Human Herpesvirus Type 6 and Multiple Sclerosis

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KEY WORDS:
HUMAN HERPESVIRUS TYPE 6 ▮ MULTIPLE SCLEROSIS ▮ AETIOLOGY ▮ INFECTIOUS AGENT ▮ THERAPY ▮ MANAGEMENT RECOMMENDATIONS

SUMMARY
The aetiology of multiple sclerosis (MS) is unknown, but is probably the result of an interaction between predisposing genetic and environmental factors. The potential role of an infectious agent in MS aetiology stems from evidence of a geographical risk component, with disease being more prevalent in temperate regions. Also, several epidemics of MS have been recorded, suggesting a transmittable element to the disease. Although no one particular virus has been implicated as a causative factor, several herpesviruses have been incriminated due to their neurotropism, ubiquitous nature and ability to establish latency. The majority of evidence supporting a link between MS and human herpesvirus-6 (HHV-6) comes from case control studies comparing the presence of HHV-6 in brain tissue, cerebrospinal fluid (CSF) and peripheral blood mononuclear cells (PBMCs) in patients with and without MS. HHV-6 was detected in a high proportion of individuals without MS, indicating that HHV-6 polymerase chain reaction (PCR) positivity in itself is not sufficient for its causality in the development of MS. Studies of the association between HHV-6 and MS have been contradictory and the *International Herpes Management Forum* (IHMF) recommends that any future studies should use validated, sensitive methods and that masked samples should undergo independent analyses. If therapy trials are indicated, it is recommended that drugs with improved activity against HHV-6 are evaluated.

Introduction

THE AETIOLOGY OF multiple sclerosis (MS) is unknown and is probably the result of an interaction between predisposing genetic and environmental factors. The clinical and pathological features of MS implicate viruses as a factor or co-factor in the development or progression of the disease. While no particular agent has been identified, much attention has recently focused on the role of human herpesvirus (HHV)-6 in the development of MS. This paper reviews the evidence for the contribution of HHV-6 to MS.

Features of MS

Multiple sclerosis is characterized by multifocal plaques of demyelination in the brain and histological indicators of active inflammation which eventually lead to motor and sensory disability.[1] MS is usually classified as either relapsing–remitting or chronic progressive (primary or secondary). Of patients with MS, 80% present with the relapsing–remitting form of the disease. Signs and symptoms generally evolve over a period of days and stabilize before improving (either spontaneously or in response to corticosteroid therapy). Patients usually present with the relapsing–remitting form in their twenties or thirties and it is more commonly seen in women than in men.[1] Primary chronic progressive MS affects men and women to an equal extent and is characterized by a gradual deterioration of clinical symptoms without periods of remission. The disease may also progress between episodes of relapse, when it is classified as secondary progressive MS.[1]

In 1992, the estimated prevalence of MS in the USA was between 250 000 and 300 000 cases.[2] Using a different measure, there was an estimated annual incidence of 10–12/100 000 in Scotland in 1995.[3] The natural course of MS indicates that 10% of patients are well for more than 20 years and approximately 70% will have secondary progression. Life expectancy may be shortened, although not to a large extent.[1]

Genetic versus Environmental Factors in the Aetiology of MS

The existence of genetic factors that determine MS susceptibility is indicated by differences in frequency among races, the existence of resistant groups or populations, familial studies, concordant rates for twins with MS and associations with some proposed susceptibility genes.[4] The risk of developing MS in monozygotic and dizygotic twins is approximately 30% and 5%, respectively. The absolute risk is less than 5% in those individuals with a first-degree relative; however, this is 20- to 40-fold greater than in the general population.[5,6] The higher degree of concordance in monozygotic twins indicates that there is a substantial genetic component to MS. However, as the concordance among twin pairs is less than complete, genetic factors alone are not the sole determinants of the disease. This suggests that an environmental factor or factors may increase the risk of developing MS in a genetically susceptible host. An argument against this is that half-siblings have a similar risk of developing MS whether raised together or apart,[7] but this may reflect the ubiquity of an agent.

The importance of genetic background is further supported by the differences in the incidence of MS in disparate genetic groups living in the same region. For example, the prevalence of MS among people of Japanese descent living on the Pacific Coast of the USA (6.7/100 000) is considerably lower than that of Caucasians living in California.[8] However, individuals of Japanese descent living on the Pacific Coast of the USA have a higher prevalence of MS than Japanese individuals living in Japan (2/100 000), suggesting an influencing environmental factor.[8]

The geographically uneven distribution of MS, which shows an increased prevalence in temperate climates compared with regions around the equator, suggests that exposure to an unidentified genetic or environmental factor, such as an infectious agent or the consequent disease, may be of importance in determining the development of MS.[9,10]

While much effort has been expended to determine the identity of the genetic predisposing factors, no one candidate has emerged. This may be because the susceptibility is controlled by more than one gene or family of genes. However, associations between MS and a number of the major histocompatability genes such as DR2 and DQw1[11] have been suggested.

RECOMMENDATIONS AND STATEMENTS

- Studies of the possible association between MS and HHV-6 infection of the central nervous system (CNS) are contradictory. Further research studies into this association are necessary, and should use validated, sensitive methods, and there must be independent analysis of samples (research need recommendation)
- If future therapy trials in MS are indicated, drugs with improved activity against HHV-6 should be tested as opposed to the currently licensed antiherpetic medications (research need recommendation)
- There is a requirement for improved assessment of disease progression in clinical trials (of antiviral therapy). This should include measurement of a range of clinical outcomes and the use of serial magnetic resonance imaging (MRI) scans (research need recommendation)
- Clinical trials of aciclovir and valaciclovir in MS did not test for a suppressive effect on HHV-6 replication. The effect of therapy on HHV-6 replication should be measured (research need recommendation)

RECOMMENDATION AND STATEMENT CATEGORIES

Category 1

Consistent evidence from controlled clinical trials. For example, for an antiviral, this would include results from at least one well-designed, randomized, controlled clinical trial, and, in the case of laboratory studies, consistent evidence from comparative studies.

Category 2

Evidence from at least one well-designed clinical trial without randomization, from cohort or case-controlled analytical studies (preferably from more than one centre), or from multiple time-series studies or dramatic results from uncontrolled experiments.

Category 3

Evidence from opinions of respected authorities based on clinical experience, descriptive studies or reports of expert committees.

Research Need

Area in which research is warranted.

A Potential Role for an Infectious Agent

The possible role of infection in the aetiopathogenesis of MS stems from epidemiological studies which have demonstrated evidence of a geographical association of disease susceptibility,[9,10] and shown that migration to areas of high incidence of MS influences the likelihood of its development.[12,13] Migration studies have shown that children, but not adults, who emigrate from an area with a high prevalence of MS (such as northern Europe) to one with a lower prevalence (such as South Africa, Australia and Israel), but not *vice versa*, acquire the risk of the new region.[14]

Several epidemics of MS have been recorded in the Faroe Islands, Denmark; the Shetland and Orkney Islands, Scotland; Key West, Florida, USA; Mossyrock, Washington, USA and Mansfield, Massachusetts, USA.[15] Its occurrence in the Faroe Islands is considered indicative of a transmissible disease, as MS had not previously been described in the islanders. Twenty Faroe Islanders developed MS in the 5-year period following occupation of the islands by British troops, with areas of the epidemic correlated with locations of troop encampments. Three successive epidemics followed this primary outbreak, each separated by 13 years.[10]

Other studies suggest that infection (with an unknown agent) later in life increases the likelihood of MS in genetically susceptible individuals. This may be because exposure to certain infections early in life is protective against MS, for example, through early development of an effective immune response.[14]

A meta-analysis of 13 case-control studies of the childhood infectious diseases measles (n=5899), rubella (n=2258), mumps (n=4365), whooping cough (n=2313) and varicella (n=4465) revealed no significant association between any of these infections and subsequent development of MS.[16] However, patients with MS have a later mean age of virus infection, particularly with measles, rubella, mumps and Epstein–Barr virus (EBV) infection.[16] Additionally, a retrospective case-control analysis from the Nurses Health Study (301 cases and 1416 matched controls) showed that development of either infectious mononucleosis or mumps after 15 years of age was associated with an approximate two-fold increase in the risk of developing MS.[17] Little association was seen between history of other common virus diseases (measles, mumps, rubella and varicella) and the risk of MS.[17] Most recently, a prospective study reported higher serum antibody titres to EBV but not cytomegalovirus (CMV) before the onset of MS.[18]

Viral Infection

A number of viruses have been associated with the development of MS and it is possible that one, or a number, of these is causally associated with the disease (Table 1).[19] Their potential role in the pathogenesis of MS may occur as a result of the initial infection or as the virus reactivates. Exposure to upper respiratory tract infections can trigger acute exacerbations of MS, with studies demonstrating a 1.3- to 3.4-fold increase in the risk of clinical exacerbations during the weeks following onset of virus infection.[20–23] Moreover, when recurrent, these are associated with neurological progression.[20–23] In addition, some viruses, including human herpesviruses, are characterized by latency and periodic reactivation, which is similar to the pattern of relapsing–remitting MS. To date, no one virus has been implicated as a causative factor of MS but certain herpesviruses, in particular, have been linked with the development of MS because they are neurotropic, establish latency and are ubiquitous.

HHV-6

HHV-6 has recently been associated with MS. The virus is neurotropic and primary infection can result in neurological complications such as meningitis and meningoencephalitis.

Table 1: Some viruses that have been associated with multiple sclerosis[19]

Virus family	Virus
Herpesviridae	Herpes simplex virus (HSV)
	Varicella zoster virus (VZV)
	Epstein–Barr virus (EBV)
	Human herpesvirus (HHV)-6
	Marek's disease
Retroviridae	Human T-cell leukaemia virus type 1
	MS-associated retrovirus (HERV-W)
Paramyxoviridae	Measles
	Mumps
	Parainfluenza virus type 1
	Canine distemper
	Simian virus type 5
Coronaviridae	Coronavirus
Papovaviridae	JC virus
Bornaviridae	Borna disease

Moreover, HHV-6 is also lymphotropic and has potential immunomodulating properties.

Detection of HHV-6 in patients with MS: The majority of evidence supporting an association between HHV-6 and MS is based on case-control studies. These compared the detection of HHV-6 in brain tissue, CSF, serum or peripheral blood mononuclear cells (PBMCs) (using PCR, immunohistochemistry or virus culture) in patients with MS and in controls. These studies have yielded conflicting results about the association of HHV-6 with MS, which may reflect either the lack of any such link or differences in study methodology.

Detection of HHV-6 in brain tissue using PCR: The first report of a potential association between HHV-6 and MS was described in 1995.[24] Representational difference analysis PCR revealed an HHV-6B DNA sequence in the brain sample from one patient with MS. Subsequent investigations reported the detection of HHV-6B antigen at a higher frequency in MS plaques than in histologically normal specimens.[24] HHV-6 DNA was found in the brains of 78% and 74% of patients with MS and controls, respectively, by PCR highlighting its neurotropism.[24] In another study utilizing PCR, HHV-6 DNA was detected in a greater proportion of active plaques than normal matter from MS cases or controls, although this also did not achieve statistical significance.[25] A study by Cermelli *et al.*[26] did achieve statistical significance, with the frequency of HHV-6 DNA reaching 57.8% in MS plaques compared with 15.9% of normal matter samples ($P<0.0005$). HHV-6 DNA was detected in 21.7% of samples from patients with non-MS neurological disorders and 26.8% of samples from patients without known neurological disease. In contrast, the study by Sanders *et al.*[27] reported no significant differences in HHV-6 PCR positivity either between MS brains compared to controls (57% versus 43%) or among active plaques, inactive plaques or normal matter in MS brains. In all of these studies examining HHV-6 in brain by PCR, the virus was also present in a high proportion of brain samples from controls (Table 2). This suggests that HHV-6 infection alone is not sufficient for the development of MS and that other factors, including genetic features, are important.

A more recent study by Goodman *et al.*[28] analysed biopsy specimens from early manifestation acute lesions in five MS patients who had not undergone immunomodulatory treatment, using a sensitive *in situ* PCR (ISPCR) method, as well as immunohistochemical staining (IHC). ISPCR revealed numerous oligodendrocytes, lymphocytes and microglia containing HHV-6 genome with all lesions sampled, whereas IHC showed only the HHV-6 glycoprotein 116 antigen in some reactive astrocytes and microglia. The study adds to the argument for a role for HHV-6 in the pathogenesis of MS, even if the virus is not the primary aetiological agent of the disease.

Detection of HHV-6 in brain tissue using immunohistochemistry: Immunohistochemical analyses of brain sections from patients with or without MS have produced inconclusive and inconsistent findings about the association between HHV-6 and MS. In the first study reporting a likely association between MS and HHV-6, HHV-6 antigens were detected using two monoclonal antibodies to early [p38/p41] and late [p101] viral proteins.[24] HHV-6 antigen was detected in the nuclei of oligodendrocytes in 12 of 15 (80%) MS cases and none of 45 controls. In grey matter, HHV-6 antigen was detected in the cytoplasm of neurons (particularly those in close proximity to plaques), although neuronal staining was not specific for MS. HHV-6 antigen was found in various other cell types in both MS cases and controls.[24] Subsequent reports have not uniformly confirmed virus involvement using similar detection techniques (Table 3).[25,28–31]

Detection of HHV-6 DNA by PCR in CSF: Increased levels of immunoglobulin (Ig)G and oligoclonal bands in the CSF are characteristic of patients with MS. Oligoclonal bands are seen in the CSF of approximately 90% of patients with MS and may represent an intrathecal immune response to an infectious agent.[32] Several case-control studies have used PCR to detect HHV-6 DNA in CSF (Table 4).[33–41] Overall, HHV-6 was detected infrequently, appearing in 0–46% of MS patients and in none of the controls, except in one study. This may be because CSF was sampled at different stages of the disease; if HHV-6 is reactivated in the CNS during periods of active disease, the virus may be detected in CSF only during these episodes. Alternatively, the different findings may be attributable to the differences in the sensitivity of the PCR assays used or because there is no association between detection of HHV-6 DNA in the CSF and MS.

Detection of HHV-6 DNA in serum by PCR: Case-control studies have used PCR to detect cell-free HHV-6 DNA in serum, which is considered to represent active infection. The incidence of HHV-6 detected in MS cases in these studies ranged between 0% and 66% (Table 5), while the virus was not found in any of the controls, with the exception of two case reports.[33–35,37,38,41–45] Therefore, in common with other investigations, these studies have shown inconsistent and inconclusive findings.

One report correlated active systemic HHV-6 infection with MS (Table 6).[29] Using rapid virus culture, 22 of 41 patients with MS (54%) showed active HHV-6 infection in blood samples compared with 0 of 61 normal controls ($P<0.0001$). While no significant differences were apparent between patients with MS who were HHV-6 viraemia positive or negative with respect to the type of disease (relapsing–remitting or chronic progressive), patients with active HHV-6 viraemia were significantly younger and experienced more aggressive disease than in HHV-6 viraemia-negative patients (Figure 1).[29] However, this requires confirmation by independent groups.

Table 2: Detection of HHV-6 DNA in MS and control brains by PCR[24–26]

Study		HHV-6 PCR positive	
		MS cases (%)	Controls (%)
Challoner *et al.*[24]*		25/32 (78)	40/54 (74)
Sanders *et al.*[27]		21/37 (57)	16/37 (43)
	Active plaques	7/22 (32)	
	Inactive plaques	5/29 (17)	
	Normal matter	9/37 (24)	
Friedman *et al.*[25]	Active plaques	6/16 (36)	3/22 (14)
	Normal matter	2/12 (17)	

*Using nested PCR.
HHV-6, human herpesvirus type 6; PCR, polymerase chain reaction; MS, multiple sclerosis.

Table 3: Immunohistochemical studies of HHV-6 in MS and control brains[25,28–31]

Study	Antibody	HHV-6 PCR positive	
		MS cases	Controls
Friedman *et al.*[25]	101 kDa mAb	7/15	0/16
Coates and Bell[30]	101 kDa mAb	0/23	
Knox *et al.*[29]	gp82/105 and gH mAbs	8/11	2/26[a]
Blumberg *et al.*[31]	p41 and 101 kDa mAbs[b]	0/?	
Goodman *et al.*[28]	P41, p101, gp116/64/54 mAbs	5/5[c]	

[a]HHV-6 leukoencephalitis cases; [b]HHV-6 detected by *in situ* PCR in MS brain tissue; [c]For some patients who had multiple samples taken, not all showed HHV-6 staining.
MS, multiple sclerosis; PCR, polymerase chain reaction; HHV-6, human herpesvirus type 6.

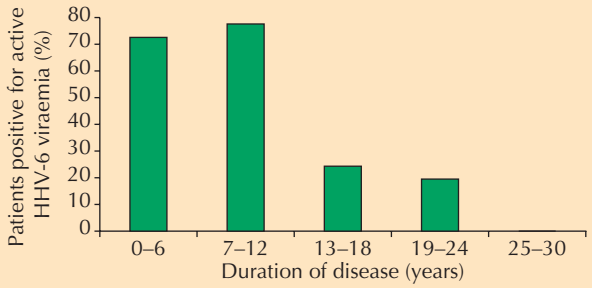

Figure 1:
Percentage of patients with multiple sclerosis who were positive for human herpesvirus (HHV)-6 viraemia expressed as a function of disease duration. Reproduced with permission from Knox KK, Brewer JH, Henry JM et al. *Human herpesvirus 6 and multiple sclerosis.* Clin Infect Dis *2000;***31:***894–903. © 2000 by The University of Chicago. All rights reserved.*

Detection of HHV-6 DNA in PBMC using PCR: In studies using PCR analysis of PBMCs, HHV-6 was detected in 3–76% of MS cases and 4–75% of controls (Table 7)[43–50,53,54] suggesting that the occurrence of the virus in these cells is not a predictor of MS. The large differences between the studies may represent differences in methodology, as the detection of HHV-6 DNA in PBMCs is primarily influenced by the amount of template DNA.[51,52] The high incidence of HHV-6 in controls recorded in the studies is likely to reflect latent HHV-6 in PBMCs, in both MS and non-MS patients.

In the study by Alvarez-Lafuente *et al.*,[50] in which there was a significant difference in the number of MS patients HHV-6 DNA PCR positive in PBMCs compared to controls, virus DNA was only detected after nested PCR. This suggests that the amounts of virus DNA were low and perhaps indicative of a latent state rather than active infection. The study by Chapenko *et al.*[54] also showed a significant difference in the number of MS patients HHV-6 DNA PCR-positive in PBMCs compared with controls.

Serological studies of the association of HHV-6 and MS: Using enzyme immunoassays or indirect immunofluorescence assays, increased serum IgG antibody titres to HHV-6 have been reported inconsistently in patients with MS.[33,46,55] When considering these studies, notably, serum antibody levels for several viruses are higher in MS patients than in controls.[11]

In some of these studies, an increased IgM response was observed, which is thought to represent recent exposure or virus reactivation, and has been associated with the early p38/p41 HHV-6 antigen in two studies involving patients with MS.[36,42] In one of these studies, an increased IgM response to the early antigen of HHV-6 was seen in 73% of patients with the relapsing–remitting form of MS compared with 18% of healthy controls ($P<0.0011$).[42] Another study also found an elevated IgM response to HHV-6 in the sera of 80% of patients with MS compared with 16% of controls but no such association was noted for anti-HHV-6 IgG.[25] An increased prevalence of HHV-6 antibody in MS CSF compared to other neurological diseases has also been reported.[36]

Other studies do not support differences in the serological response to HHV-6 between MS cases and controls. No difference was reported in antibody response to the HHV-6 late antigen (gp110) between cases and controls.[36] Similarly, in another study, serum IgM or IgG reactivities against p41 antigens did not differ significantly between groups of patients with MS (n=19), optic neuritis (n=20), other neurological

Table 4: Detection of HHV-6 in CSF from MS cases and controls by PCR[33–41]

Study	HHV-6 PCR positive	
	MS cases (%)	Controls (%)
Wilborn *et al.*[33]	3/21 (14)	0/16
Fillet *et al.*[34]	2/32 (6)	0/21
Martin *et al.*[35]*	0/20	0/20
Ablashi *et al.*[36]	2/12 (17)	0/4
Goldberg *et al.*[37]	0/6	0/14
Mirandola *et al.*[38]	0/32	0/12
Taus *et al.*[39]	0/25	0/16
Clark[40]	1/34 (3)	0/25
Tejada-Simon *et al.*[41]	14/30 (46)	6/30 (20)

*Only part of the study presented.
HHV-6, human herpesvirus type 6; CSF, cerebrospinal fluid; MS, multiple sclerosis; PCR, polymerase chain reaction.

Table 5: Detection of HHV-6 in sera from MS cases and controls by PCR[33–35,37,38,41-45]

Study	HHV-6 PCR positive	
	MS cases (%)	Controls (%)
Wilborn *et al.*[33]	0/21	0/16
Fillet *et al.*[34]	2/32 (6)	1/34 (3)
Martin *et al.*[35]	0/20	0/20
Goldberg *et al.*[37]	1/24 (4)	0/30
Mirandola *et al.*[38]	0/32	0/12
Soldan *et al.*[42]	15/50 (30)	0/47[a]
Akhyani *et al.*[43]	8/34 (23)	0/19
Tomsone *et al.*[44]	14/38 (37)	0/43
Alvarez-Lafuente *et al.*[45]	15/30 (14.6)	0/46
Tejada-Simon *et al.*[41]	22/33 (66)	7/21 (33)

[a]Some controls also had other autoimmune disorders.
HHV-6, human herpesvirus type 6; MS, multiple sclerosis; PCR, polymerase chain reaction.

Table 6: Isolation of HHV-6 from patients with MS using the rapid culture assay[29]

Virus isolation	Patients with MS (%)	Normal controls (%)
Yes	22 (54)	0 (100)*
No	19 (46)	61 (0)

*P<0.0001 versus HHV-6-positive patients with MS.
HHV-6, human herpesvirus type 6; MS, multiple sclerosis.
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Table 7: Detection of HHV-6 in PBMC from MS cases and controls by PCR[43–50,53,54]

Study	HHV-6 PCR positive	
	MS cases (%)	Controls (%)
Akhyani *et al.*[43]	26/34 (76)	15/20 (75)
Tomsone *et al.*[44]	38/56 (68)[d]	43/150 (29)
Sola *et al.*[46]	1/31 (3)	1/24 (4)
Mayne *et al.*[47]	10/40 (25)	5/24 (21)
Rotola *et al.*[48]	8/20 (40)[a]	11/30 (37)
Hay & Tenser[49]	2/29 (7)	1/7 (14)
Alvarez-Lafuente *et al.*[50]	50/102[c] (49)[b,d]	22/102 (22)
Alvarez-Lafuente *et al.*[45]	55/103 (53)	14/46 (30)
Ferrante *et al.*[53]	9/22 (42)	8/18 (46)
Chapenko *et al.*[54]	16/26 (61)[d]	6/21 (29)[e] 43/150 (29)[f]

[a]All positive samples were variant B; [b]Samples were taken from patients with relapsing–remitting MS during episodes of relapse; [c]62 patients were receiving treatment with beta-interferon; [d]P<0.01 versus controls;
[e]Patients with neurological diseases other than MS; [f]Blood donors.
HHV-6, human herpesvirus type 6; PBMC, peripheral blood monomuclear cells; PCR, polymerase chain reaction; MS, multiple sclerosis.

diseases (n=20) and healthy age-matched controls (n=13).[56] Additionally, reactivities against both A and B variants were similar.[56]

Lymphoproliferative response to HHV-6 in patients with MS: Two studies have compared lymphoproliferative responses to HHV-6A and -6B in MS patients and healthy controls (Table 8).[57,58] In the first study, the lymphoproliferative response to HHV-6A was significantly increased in 21 patients with MS compared with 18 healthy controls (P=0.015); responses to HHV-6B and HHV-7 did not differ appreciably.[57] However, in a second study, no significant differences were apparent between 14 patients with MS and 29 healthy controls with regard to lymphoproliferative response to HHV-6.[58] There is, therefore, no consistent evidence of an altered lymphoproliferative response to HHV-6 in MS patients.

Table 8: Lymphoproliferative responses to HHV-6 and HHV-7 in MS cases and controls[57,58]

	Lymphoproliferative responses (stimulation index >2)[b]		
	HHV-6A (%)	HHV-6B (%)	HHV-7 (%)
Soldan *et al.*[57]			
MS patients	12/18 (67)[a]	14/18 (78)	5/18 (28)
Healthy controls	7/21 (32)	15/21 (71)	5/21 (24)
Enbom *et al.*[58]			
MS patients	5/14 (36)	8/14 (57)	NA
Healthy controls	6/29 (20)	14/29 (48)	NA

NA=not applicable; [a]P=0.015 versus control group.
[b]Defined as the counts per minute obtained after antigen stimulation by the counts per minute obtained after stimulation with the corresponding control antigen. A response was considered positive if the stimulation index was >2 and the net counts per minute were >1000.

T-Cell immune response studies: Studies of the T-cell immune response to HHV-6 in MS patients have been conducted in an attempt to elicidate more fully the possible aetiological role of HHV-6 in MS and to correlate virological evidence for HHV-6 activity with the state of host immunity to HHV-6 in these patients. A study by Tejada-Simon *et al.*[41] demonstrated that T-cells recognizing the recombinant 101 kDa protein corresponding to the major immunoreactive protein unique to HHV-6 occurred at significantly lower precursor frequency in MS patients than in control subjects. The resulting HHV-6-specific T-cell lines obtained from MS patients exhibited skewed cytokine profiles characterized by the inability to produce interleukin (IL)-4 and IL-10. The decreased T-cell responses to HHV-6 and the altered cytokine profile were consistent with significantly declined serum IgG titres for HHV-6 of MS patients compared with those of control subjects. The findings suggest that the decreased immune responses to HHV-6 may be responsible for the ineffective clearance of HHV-6 in MS patients.

A later T-cell response study by the same group[59] added to the argument that HHV-6 may carry amino acid sequence homology with myelin basic proteins (MBP) and thus induce activation of autoreactive T-cells recognizing myelin antigens through molecular mimicry. In the study, 50% of T-cells recognizing MBP (residues 93–105) cross-reacted with and could be activated by a synthetic peptide corresponding to residues 1–13 of HHV-6 U24 in MS patients. The estimated precursor frequency of these cross-reactive T-cells recognizing peptides MBP (93–105) and HHV-6 (U24) (1–13) was significantly elevated in MS patients compared with that in healthy controls.

Summary of Evidence of HHV-6 as an Aetiopathogenic Agent in MS

- Studies of the possible association between MS and HHV-6 infection of the CNS are contradictory. Further research studies into this association are necessary, and should use validated, sensitive methods, and there must be independent analysis of samples (research need recommendation)

Human herpesvirus-6 is frequently detected by PCR in the brains of patients with MS, healthy controls and those with other neurological diseases. There is no consistent finding of the higher occurrence of HHV-6 in patients with MS. Similarly, contradictory findings are reported for:

- HHV-6 immunohistochemistry in the brain;
- HHV-6 DNA in serum by PCR;
- HHV-6 antibody responses;
- HHV-6 specific lymphoproliferative responses.

The findings that people with MS have a higher incidence of HHV-6 infection are equivocal and may be because HHV-6 does not play a role in the aetiology of MS. It is, therefore, possible that the inconsistency between studies may be due to chance. Alternatively, the lack of an association may reflect methodological issues. For example, there may be wide variation in the sensitivity of the assays used, or differences in the characteristics of the case and control groups used in the various studies. To establish whether or not there is a link between HHV-6 infection and MS requires standardization of methodology and patient selection.

HHV-6 Variants and MS

There are two variants of HHV-6: HHV-6A and HHV-6B, which display distinct genomic, antigenic and biological differences.[60] HHV-6B accounts for most cases of exanthem subitum, whereas no known pathology is definitely associated with HHV-6A.[61] Just as there is uncertainty whether or not HHV-6 is an aetiological agent for MS, there are conflicting reports as to which HHV-6 variant is primarily associated with MS. While some studies suggest that variant A is predominantly responsible for this association,[43,57,62] others indicate that it is variant B.[24,36,63]

Randomized Controlled Trials of Antiviral Therapy in MS

There is much speculation about the role of HHV-6 in MS. One way to test the hypothesis that the virus is directly involved is to consider trials of antiviral therapy that target it. Two randomized, double-blind, placebo-controlled trials have described the results of treatment with antiviral therapy (either oral aciclovir or valaciclovir) in patients with relapsing–remitting MS.[64,65]

- If future therapy trials in MS are indicated, drugs with improved activity against HHV-6 should be tested as opposed to the currently licensed antiherpetic medications (research need recommendation)
- There is a requirement for improved assessment of disease progression in clinical trials (of antiviral therapy). This should include measurement of a range of clinical outcomes and the use of serial MRI scans (research need recommendation)

ACICLOVIR

Aciclovir was found to have no effect on neurological function of patients with MS in a placebo-controlled trial. Sixty patients with MS were randomized to receive either oral aciclovir 800 mg or placebo (both n=30) three times daily for 2 years.[64] After 2 years' treatment, the mean annual exacerbation rate was 1.03 and 1.57 in the aciclovir and placebo groups, respectively. Despite this 34% reduction in exacerbations in patients who received aciclovir compared with placebo, statistical significance was not achieved. However, when stratified into groups

who experienced low, medium or high exacerbation rates during the study period (0–2, 3–5 and 6–8 exacerbations yearly, respectively), a significant benefit of treatment with aciclovir versus placebo was evident (P=0.017), with patients experiencing higher exacerbation rates deriving greater benefits from antiviral therapy.[64]

In a further subgroup analysis, patients with duration of disease of greater than 2 years at baseline who were randomized to receive aciclovir therapy (n=19) also showed a significant change in the mean annual exacerbation rate compared with those who received placebo (n=20; –0.44 versus 0.37; P=0.024). Furthermore, 84% and 40% of patients in the aciclovir and placebo subgroups, respectively, showed a reduction, or no change, in the frequency of exacerbations.[64]

No effect of aciclovir treatment was seen on neurological function. Neurological deterioration was significant in both groups when pre- and post-treatment Regional Functional Scoring System (RFSS) deficits were compared (P<0.002).[64]

VALACICLOVIR

Valaciclovir reduces disease progression in patients with high levels of MRI-evident disease activity but, in most patients with MS, this effect is not seen.[65] In a study of valaciclovir, 36 patients with relapsing–remitting MS who received valaciclovir (1000 mg three times daily) showed a similar median number of new lesions (the primary end-point determined by MRI scans) after 24 weeks' treatment to that of 34 patients who received placebo. The mean number of new active lesions in the antiviral treatment group was 11.9±17.6 compared with 14.5±21.4 in the placebo group.[65] However, valaciclovir treatment was associated with a reduced number of new active MRI-evident lesions in a subgroup of patients with high levels of disease activity at baseline.[65] For patients with high-level disease (defined as >1 active lesion during the 4-week run-in period), the median number of active lesions per 4 weeks during the treatment period was significantly reduced in the valaciclovir arm (median of two lesions) compared to the placebo group (median of 6.5 lesions; P=0.025). This suggests that valaciclovir may be of therapeutic benefit in patients with high levels of MRI-evident disease activity.[65] The lack of overall efficacy of valaciclovir demonstrated in this clinical trial may be due to the relatively short treatment period in what is a long-term, progressive disease, and because of the small number of patients involved.

- Clinical trials of aciclovir and valaciclovir in MS did not test for a suppressive effect on HHV-6 replication. The effect of therapy on HHV-6 replication should be measured (research need recommendation)

If HHV-6 does have a role in MS, then the lack of effect of aciclovir and valaciclovir may be explained by the susceptibility of HHV-6 to aciclovir. The mean IC_{50} (median inhibitory concentration) ranges for aciclovir are: HSV, 1–3.0 µM; VZV, 1.6–5.1 µM; EBV, 6.0–7.0 µM; HHV-6, 13.3–111.0 µM.[65] The dosage of aciclovir used in the study is likely to have achieved mean trough and peak serum levels of 2.47 and 4–8 µM, respectively. In the CSF of MS patients, mean aciclovir levels were 0.83 µM. Therefore, the CSF concentrations of aciclovir were probably sufficient only to suppress reactivation of HSV.[66] Valaciclovir, which at a dosage of 3000 mg/day achieves a serum level of 15 µM, would also have suppressed VZV and, to a lesser extent, EBV in the serum. However, neither antiviral would have suppressed replication of HHV-6 in the serum. Moreover, if the penetration of aciclovir into the CSF is assumed to be 31% of that in the serum, then it is likely that VZV but not EBV replication, and almost certainly not HHV-6 replication, would have been inhibited. However, the effect of valaciclovir in patients with high levels of MRI-evident disease activity suggests that antiviral treatment may be worth investigating if agents that can achieve CSF levels capable of suppressing HHV-6 are available.

Conclusions

Multiple sclerosis is a chronic progressive disease which probably has a multifactorial aetiogenesis involving genetic and environmental factors. Viruses have been proposed as triggers of MS, with much attention focused on HHV-6. Studies have compared the prevalence of HHV-6 infection in patients with MS and controls. The evidence for an association of HHV-6 with MS in these studies is not conclusive; with some studies reporting higher incidences of HHV-6 infection in people with MS while others did not. To establish whether or not there is a link between HHV-6 infection and MS requires standardization of methodology and patient selection.

Overall, aciclovir did not reduce the number of MS exacerbations compared with placebo, but appeared effective in patients with duration of disease of greater than 2 years. Valaciclovir had no effect on new lesions in patients with MS but reduced the evidence of active lesions in patients with high levels of MRI-evident disease activity. Further long-term studies are required to elucidate the potential role of antivirals, such as aciclovir and valaciclovir, in the treatment and management of MS.

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Received for publication: 29 September 2003
Accepted for publication: 24 November 2003

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