Koch’s postulates, are often not practical when examining viral triggers, as it is rare that any single line of evidence is sufficient to prove an infectious nature of chronic disease. Proof is often realized only with the accumulation of sufficient and supportive data by multiple sources and techniques. Although it is extremely important to detect the suspected pathogen in diseased tissue, detection does not necessarily prove causality, as the organism could be an innocent bystander or endogenous flora—present but not a factor in the disease. Additionally, a lack of detection does not necessarily mean an association does not occur, as the method used may not be sensitive enough to detect low levels of virus. Any association must include evidence from multiple sources, including genetic, epidemiological, microbiological, and pathological components (Carbone et al., 2005).

Animal models can be helpful in generating hypotheses and can be useful to support viral associations with disease, but proving a link between an organism and a human chronic illness in an animal model again does not prove causality. Therefore animal models cannot always be used to draw conclusions regarding viral association. Obviously, viral associations in human disease would be more compelling, using methods such as the polymerase chain reaction (PCR) to detect viral nucleic acid in human tissue or by measuring antibody levels in serum, plasma, or CSF. Often, prospective and retrospective serological studies are used to help elucidate the cause of chronic illness; but here too lie problems, as evidence of past exposure does not imply current infection, seronegative individuals could have past infection, antibody levels could decline over time, and often tests are not sensitive enough to detect low levels of virus present in chronic infection.

A viral trigger of disease is any virus that sets into motion or expedites the disease process and can be involved in the development of disease in a number of ways, including persistence as a chronic infection and induction of destructive host immune responses in genetically susceptible individuals (Carbone et al., 2005).

4 Viruses Implicated in Multiple Sclerosis

The large body of evidence that points to a role for viral infection(s) in MS pathogenesis has led to numerous possible viral candidates for association with this disease. Viral triggers have been suggested to be involved in MS for more than 100 years (Marie, 1884), and since then numerous viruses have been suggested as causative agents in the pathogenesis of MS (Table 11.1). Some viruses that were

initially thought to be associated with MS have, upon further analysis, shown less likelihood of association. This long-list of viruses can be interpreted in one of three ways: (1) viruses have nothing to do with MS pathogenesis, as none of these observations have stood the test of time; (2) the list is incomplete and the “MS virus” has yet to be discovered; (3) some but not all of these viruses are associated with disease in a subset of MS patients. This review argues for the latter interpretation by focusing on three ubiquitous candidate agents: human endogenous retroviruses and the herpesviruses Epstein-Barr virus (EBV) and human herpesvirus-6 (HHV-6), whose association with MS is currently being extensively explored.

4.1 Human Endogenous Retroviruses

Retroviruses were named for the presence of the viral enzyme reverse transcriptase (RT), an RNA-dependent DNA polymerase; the assessment of RT activity is the main criterion for distinguishing retroviruses. Most human endogenous retroviruses (HERVs) are endogenized exogenous retroviruses; they were incorporated into our genome during primate evolution and now make up 8% of the human genome. Additionally, retroviruses have been implicated in immunodeficiency and neurological disorders, and RNA for HERVs may be upregulated during inflammation (Christensen, 2005). As HERVs are ubiquitous, their pathogenicity must be modulated by genetic and environmental factors. The diversity of HERVs in the human genome is the result of homologous recombinations, as seen in the MHC region (Christensen, 2005).

Several human endogenous retroviruses have been implicated in MS based on the presence of activated HERVs in MS blood, increased RNA expression of HERV in brain tissue from MS patients, and elevated levels of HERV antibodies in the serum and CSF of MS patients (Brudek et al., 2004). Some studies have found that MS patients have increased concentrations of antibody to RT from HERVs compared to control patients, although other studies have had contradictory findings (Gilden, 2005). Retrovirus-like particles (RVLPs) have been found in T cell cultures from patients with MS, and cultures also express EB virus-encoded proteins (Haahr et al., 1991). Multiple sclerosis-related virus (MSRV, previously known as LM7), a HERV, was first isolated from MS patients, and its expression has been associated with disease severity and duration, although increased expression has also been observed in other inflammatory neurological diseases (Dolei et al., 2002; Zawada et al., 2003). It is unknown if HERVs are a causal factor in MS; and if not causal they could be involved as a synergistic player, an immune activator or suppressor; alternatively, HERVs could be an epiphenomenon (Christensen, 2005). HERVs or HERV-activated proteins could act as insertional mutagens, as regulators of gene expression, or they could interact with exogenous viral RNA or elicit abnormalities as viruses.

HERVs could also be transactivated by human herpesviruses, as it has been found that herpesviruses and retroviruses are multiplicatively synergistic in stimulating cell-mediated immune responses (Brudek et al., 2004). The

combination of HERV-H and either HHV-6 or HSV-1 increased cell proliferation in MS patient cell cultures over that of healthy controls, although the increase was not significant (Brudek et al., 2004). Regardless of the significance, the cell-mediated immune response of HERV and herpesvirus antigens in conjunction has a stimulatory effect (Christensen., 2005). The interaction of HERVs and herpesviruses is similar to situations found in vivo, as HERVs are ubiquitous in the genome and herpesviruses are highly prevalent throughout the human population; therefore an interaction between them is plausible. The herpesviruses could also function to induce aberrant expression of HERVs, as it has been found that the retroviruses human immunodeficiency virus (HIV) and human lymphotropic virus (HTLV) in the presence of herpesviruses encode transactivating factors that can exacerbate disease (Christensen, 2005). It is clear that further studies are needed to better elucidate the role of HERVs in the development of MS.

4.2 *Epstein-Barr Virus*

Epstein-Barr virus (EBV) is a herpesvirus believed to infect 95% of the world's population. EBV is trophic for B cells and can establish latency and persist throughout life. Primary infection can be asymptomatic or develop into infectious mononucleosis (IM), which is associated with lymphoproliferation in an immunocompromised host.

Whether EBV has a role in MS has yet to be elucidated, but virtually all patients with MS have antibodies against EBV versus 86% to 95% of healthy individuals (Gilden, 2005). Individuals who had IM in the past have an increased risk of developing MS (Lindberg et al, 1991). Prospective samples from MS patients have been shown to be positive for EBV antigens EBNA-1 (Epstein-Barr nuclear antigen-1) and VCA (viral capsid antigen); high activity to EBNA-1 significantly increased the risk for MS, whereas VCA was not associated with MS development (Sundstrom et al, 2004). Antibodies to EBNA-1 have been found in 85% of MS patients compared to only 13% of EBV-seropositive controls (Bray et al, 1992). Increases in serum titers of EBV antibodies have been found before the onset of MS in prospective studies, with the strongest predictor of MS the serum levels of IgG to EBNA-1, supporting a role for EBV as a risk factor in MS (Ascherio et al., 2001; Levin et al., 2005). While oligoclonal IgG bands in brain and CSF of MS patients have been well documented, but there is no indication that these bands are directed against EBV (Gilden, 2005).

Although many studies have pointed toward a role of EBV in MS, EBV RNA and DNA have not been found in MS brain tissue (Challoner et al., 1995; Hilton et al., 1994; Morre et al., 2001; Sanders et al., 1996) or in CSF of MS patients (Martin et al., 1997; Morre et al., 2001), and studies examining EBV DNA in blood and serum have produced mixed results. EBV could lead to MS though molecular mimicry, as EBNA-1 has been shown to have homologies with MBP, and EBV peptides may activate HLA-DR2-restricted MBP-specific T cells (Sundstrom et al., 2004).

4.3 *Human Herpesvirus-6*

Human herpesvirus-6 was first isolated in 1986 from immunosuppressed patients with lymphoproliferative disorders and HIV infection (Salahuddin et al., 1986). HHV-6 is classified as a hematotropic or beta-herpesvirus, along with cytomegalovirus (CMV) and human herpesvirus-7 (HHV-7); HHV-6 bears 67% sequence homology with CMV (Lawrence et al., 1990). HHV-6 infects most individuals between 6 and 12 months of age (Okuno et al., 1989), and more than 90% of the general population is seropositive for HHV-6 (Kimberlin, 1998). Primary HHV-6 infection has been identified as the causative agent in exanthum subitum (roseola infantum), a febrile illness sometimes resulting in seizures and neurological complications including meningitis and encephalitis (Hall et al., 1994; Yamanishi et al., 1988; Yoshikawa and Asano, 2000).

Two variants of the virus have been identified, variant A and variant B, exhibiting a nucleotide sequence homology of between 88% and 96%. HHV-6A has been implicated in multiple sclerosis and is associated with viral persistence and reactivation in the CNS (Akhyani et al, 2000; Hall et al, 1998). The HHV-6B variant is primarily associated with symptomatic infections during infancy and limbic encephalitis and is the variant implicated in exanthem subitum. HHV-6B appears to be the predominant strain of the virus, as it is detected more frequently, although the 6A variant has been suggested to have a greater neurotropic potential than 6B (Dewhurst et al., 1993; McCullers et al., 1995; Wainwright et al., 2001).

4.3.1 Latent Infection

After primary infection, HHV-6 can establish a lifelong latent infection, with the viral genome persisting in peripheral blood mononuclear cells (PBMCs) and in the salivary glands (Campadelli-Fiume et al., 1999). HHV-6 DNA has been observed in the CSF of children not only during primary infection but also subsequent to infection (Caserta et al., 1994). Reactivation of latent virus is often seen in immunocompromised patients, as half of all patients who undergo stem cell or bone marrow transplant develop active HHV-6 infection (Caserta et al., 2001; Singh et al., 2000) and may contribute to disease in HIV infection and chronic fatigue syndrome. HHV-6 is suggested to be an immunosuppressive agent, although the mechanism of immunosuppression is unknown. Even in immunocompetent subjects HHV-6 has been shown to invade the CNS directly, creating a persistent, latent infection (Saito et al., 1995).

4.3.2 HHV-6 in Neurological Diseases

HHV-6 has been indicated as a cofactor in neurological diseases, most notably demyelinating diseases including multiple sclerosis (Challoner et al., 1995; Soldan et al., 1997). Progressive multifocal leukoencephalopathy (PML), another demyelinating disease of the CNS, is generally thought to be caused by reactivation of the human polyoma virus, JC virus, although it is now suggested that HHV-6 activation, in conjunction with the JC virus, is associated with demyelination

in PML (Mock et al., 1999). In addition to demyelinating diseases, HHV-6 has also been implicated as a cause of epilepsy, encephalitis, encephalomyelitis, and meningitis. HHV-6 has been shown to infect numerous CNS cell types, including T cells, oligodendrocytes, and astrocytes, and has been shown to infect primary human fetal astrocytes in vitro (Albright et al., 1998; He et al., 1996; Lusso et al., 1988). HHV-6 DNA has been detected in CSF of patients with limbic encephalitis, and astrocytes positive for HHV-6B DNA were the most commonly infected cell type in the hippocampus (Wainwright et al., 2001).

5 Human Herpesvirus-6 and Multiple Sclerosis

The case for HHV-6 as one of many potential viral triggers in MS is supported by a large number of studies that are consistent with the following observations: First, the initial HHV-6 infection during infancy is compatible with the epidemiology of MS that suggests exposure to a microbial agent during childhood. Second, HHV-6 has been shown to infect numerous cell types in the CNS, including lymphocytes and glial cells (He et al., 1996). Third, herpesviruses are highly neurotropic and neuroinvasive, and they are often implicated in neurological diseases and other CNS complications (Wilborn et al., 1994). Fourth, the high seroprevalence of HHV-6 throughout the world is consistent with the high prevalence of MS. Lastly, herpesviruses are able to establish persistent, latent infections in the CNS and can be reactivated as a result of stress or other infections, the same stressors that have also been associated with MS exacerbations. Although an association between HHV-6 and MS remains to be definitively proven, the large numbers of reports that demonstrate a role of this virus in MS pathogenesis (Fig. 11.2) are compelling evidence that suggest that this may be an important area of investigation.

Supportive proof for an infectious etiology of disease can be realized only through the accumulation of data by multiple approaches. For example, the association of HHV-6 with MS has been made based on: (1) immunological detection: differential antibody and virus-specific lymphoproliferative responses between patients and controls; (2) molecular detection: demonstration of virus-specific DNA and RNA sequences in cell-free and cell-associated compartments; (3) pathological detection: expression of viral antigen and/or viral DNA in affected brain tissue from diseased patients; (4) clinical and radiological correlations: temporal association of virus specific responses with clinical and MRI parameters of MS disease. This review highlights the findings from these diverse approaches that have been used to correlate HHV-6 infection and MS as an example of how an association of a ubiquitous virus with MS is currently being explored.

5.1 Immunological Detection

Viral associations in MS have been suggested for over 50 years with numerous representations from many viral families including both DNA and RNA viruses

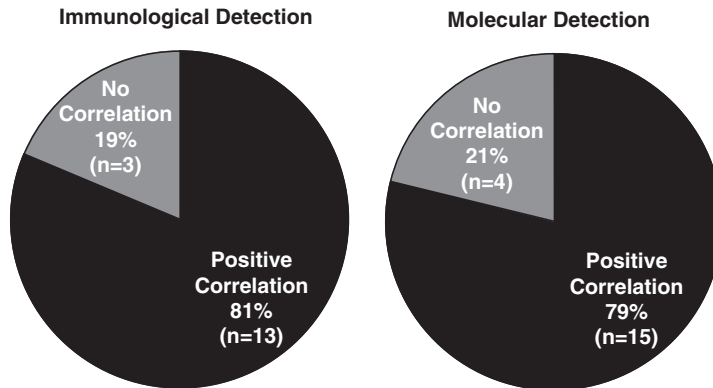


FIGURE 11.2. Studies correlating HHV-6 infection and multiple sclerosis (MS).

Immunological detection—positive correlation (Ablashi et al., 1998, 2000; Caselli et al., 2002; Derfuss et al., 2005; Friedman et al., 1999; Liedtke et al., 1995; Ongradi et al., 1999; Sola et al., 1993; Soldan et al., 1997, 2000; Tejada-Simon et al., 2002; Villoslada et al., 2003; Wilborn et al., 1994). *No correlation* (Enbom et al., 1999; Nielsen et al., 1997; Xu et al., 2002).

Molecular detection—positive correlation (Ablashi et al., 1998; Akhyani et al., 2000; Alvarez-Lafuente et al., 2002b, 2004b; Berti et al., 2002; Chapenko et al., 2003; Clark, 2004; Fillet et al., 1998; Goldberg et al., 1999; Liedtke et al., 1995; Rotola et al., 2004; Soldan et al., 1997; Tejada-Simon et al., 2002; Tomsone et al., 2001; Wilborn et al., 1994). *No correlation* (Al-Shammari et al., 2003; Martin et al., 1997; Mirandola et al., 1999; Taus et al., 2000).

(Berti et al., 2000; Cermelli and Jacobson, 2000; Johnson, 1994) largely based on increased antibody detection in patients versus controls. These studies typically have measured long-term IgG titers in either serum or CSF as a measure of past exposure. By contrast, a number of compelling studies (Ablashi et al., 1998, 2000; Friedman et al., 1999; Ongradi et al., 1999; Soldan et al., 1997; Villoslada et al., 2003) have demonstrated significant elevations of HHV-6-specific IgM in the serum of MS patients compared to patients with other neurological diseases, other inflammatory diseases, and healthy controls (Soldan et al., 1997). Detection of an early antibody response (IgM) to an early antigen of HHV-6 (p41/38) has suggested that active HHV-6 replication may be occurring in a subset of MS patients. Consistent with these HHV-6-specific IgM responses detected in MS sera, other studies have found an increased percentage of MS patients with anti-HHV-6 IgM in CSF. For example, Ongradi et al. demonstrated anti-HHV-6 IgM in 57% of MS patients and 0% of controls (Ongradi et al., 1999). In addition to IgM responses, increased IgG antibodies to HHV-6 has been one of the most consistent findings in most of the studies (Fig. 11.2) examining the role of HHV-6 in MS serum (Ablashi et al., 1998, 2000; Caselli et al., 2002; Liedtke et al., 1995; Ongradi et al., 1999; Sola et al., 1993, 1997; Wilborn et al., 1994) or CSF

(Ablashi et al., 1998; Derfuss et al., 2005; Ongradi et al., 1999). Although some reports have failed to support these findings (Enbom et al., 1999; Nielsen et al., 1997; Taus et al., 2000; Xu et al., 2002) the wide variety of assay platforms, use of different viral preparations, and variations in selection of patients and controls make it difficult to compare results among studies. There is a clear need to define a “gold standard” for HHV-6-specific IgG and IgM responses in both serum and CSF to better characterize the antibody response to HHV-6 in MS.

Immune responses to HHV-6 have also been investigated by examining virus-specific T cell proliferation. In a study from our own group, although there was no difference between MS patients and controls in response to HHV-6B or HHV-7, a significantly higher percentage of MS patients demonstrated proliferative responses to HHV-6A (Soldan et al., 2000). Increased frequency of HHV-6A specific T cells in MS is of interest because the HHV-6A variant has been suggested to be more neurotropic (Hall et al., 1998) and to have a greater propensity for latency and reactivation (Dewhurst et al., 1993), and HHV-6A sequences are more often detected in MS sera (Akhyani et al., 2000; Alvarez-Lafuente et al., 2002b) and CSF (Rotola et al., 2004) than variant B. More recently, T cells recognizing the recombinant 101-kDa protein of HHV-6 that corresponds to an immunodominant region of the virus occurred at a significantly lower precursor frequency in MS patients than controls (Tejada-Simon et al., 2002). These responses were associated with a skewed cytokine profile characterized by the inability to produce IL-4 and IL-10. The authors concluded that the diminished T cell response to HHV-6 and skewed Th2 cytokine profile was associated with ineffective clearance of HHV-6 in MS, suggesting a role for this virus in MS disease pathogenesis (Tejada-Simon, et al., 2002).

Although not universally accepted (Nielsen et al., 1997; Xu et al., 2002), most reports employing immunological detection methods consistently demonstrate HHV-6-specific responses in MS patients (Fig. 11.2). Collectively, these studies continue to support a role for HHV-6 as a reasonable candidate for an etiological agent in MS.

5.2 *Molecular Detection*

Detection of HHV-6 DNA and RNA sequences by primary and nested PCR has been used to demonstrate the presence of HHV-6 in MS patients compared to controls. The detection of HHV-6 DNA in cell-free compartments (i.e., serum, CSF, urine) has been suggested to reflect potentially active HHV-6 replication, whereas detection of cell-associated (e.g. PBMCs) HHV-6 sequences may not be able to distinguish latent from active virus. Moreover, using HHV-6 variant-specific primers and probes, it is possible to distinguish HHV-6A from HHV-6B infection. Recently, real-time quantitative PCR methods have been used to measure HHV-6 viral loads accurately from PBMCs and CSF lymphocytes; and RT-PCR has been used to amplify HHV-6 mRNA sequences. Demonstration of HHV-6-specific RNA in PBMCs is also suggestive of active HHV-6 replication (Alvarez-Lafuente et al., 2004b).

The results from these molecular analyses support the HHV-6 immunological observations found in MS. In both serum and CSF, HHV-6 DNA has been found in significantly more MS patients than controls (Ablashi et al., 1998; Akhyani et al., 2000; Alvarez-Lafuente et al., 2002b; Berti et al., 2002; Chapenko et al., 2003; Clark, 2004; Fillet et al., 1998; Goldberg et al., 1999; Liedtke et al., 1995; Rotola et al., 2004; Soldan et al., 1997; Tejada-Simon et al., 2002; Tomsone et al., 2001; Wilborn et al., 1994). We have demonstrated HHV-6 DNA in serum from approximately 25% of MS patients compared to 0% of controls including patients with other inflammatory diseases, other neurological diseases, and healthy subjects (Akhyani et al., 2000; Berti et al., 2002; Soldan et al., 1997). Although we find that most MS patients and controls have detectable HHV-6 DNA in the PBMCs, others have demonstrated HHV-6 DNA sequences in PBMCs more frequently in MS patients (Fig. 11.3) (Chapenko et al., 2003; Tomsone et al., 2001). Importantly, the increased frequency of detection in MS patients was HHV-6-specific, as no differences were observed between MS patients and controls to seven other human herpesviruses tested (Fig. 11.3).

More recently a number of studies have focused on HHV-6 RNA in PBMCs of MS patients. Alvarez-Lafuente and colleagues demonstrated HHV-6 mRNA for three immediate early (IE) genes by quantitative real-time RT-PCR in a substantial number of RRMS patients and not in healthy blood donors (Alvarez-Lafuente et al., 2002b). As a method of distinguishing active from latent infection, this study compared mRNA expression of the IE genes U89/90, U16/17, and U94 with the expression of U94 alone. Presence of U94 in the absence of other IE gene transcripts has been associated with latent HHV-6 infection (Mirandola et al., 1998). More MS patients were found to have mRNA for all three IE genes

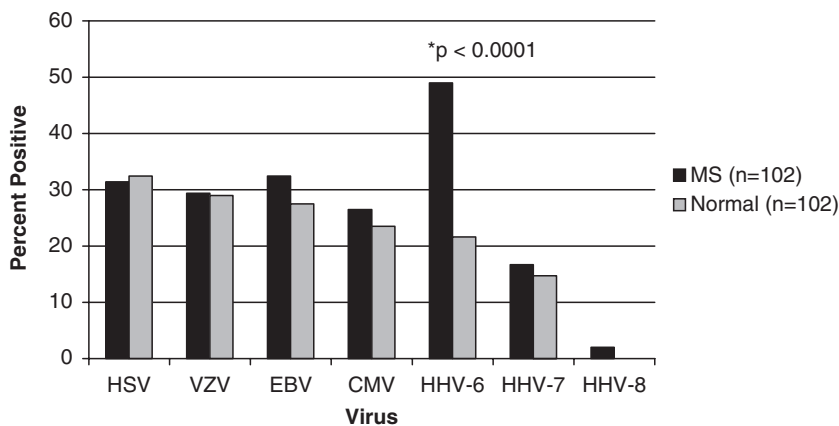


FIGURE 11.3. Prevalence of herpesvirus DNA in peripheral blood monocyctic cells of MS patients. HSV, herpes simplex virus; VZV, varicella-zoster virus; EBV, Epstein-Barr virus; CMV, cytomegalovirus; HHV, human herpesvirus. (Adapted from Alvarez-Lafuente et al., 2002a.).

than U94 alone, indicating active HHV-6 infection in this subset of MS patients (Alvarez-Lafuente et al., 2002b). No significant difference in U94 expression was found between MS patients and controls.

Similar to the observations using immunological detection methods that suggest a role of HHV-6 in MS, most reports based on molecular methods support these findings (Fig. 11.2), although with greater variability among these studies (Al-Shammari et al., 2003; Martin et al., 1997; Mirandola et al., 1999; Taus et al., 2000). This is not unexpected as it is well appreciated in molecular PCR-based assays that there is considerable variability in the use of these methods. Different regions of the virus are amplified with different sets of primers and probes having varying degrees of sensitivity and specificity that are used in either primary or nested PCR conditions. Only through standardized PCR assays can these difficulties be overcome.

5.3 Pathological Detection

Detection of HHV-6 by immunological and molecular means are important observations in associating this virus with MS. However, the demonstration of this (or any infectious) agent in diseased MS brain material would be even more compelling. As access to MS brain tissue is limited, only a few studies demonstrating HHV-6 in MS brains have been reported. Indeed, the first report of an association of HHV-6 with MS was based on the immunohistochemical detection of HHV-6 antigen in oligodendrocytes from MS plaques (Challoner). This landmark study using an unbiased subtractive hybridization approach (representational differential analysis) demonstrated HHV-6-specific sequences in MS plaque material compared to controls. A more recent report also localized HHV-6 to oligodendrocytes in MS brains (Opsahl and Kennedy, 2005). Although HHV-6 mRNA was detected in both MS and control brain tissue, higher levels of HHV-6 viral activity as determined by percentage of HHV-6 mRNA-positive oligodendrocytes were demonstrated in MS patients compared to controls (Opsahl and Kennedy, 2005). In this study, quantitatively more HHV-6 mRNA to both immediate early and late genes was detected in MS lesions versus controls, suggestive of an active HHV-6 infection (Opsahl and Kennedy, 2005).

The demonstration of HHV-6 in MS brain is also supported by studies examining HHV-6 DNA in brain tissue by PCR. Cermelli et al., using laser microdissection, found statistically more HHV-6 DNA in active MS plaques than in normal-appearing white matter (NAWM) from the same MS patients and brain material from patients with other neurological diseases including inflammation (Cermelli et al., 2003). These findings are consistent with previous studies that demonstrated HHV-6 DNA by PCR more often in brain tissue from MS patients than in controls (Friedman et al., 1999; Sanders et al., 1996). In a case report of brain biopsies from five MS patients, all sections demonstrated high levels of HHV-6 DNA-positive cells by *in situ* PCR, most of which were oligodendrocytes (Goodman et al., 2003). Although HHV-6 DNA was detected in oligodendrocytes, HHV-6 antigen was not, using immunohistochemical analysis for HHV-6

p41, p101, or gp116. However, HHV-6 antigen was detected in hypertrophic astrocytes to the HHV-6 gp116 protein staining in two of the five patients (Goodman et al., 2003). The authors concluded that the prevalence of HHV-6 genome-containing cells in MS lesions support the hypothesis that HHV-6 plays a role in the demyelination pathogenesis of MS. The demonstration of HHV-6 in pathological material is crucial to support the association of this agent in this disorder. Although studies using different antibodies have found HHV-6 antigen more often in MS patients than controls (Friedman et al., 1999; Knox et al., 2000; Virtanen et al., 2005), others have not (Blumberg et al., 2000; Coates and Bell, 1998). Clearly, more studies are needed to qualitatively and quantitatively detect both HHV-6 genome and HHV-6 antigen(s) in the CNS of MS patients.

5.4 *Clinical Correlation*

Immunological, molecular, and pathological detection of HHV-6 infection in MS have supported an associative role of HHV-6 in this disease. Even more compelling are the clinical correlative studies between virus and MS disease development or progression. As most MS patients are clinically defined by relapses and remissions, a number of reports have investigated whether HHV-6 can be differentially detected during these phases of disease. A significant correlation was demonstrated between serum HHV-6 DNA and the number of MS patients with clinical exacerbations, diagnosed by patient complaints and neurological examination (Berti et al., 2002). The detection of HHV-6 DNA more often in patient serum during exacerbation than remission suggests that active HHV-6 infection may play a role in the development and/or progression of MS (Berti et al., 2002). These findings were supported by others who found a higher viral load of HHV-6 in MS patients during exacerbations than during remissions for three different IE HHV-6 genes: U89/90, U16/17, and U94 (Alvarez-Lafuente et al., 2004b). Similarly, Chapenko et al. identified periods of HHV-6 viremia by detecting HHV-6 DNA in plasma only during periods of new MS activity (exacerbations), as indicated by the presence of gadolinium (Gd)-enhanced lesions, and not during periods of relative remission, as indicated by an absence of Gd-enhanced lesions (Chapenko et al., 2003). The study concluded that the risk of exacerbation was 2.5 times greater in patients with active HHV-6 infection than in those with latent infection. We have also observed that serum HHV-6 levels cycle over time by a longitudinal analysis of monthly serum samples (24-month time course) (Berti et al., 2002). As it is known that Gd-enhanced MRI lesions also cycle, more patients must be evaluated to determine if there is a correlation between HHV-6 and these lesions.

Of interest is the use of IFN β , an established therapy for MS based on its ability to reduce the frequency and severity of exacerbations, disability, and brain lesions in patients with MS (IFNB, 1993). IFN β has become one of the most commonly used treatments for RRMS, and the success of IFN β in MS is thought to be not only a result of its antiinflammatory properties but may also be due in part to its antiviral activity (Alvarez-Lafuente et al., 2004a; Hong et al., 2002).

IFN β has been demonstrated to inhibit significantly the viral replication of HHV-6, as treatment with IFN β decreased the amount of HHV-6 DNA in the serum of MS patients as compared to untreated MS patients (Hong et al., 2002). In another study, IFN β was not found to diminish HHV-6 DNA in the serum of MS patients during either relapse or remission (Alvarez-Lafuente et al., 2004a). Although DNA in serum was not reduced, they did find that the viral load in the serum of patients undergoing an acute attack was significantly lower in the IFN β RRMS group than in the untreated RRMS group (Alvarez-Lafuente et al., 2004a).

Collectively, these clinically correlative studies, together with the immunological, molecular, and pathological detection of HHV-6 in MS, continue to support a role for the involvement of HHV-6 in this disorder.

6 Conclusion and Future Directions

Further research is needed to associate HHV-6 infection with the immunopathogenesis of MS definitively. Although more data are needed to make an association, it is clear from the breadth of research in the field that most studies find a positive correlation between infection and disease. However, more studies focusing on associations with HHV-6 and MS may get us no closer to proving a causal role for this agent. In all of the studies to date demonstrating the presence of HHV-6 in MS patients, it is difficult to conclude whether the virus is the “cause” of the disease or a mere bystander that results from other immunological events that nonspecifically reactivate the virus. Although some studies have controlled for this (Fig. 11.3), the argument of HHV-6 (or any infectious agent) as an epiphenomenon with little to do with the pathogenesis of MS is valid.

To address this crucial issue, a growing number of investigators believe that only through well controlled interventional clinical trials with effective and safe antiviral agents can a causal role be made for any infectious agent in MS. To date, only a handful of reports have attempted to intervene in MS with anti-beta herpesvirus drugs. Although no compound has been formally approved as an antiviral for the treatment of HHV-6 infection, antiviral agents used for CMV infection or other herpesvirus treatment, including ganciclovir, acyclovir, cidofovir, and foscarnet, are often used (De Bolle et al., 2005). Antiviral drugs used for the treatment of herpesvirus infections act by targeting virus-specific kinases and inhibiting viral DNA polymerases (Bech et al., 2002).

Several case studies of the successful use of ganciclovir for HHV-6 encephalitis in bone marrow transplant (BMT) patients have been published, and prophylactic therapy with ganciclovir has also been shown to be effective in preventing HHV-6 reactivation in BMT patients (Johnston et al., 1999; Mookerjee and Vogelsang, 1997; Rapaport et al., 2002; Rieux et al., 1998; Tokimasa et al., 2002; Wang et al., 1999; Yoshida et al., 2002). Case studies of foscarnet for the treatment of HHV-6 encephalitis have been mixed, with some showing successful results (Bethge et al., 1999; Zerr et al., 2002) and others yielding less success (Rossi et al., 2001; Tiacci et al., 2000). Clinical reports of cidofovir are more

limited owing to the risk of nephrotoxicity; and one report found ganciclovir more successful than cidofovir in treating HHV-6 encephalomyelitis in an immunocompromised patient (Denes et al., 2004).

There have been limited studies in the use of antivirals in MS. One study found that valacyclovir, the valine ester of acyclovir with increased bioavailability, did not reduce the formation of active lesions over the 24-week course of treatment (Bech et al., 2002). However, a subgroup of MS patients with high disease activity, as measured by more than one active MRI lesion, valacyclovir was found to have reduced numbers of new active MRI lesions (Bech et al., 2002). In a more recent clinical trial in MS patients, valacyclovir, though the results were not statistically significant, was found to have a stabilizing effect on clinical progression of disease (Friedman et al., 2005).

These clinical trials serve to highlight the challenges in designing and interpreting an antiviral trial in MS. First, the choice of drug is critical, particularly with respect to HHV-6, as it is not clear what is the most effective anti-HHV-6 compound to use. Is the intent to interfere with HHV-6 replication in the periphery or CNS—and in which cell type (e.g., virus-infected lymphocytes or glial cells)? Second, which group of MS patients should one select? If HHV-6 plays a role in only a subset of patients, how is this group to be selected and what assay(s) should be used to monitor patients? Lastly, what clinical and/or radiological measures are to be used as a primary outcome measure of treatment efficacy? The MS trial design has made significant advances over the years with a number of drugs approved for the treatment and many more in the pipeline (McFarland and Reingold, 2005; Mouzaki et al., 2004). If antiviral drugs are to be part of the armamentarium for MS, these drugs must be shown to interfere with virus growth or replication in patients with detectable levels of virus. If these criteria are met, coupled with clinical and/or radiological improvement with antiviral therapy, there can be confidence for an etiological role of a virus in MS.

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