Human Herpesvirus 6 and Multiple Sclerosis: The Continuing Conundrum

Human herpesvirus 6 (HHV-6) is one of the latest in a long list of infectious agents postulated to play a role in the etiopathogenesis of multiple sclerosis (MS). It is essential in critically evaluating the still exceedingly controversial association between MS and HHV-6 to understand the basic biology of the virus. HHV-6 was first isolated in 1986 from human peripheral blood mononuclear cells (PBMCs) of patients with lymphoproliferative disorders [1]. Two “variants,” HHV-6A and HHV-6B, that differ enough in their epidemiology, pathogenesis, and genomic sequence to essentially be considered separate viral species have been identified, although not yet taxonomically recognized as such. In 1988, HHV-6B was etiologically associated with a human disease, exanthema subitum (ES) [2]. HHV-6A is still essentially an orphan virus, although it is likely to induce disease similar to HHV-6B [3]. HHV-6A and HHV-6B, along with HHV-7, are members of the Roseolovirus genus of the β-herpesvirus subfamily. These viruses share the common properties of ubiquitous prevalence in human populations, a propensity to cause illnesses characterized by fever and rash, and the capacity to grow in lymphoid cells.

The basic structure of HHV-6 is similar to that of other herpesviruses and includes a double-stranded DNA genome enclosed in an icosahedral protein capsid surrounded by a tegument. This entire structure is in turn contained within a host-cell-derived lipid envelope into which viral proteins are inserted. Infection is initiated when virus binds to CD46 [4], a 57–67-kDa type I transmembrane glycoprotein expressed on the surface of all nucleated human cells. CD46 is 1 of at least 6 glycoproteins belonging to the regulator of complement activation (RCA) protein family, several of which can serve as viral receptors. Interestingly, both HHV-6 and measles can use CD46 as a receptor, although they bind to distinct domains [5]. Increased levels of soluble CD46 can be detected in serum and cerebrospinal fluid (CSF) of patients with MS and other inflammatory diseases, compared with that in control subjects, although the pathogenetic significance of this remains uncertain [6].

The cell attachment protein of HHV-6 has not been definitely identified, although mapping of neutralization epitopes by monoclonal antibodies indicate that multiple envelope glycoproteins, including gp82-gp105 and the gH-gL complex, are likely candidates. Entry occurs through receptor-mediated endocytosis after which the virus envelope is removed, and the nucleocapsid is transported to the nucleus. The subsequent stages of viral replication are likely to be substantially similar to those of human cytomegalovirus (CMV), another β-herpesvirus [7, 8].

The HHV-6 genome has been completely sequenced [9]. It is between 159 and 170 kb long. Eighteen open-reading frames are unique to either HHV-6A or HHV-6B. The genome encodes a number of potential immunomodulatory molecules, including a CCR2 chemokine agonist (pU83), 2 functional β-chemokine receptors (pU12 and pU51), and a homologue of OX-2/CD100 (pU85) [10, 11]. HHV-6 also contains a gene encoding a protein (pU24) with a sequence of 7 aa (“PRTPPS”) that are identical to aa 96–102 of human myelin basic protein (MBP), and recent studies suggest that T cells recognizing this peptide sequence occur with significantly higher frequency in patients with MS than among healthy control subjects [12]. T cells that react with this peptide after priming with MBP also were more frequent in patients with MS than among healthy control subjects, but the difference was not significant [12]. The molecular mimicry between pU24 and MBP raises the intriguing possibility that HHV-6 infection could contribute to the pathogenesis of MS by altering host immune responses to MBP. However, these results need to be interpreted with caution, because another study of T cell lines derived from patients with MS and control subjects did not show any significant differences in the ability of HHV-6 to activate MBP-reactive T cells [13].

In addition to the ability to cause lytic infections, HHV-6 shares with other herpesviruses the ability to become latent [7, 8]. Latency is generally inferred from the ability to detect viral genome, but not viral antigens or infectious virus, in cells or tissues at times remote from the initial primary infection. It is important to recog-
nize that not all cells harboring "latent" HHV-6 DNA may be able to support re-activation of infectious virus. Potential sites of HHV-6 latency include lymphoid cells, bone marrow, salivary glands, kidney, lung, and the central nervous system (CNS). During pregnancy, immunosuppression, hypersensitivity syndromes, and acute infection with some other viruses (e.g., dengue, measles, and possibly influenza), HHV-6 can reactivate, an event that can also occur even in critically ill immunocompetent patients [14].

Understanding of the pathogenesis of HHV-6 infection has been hampered by the lack of a suitable animal model, although the virus does infect primates other than humans. Seroprevalence studies indicate that primary infection is acquired during the first 6 months of life, with <10% of infants seropositive at age 1 month, >66% by age 1 year, and >95% by adult life [15, 16]. Primary HHV-6 infection occurs predominantly postnatally, with the likely source of infection virus shed in saliva and, possibly, in the female genital tract.

Primary HHV-6 infection most commonly produces an acute nonspecific febrile illness and may account for up to 20% of emergency department visits for febrile illness in children aged 6–8 months [16]. Primary HHV-6 infection also frequently presents as ES (roseola infantum), an illness characterized by several days of high fever followed by the appearance of a maculopapular rash starting on the trunk and spreading centrifugally to the face and limbs. Primary HHV-6 infection in adult life is unusual and, when documented, has typically presented as a mononucleosis-like syndrome rather than as ES [17]. CNS infection appears to be an integral feature of primary HHV-6 infection, with virus likely reaching the CNS through the bloodstream in association with infected lymphocytes and monocytes. The consequences of HHV-6 CNS infection are extremely variable, ranging from asymptomatic infection through febrile convulsions to rarer cases of severe meningoencephalitis [16, 18–20]. Asymptomatic CNS infection is probably the most common scenario, and HHV-6 DNA can be detected by polymerase chain reaction (PCR) in the CSF of ∼25% of patients with ES, even in the absence of seizures or associated neurological manifestations. The next most common presentation is with febrile seizures, which occur in up to one-third of children with primary HHV-6 infection. HHV-6 infection probably accounts for one-third of all cases of febrile convulsions. In rare instances, primary infection is associated with more-severe CNS manifestations, including focal or diffuse meningoencephalitis with or without associated demyelination.

Both HHV-6A and HHV-6B are found more often in CSF specimens than in blood specimens, but the difference in frequency is much greater for HHV-6A, which has led to the suggestion that HHV-6A is more neurotropic and more likely than HHV-6B to persist in the CNS [21]. HHV-6A is also the variant most commonly isolated from brain tissues of patients with MS [22]. However, HHV-6B has been linked to encephalitis in adult bone marrow transplant recipients [23], indicating that both HHV-6 variants are extremely neurotropic. Not only does CNS involvement occur during primary infection but several cases of fatal encephalitis have been reported in immunocompromised individuals, predominantly bone marrow transplant recipients, although the source of viral reactivation (i.e., CNS or peripheral sites) has not been definitely established [23].

Interest in the association between HHV-6 infection and MS was triggered in 1995, when Challoner et al. [24] reported that they had detected a nucleotide fragment that was >99% identical to the major DNA-binding protein gene (MDBP) of HHV-6B (U41) by using representation-based difference analysis to search for potential pathogens in brain specimens from patients with MS. HHV-6 MDBP gene DNA was detected in 25 (78%) of 32 brain specimens from patients with MS and 40 (74%) of 54 brain specimens from control subjects by nested PCR, with all amplicons typed as HHV-6B. Although the prevalence of amplifiable DNA was similar between brain specimens from patients with MS and control subjects, staining for HHV-6 antigen was found in oligodendrocyte nuclei of 12 (80%) of 15 brain specimens from patients with MS, predominantly in cells associated with MS plaques rather than normal white matter, and in none of 45 brain specimens from control subjects, suggesting that virus was actively replicating and not merely latent in brain specimens from patients with MS. A variety of other cell types, including neurons, astrocytes, macrophages, epithelial cells, choroid plexus epithelial cells, and endothelial cells, also showed positive staining, although this was essentially similar in brain specimens from both patients with MS and control subjects. Staining was particularly striking in cases with inflammatory CNS disease, with antigen-positive cells usually being macrophages rather than lymphocytes. This finding and the known affinity with which HHV-6 replicates in many immune cells indicate the importance of considering the possibility that any association between HHV-6 and MS might simply reflect the presence of infiltrating inflammatory cells harboring HHV-6.

Subsequent attempts to confirm the association between MS and HHV-6 have taken several forms. Many of the available studies have recently been subjected to a systematic review [25]. One strategy has been to examine the relative prevalence or intensity of HHV-6–specific antibody responses in serum and CSF from patients with MS compared with control subjects. Not surprisingly, this approach has been of rather limited utility, given the almost universal prevalence of HHV-6 antibodies. The best-designed studies have not provided convincing evidence of differences between patients with MS and control subjects [25]. A second strategy, searching for differences in the prevalence of amplifiable HHV-6 DNA in serum or purified PBMCs from patients with MS and con-
precise sampling of specific brain microregions (e.g., plaque-containing vs. normal-appearing white matter) is essentially impossible. Two articles in this issue of The Journal of Infectious Diseases address both of these problems. The first article [30] uses a refinement of PCR technique, in situ–PCR (ISPCR), which can be performed directly on formalin-fixed tissue sections and therefore allows individual cells containing the HHV-6 genome to be identified. This group previously used this technique on autopsy brain tissue specimens from patients with established MS and found ISPCR-positive cells in 11 of 13 sections from 8 brain specimens, predominantly in oligodendrocytes [31]. By contrast, HHV-6 antigen was not detectable in oligodendrocytes, despite the use of antisera recognizing 3 different HHV-6 proteins (p41, p101, and gp116). Their current article [30] is essentially a repeat of the original study but now performed on surgical biopsy specimens from a subset of patients with MS presenting with new onset acute disease and therefore free from the potential confounding issues of drug therapy and the effects of chronic disease. Both studies [30, 31] suffer from the same potential methodological issues, including the lack of blinding, the use of historical controls with no definition of how they were selected, and the dependence on morphological criteria (rather than immunostaining for specific markers) to identify oligodendrocytes as the predominant cell infected. In this new study [30], 9 of 9 sections from the 5 brain specimens from patients with MS examined showed cells positive for the HHV-6 genome by ISPCR. As noted, the majority of positive cells were considered to be oligodendrocytes on the basis of their appearance and lack of staining for an immune cell marker (CD45). Some microglial (CD68 +) cells also were positive. In contrast to the ISPCR result, no evidence of the HHV-6 antigen was found in any of the MS tissue specimens. The failure to detect both genome and antigen is unfortunate, because their concomitant presence would have provided strong support for the validity of both detection assays (immunocytochemistry and ISPCR), as well as independent corroboration of the presence of HHV-6 infection. The presence of both genome and viral proteins is characteristic of lytic viral infection; however, during the latent state, in which virus is not replicating, only the genome and not the antigen would be detectable, and the results reported are certainly consistent with this interpretation.

In the second study in this issue [32], laser microdissection was used to specifically isolate tissue from plaque-containing and normal-appearing white matter from 13 patients with MS. The isolated material was then subjected to nested PCR to amplify the HHV-6 major capsid protein gene. The number of samples in which the HHV genome was detected (16%–27%) did not differ among healthy brain specimens, normal-appearing white matter in brain specimens from patients with MS, and brain tissue samples from patients with non-MS neurological disease. However, the HHV-6 genome was detected in 57% of samples from plaque-associated white matter in brain samples from patients with MS, a difference that was highly significant when compared with the other groups. The assays were done in a blinded fashion, although the mechanism for selection of “control” cases was not defined. There also was a striking and unexplained amount of variation between the results obtained from different plaques from the same brain and from different samples of the same plaque. If this sample variation is excluded by simply counting any patient with a positive sample as positive, the differences between groups are less impressive: 12 (92%) of 13 MS plaques had at least 1 positive sample, compared to 9 (75%) of 12 healthy brain specimens, 8 (62%) of 13 brain specimens from patients with non-MS disease, and 5 (50%) of 10 normal-appearing white matter brain samples from patients with MS. These differences are not statistically significant.

Where do these new studies leave us? They provide evidence that the HHV-6 genome is present in cells, including ol-
igodendrocytes, from brain specimens from patients with MS and may be more frequently detectable in areas with MS plaques than in normal-appearing white matter. The failure to detect viral antigen, especially in oligodendrocytes, in contrast to findings in some previous studies [24, 33], suggests that brains of patients with MS, like those of their unaffected counterparts, harbor latent, rather than actively replicating, virus and argues strongly against a model in which direct virus-induced oligodendrocyte injury contributes to the pathogenesis of MS. The high frequency with which the HHV-6 genome is detected in brain tissue specimens from patients with non-MS neurological disease suggests that the virus should be considered part of "normal brain flora," and the current studies do not explain how this ubiquitous virus might be involved in the etiopathogenesis of MS in some individuals while seemingly having no effect in others. The diverse immunomodulatory effects of HHV-6, including the potential for molecular mimicry, raise the possibility that it is not viral infection itself but rather unique aspects of the host response to this infection that may be key.

If HHV-6 does play a role in MS, the potential to alter the course of disease with antiviral therapy for HHV-6 would be of great interest. Most HHV-6 infections are benign self-limited illnesses and do not require specific antiviral therapy. Unfortunately, there are no controlled clinical trials of the use of antiviral agents in HHV-6 infection. In vitro, HHV-6A and HHV-6B have a similar pattern of antimicrobial sensitivity that closely resembles that of CMV, with isolates being resistant to acyclovir, famiciclovir, and valacyclovir, and sensitive to foscarnet, ganciclovir, and valganciclovir. It should be noted that HHV-6 is less sensitive to ganciclovir than CMV, which is probably related to a lessened capacity of the HHV-6-encoded pU69 kinase to phosphorylate ganciclovir [34]. As a result of this pattern of susceptibility, the failure or limited efficacy of valacyclovir in MS [35] would not be unexpected, even if HHV-6 had a role in the etiopathogenesis of MS.

Uncontrolled trials of treatment of HHV-6 CNS infection in hematopoietic stem cell recipients indicate that both ganciclovir and foscarnet therapy are associated with reduction in virus load in CSF and plasma, although the response was not universal and not always statistically significant [23, 36]. Ganciclovir also has been reported to be of benefit in prophylaxis against HHV-6 reactivation in patients receiving stem cell transplantation [36], which suggests that this drug would be a far better choice than acyclovir derivatives to target active HHV-6 infection. Interestingly, interferon (IFN)–β, which is frequently used for the treatment of MS, significantly reduces HHV-6 replication in T cell lines in vitro. Patients with MS being treated with IFN-β also have decreased levels of serum HHV-6 DNA, compared with that in untreated control subjects, or their own pretreatment serum, which suggests a possible antiviral effect in vivo [37]. It is important to recognize that any antiviral treatment strategy would only be useful if reactivation and active replication of HHV-6 were key events in the pathogenesis of MS. If virus is only present in a latent state, as suggested by the failure of some [30, 31], but not all [24, 33], studies to detect viral antigen, then no currently available antiviral agent would be expected to be of benefit in MS treatment.

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References