Human Herpesvirus 6 and Multiple Sclerosis: Potential Mechanisms for Virus-induced Disease

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KEY WORDS

▮ *HUMAN HERPESVIRUS 6* ▮ *MULTIPLE SCLEROSIS*
▮ *MOLECULAR MIMICRY* ▮ *COMPLEMENT ACTIVATION*

SUMMARY

Multiple sclerosis (MS) is a debilitating neurological disease of unknown cause that affects people between 20 and 40 years of age. While several viruses have been associated with MS, none have proven causative. Human herpesvirus 6 (HHV-6) is one agent that may play a role in MS. Some studies have demonstrated an association between HHV-6 and MS based on immunological and molecular data, suggesting that a subset of MS patients may have reactivation of this widespread herpesvirus. New studies investigating the biology of HHV-6 have given insights towards understanding how HHV-6 may play a role in MS pathology. By inducing molecular mimicry or excessive complement activation, HHV-6 reactivation may have the potential to trigger autoimmunity and tissue damage associated with MS lesion development.

Introduction

MULTIPLE SCLEROSIS (MS) is a demyelinating disease that most commonly affects people between 20 and 40 years of age. Significant neurological impairments can occur, ranging from blurred vision to paralysis. MS has often been considered an autoimmune disease, a concept that involves lesions caused by CD4+ Th1 cells that recognize and attack components of the myelin sheath. Recent theories suggest that more complicated and varied mechanisms are involved in MS pathology. Hans Lassmann and colleagues have proposed new pathological classifications of MS lesions, where lesions are designated as having one of four distinct patterns.[1] The new designations are based on the characteristic heterogeneity of MS with respect to clinical course, magnetic resonance imaging findings and therapeutic response. Pattern I is based on monocyte and macrophage involvement in central nervous system (CNS) injury; pattern II involves complement activation and antibody production against components of myelin; pattern III is characterized by loss of myelin-associated glycoprotein; and pattern IV is characterized by non-apoptotic loss of oligodendrocytes.[1] Understanding the pathological mechanisms involved in these four distinct patterns can help with targeting therapies more specifically, and may increase effectiveness of MS drug treatments.

Recent studies have focused on the presence of CD8+ cells within MS lesions, as the clonal expansion of these cells is more prominent than for CD4+ cells.[2–6] The presence of CD8+ cells, rather than CD4+ cells, correlates better with axonal injury. The shift of focus to CD8+ cells is suggestive of viral infection associated with MS lesions, which may be particularly important for the subset of MS patients with virus-associated disease. The β-herpesvirus human herpesvirus 6 (HHV-6) is one of numerous agents to be associated with MS, and although several studies associate HHV-6 with MS (summarized in Tables 1 and 2),[7–39] not all research supports a link between this virus and MS. Some studies have shown that HHV-6 viral DNA can be detected in brain tissue,[20,31,32,34] cell-free serum[11,18,21,22] and cerebrospinal fluid (CSF) specimens[8,9,12,21,24] from MS patients. Although HHV-6 viral protein has been detected in MS lesion tissue, anti-HHV-6 antibody has been inconsistently detected in sera and CSF samples from MS patients (Table 1).

Human herpesvirus 6 was first associated with MS in 1995.[36] Using an unbiased representation differential analysis, this study found that 78% of MS and 74% of control patient brains harboured HHV-6 DNA, but viral protein expression was restricted to MS brains and was detected in oligodendrocytes and neurons.[36] The presence of viral antigen in MS brain suggested active viral replication in patients with this disease, whereas viral DNA detected in the absence of viral antigen implicated a latent infection in control patients.

Human Herpesvirus 6

Human herpesvirus 6 is an enveloped, double-stranded DNA β-herpesvirus that is related to HHV-7 and cytomegalovirus (CMV). HHV-6 was first isolated in 1986 by Salahuddin and colleagues from patients with lymphoproliferative disorders.[40] Although originally considered lymphotropic, HHV-6 infects neural cells[41,42] and may contribute to the pathology of neurological disease. Two HHV-6 variants have been identified – HHV-6A and HHV-6B[43] – which differ in genome, tropism and aetiology, although the genomes of HHV-6A and HHV-6B have 90% nucleotide sequence homology.[44,45] HHV-6B infection causes the common childhood disease exanthema subitum, which is also known as roseola,[46] whereas disease associations of HHV-6A are not well defined. Roseola is characterized by a high fever and rash that develops after resolution of fever, and infection is common within the first 2 years of life; clinical disease in adults is more commonly associated with immunosuppression.

Recent interest has focused on HHV-6 and neurological disease: HHV-6 infects glial cells in the central nervous system and has recently been associated with mesial temporal lobe epilepsy (MTLE). This study detected HHV-6B DNA in MTLE resected tissue, and viral antigen was detected in astrocytes,[47] whereas HHV-6 association with MS primarily involves infection of neural cells with HHV-6A.

Studies Associating HHV-6 with MS

Results from several immunological studies summarized in Table 1 highlight the difficulty in studying associations between a ubiquitous agent and a disease. For example, while five of nine studies

Table 1: HHV-6 antibody detection in samples from multiple sclerosis (MS) patients and controls

Sample	MS (%)	Control (%)	Reference
Serum IgG	71	41	7
	high titres	low titres	8
	39	18	9
	equal titres	equal titres	10
	85	72	11
	69	28	12
	100	100	13
	higher titres	lower titres	14
	90	75	15
	30	25	16
	equal titres	equal titres	17
Serum IgM	3	2	9
	73	18	11
	56	19	12
	80	16	18
	higher titres	lower titres	14
	71	15	15
	2	NT	19
	0	0	16
	equal titres	equal titres	17
CSF IgG	7	NT	7
	0	0	8
	39	7	12
	94	100	18
	43 (6A)	17 (6A)	14
	87 (6B)	0 (6B)	14
	4	NT	15
CSF IgM	0	0	18
	57	0	14
	0	0	15
	0	NT	15

Ig, immunoglobulin; CSF, cerebrospinal fluid; NT, not tested

Table 2: HHV-6 DNA detection in multiple sclerosis (MS) patients and controls

Sample	MS (%)	Control (%)	Reference
Serum DNA	0	0	8
	0	NT	20
	30	0	11
	6	0	21
	4	0	22
	0	0	23
	67	33	24
	0	0	25
	83	55	26
PBMC DNA	3	4	7
	3	22	27
	5	0	28
	25	24	29
	41	29	30
	75	60	15
	7	14	31
	21	0	32
	40	37	33
	14	0	16
	62	29	34
Plasma DNA	31	0	34
CSF DNA	14	0	8
	11	5	9
	0	0	20
	17	0	12
	6	0	21
	6	6	13
	0	0	22
	0	0	23
	0	0	16
	47	20	24
	78	NT	35
Brain DNA	78	74	36
	57	38	37
	0	50	28
	equal levels	equal levels	38
	36	14	18
	58	27	39

PBMC, peripheral blood mononuclear cell; CSF, cerebrospinal fluid; NT, not tested

measuring serum HHV-6 IgM levels support the observation of active HHV-6 infection in MS patients, few studies have been able to detect HHV-6 antibody in CSF. Many of these studies measured antibody levels by immunofluorescence, which is generally considered a semi-quantitative technique. Improvements in technology for quantitatively determining HHV-6 antibody levels in serum and CSF will help to characterize the immune response to HHV-6 in MS patients. Studies investigating lymphoproliferation in MS modestly support an immune response towards HHV-6A in MS patients.[13,48]

A summary of results from several molecular studies is included in Table 2. Of 29 studies summarized, 14 support higher levels of HHV-6 DNA in MS patients compared with controls. Although Challoner *et al.*[36] found no difference in HHV-6 DNA between MS and controls, HHV-6 antigen was detected exclusively in oligodendrocytes from MS patients, which supports a hypothesis of active HHV-6 infection in these people. Many of the earlier studies measured DNA levels using standard or nested polymerase chain reaction (PCR), but the development of quantitative PCR will help to clarify the presence of HHV-6 DNA in MS patient samples in future studies. Of note is that four of six studies summarized in Table 2 support the detection of HHV-6 DNA in brain from MS patients.[18,36,37,39] In particular, the study by Cermelli *et al.*[39] detected viral DNA in 58% of MS plaques tested, compared with 16% of normal-appearing white matter samples, using laser microdissection. Such techniques allowed the authors to investigate the distribution of HHV-6 DNA throughout the brain.

Goodman *et al.*[49] detected HHV-6 DNA by *in situ* PCR localized to oligodendrocytes, microglia, lymphocytes and macrophages, which supports a hypothesis of viral infection both in infiltrating immune cells and in brain-resident glial cells. The possibility that detection of HHV-6 DNA in MS patients may merely reflect brain infiltration of inflammatory cells harbouring HHV-6 needs to be considered. Indeed, we have always exercised great caution on the interpretation of virus-associative studies in MS.[50] A more recent review supports this guarded position by stressing the need to assess human brain material directly for the presence of HHV-6.[51] Since the studies that investigated the frequency of HHV-6 detection in MS brain (summarized in Table 2) were able to detect HHV-6 DNA in control brain material, HHV-6 has been proposed to be a natural component of CNS flora, establishing latency. Therefore, quantitating viral DNA and measuring indicators of active versus latent infection become important when studying the association between a neurological disease and a latent CNS virus.

Recently HHV-6 RNA was found in blood from 65%

of patients with relapsing–remitting MS, and relapse correlated with higher viral load in PBMC; this suggests that relapse may be associated with active HHV-6 infection.[52] Studies that find higher DNA levels and detection of antigen in MS brain suggest that HHV-6 may be reactivated from latency during disease.[36,40,53] Indeed, research investigating the presence of HHV-6 antigen in brain from MS patients supports an association between active HHV-6 infection and MS. Four studies that investigated the presence of viral DNA also found HHV-6 viral antigen expression that was localized to glial cells.[18,36,49,453] Studies to examine antigen and viral mRNA expression in more detail in both MS and control brains will help to delineate the relationship between latent and active virus and this disease. A recent report suggests that HHV-6 may be constitutively active in glial cells, and that infectivity is controlled in normal brain while the dysregulated immune system of MS[1] is unable to control periodic flare-ups of the virus, which possibly contributes to disease pathology.[53] While an association between HHV-6 and MS has not been conclusively proven, the possibility of reactivated latent virus, or of virus trafficked into the brain by inflammatory cells in MS, suggests that HHV-6 may play a role in disease development where viral infection may affect neural cell function.

While improved quantitative techniques for measuring viral antibody and DNA levels – and more studies investigating the presence of viral antigen – will clarify the proposed association between HHV-6 and MS, associative studies cannot implicate HHV-6 as a causative agent. Virological and MS research can, however, be advanced by better understanding of the effects of viral infection on cell function and potentially on disease pathology.

HHV-6 Infection

The widely expressed complement regulatory protein CD46 (membrane cofactor protein) is used as a cellular receptor by HHV-6[54] and other viruses, including measles virus and adenovirus.[55,56] CD46 interacts with a complex of envelope glycoproteins on the HHV-6 virion (gH, gL, gQ), which facilitates infection of the host cell with virus.[57–59] Normally, CD46 is expressed as a soluble and membrane-bound protein that functions as an inactivating factor for C3b and C4b complement products, protecting healthy cells from lysis by autologous complement. Constitutive expression of CD46 emphasizes its importance in protecting healthy cells from immune-mediated damage. CD46 signalling can affect CD8+ T-cell cytotoxicity, CD4+ T-cell proliferation, IL-2 and IL-10 production, depending on the isoform expressed.[60] For example, activation of the CD46 isoform dominant in brain is associated with increased CD8+ T-cell cytotoxicity, decreased CD4+ T-cell proliferation and decreased IL-10 and IFN-γ production by CD4+ T-cells,[60] which suggests a cytotoxic T-lymphocyte-driven immune response.

Mechanisms of Virus-induced Neurological Disease

Studies on basic HHV-6 biology are giving insight into the mechanisms involved in virus-induced disease. Potential disease mechanisms include:

- Molecular mimicry;
- Direct toxic actions of virus on infected cells;
- Incorporation of host proteins into virus particles, which triggers antibody production directed against the host proteins.

MOLECULAR MIMICRY

Two studies have addressed cross-reactivity of HHV-6 viral antigen with myelin basic protein (MBP); both have suggested the potential for molecular mimicry.[26,35] Molecular mimicry involves cross-reactivity between viral and host antigen, triggering an autoimmune response. The studies highlight the potential for HHV-6 infection to induce autoreactive T-cells that recognize host MBP. T-cells recognizing MBP can induce experimental autoimmune encephalomyelitis (EAE), a model of MS. Clonal expansion and activation of T-cells recognizing MBP in MS may be triggered by viral infection and molecular mimicry.[61–65]

The first study investigated T-cell cross-reactivity between HHV-6 antigen and MBP in MS patients and healthy controls, discovering that T-cells from both MS patients and controls cross-reacted with HHV-6 and MBP, although these cells were found more frequently in MS patients (36% of MS patients versus 26% of controls).[35] When T-cells were stimulated first with MBP, MS patients had higher levels of cross-reactive cells than healthy controls (40% of MS patients versus 21% of controls).[35] Seven of the nine MS patients studied were positive for HHV-6A viral DNA in CSF and there was no difference in HHV-6 IgG antibody titres between the MS and control patients.[35] Although specific HHV-6 antigens involved in MBP cross-reactivity were not identified, this study supports molecular mimicry between HHV-6 antigen and components of myelin as a potential mechanism for virus-induced disease.

A second study identified an HHV-6 antigen involved in T-cell cross-reactivity with MBP. The 1–13 region of U24, a viral protein expressed by both HHV-6A and HHV-6B, shares amino acid sequence homology with the 93 to 105 region of human MBP.[26] A subset of T-cells from MS patients that recognize this MBP sequence proliferated in response to U24 peptide.[26] Antibody titres for U24 and the homologous sequence on MBP were elevated in MS patients compared with controls, although the differences were not statistically significant.[26] The authors argue that active replication of HHV-6, indicated by elevated cell-free viral DNA in serum and CSF from MS patients, may sensitize the immune system to the region of human MBP homologous with $U24_{1–13}$ and may contribute to molecular mimicry by increasing sensitivity to other MBP epitopes through epitope spreading.

Although both studies[26,35] provide intriguing data to support molecular mimicry as a potential mechanism of virus-induced disease for HHV-6 and MS, they also highlight the extent of further work necessary. Cross-reactivity between U24 and MBP may only play a role in a subset of MS patients with active HHV-6 infection. Cross-reactivity may also exist between HHV-6 antigen and other components of myelin, including myelin oligodendrocyte glycoprotein and proteolipid protein, or perhaps other host proteins expressed on oligodendrocytes or neurons. Other potential mechanisms for virus-induced disease remain to be studied, including incorporation of host protein into HHV-6 virions and direct toxicity to neural cells following active HHV-6 infection. Incorporation of host protein into virions has been shown following infection with CMV. CMV can incorporate host CD13 into its envelope and give rise to generation of anti-CD13 antibody.[66–68] Infection of oligodendrocytes with HHV-6 and active viral replication may generate antibodies recognizing components of host myelin in a similar fashion. HHV-6 antigen has been detected in oligodendrocytes in MS lesions[36] and HHV-6 can infect oligodendrocytes *in vitro*.[41] Further *in vitro* studies investigating how HHV-6 infection alters glial cell function will help to clarify whether there is the potential for active glial cell infection with HHV-6 to participate in the induction or propagation of MS.

COMPLEMENT ACTIVATION

CD46 may play a role in MS pathogenesis: increased serum and CSF levels of soluble CD46 have been detected in MS patients and have correlated with the

presence of HHV-6 DNA.[69] This finding is further supported by a recent report that four of 42 MS patients with elevated soluble CD46 had elevated serum HHV-6 DNA typed as HHV-6A.[70] The authors of the initial CD46 study postulated that increased soluble CD46 levels indicate activation of the complement system in MS. Due to its role in regulating complement-induced damage to healthy cells, increased complement system activation may lead to tissue damage in MS. A role for CD46 in MS is further supported by a study that found increased levels of antibodies to both CD46 and another complement regulator, CD59, in MS sera.[71] Viruses can incorporate complement regulatory proteins including CD46 and CD59,[72–74] which may give rise to antibodies against these proteins and may account for high anti-CD46 and anti-CD59 antibodies detected in MS.[71] Finally, HHV-6 infection of a host cell occurs through interaction of a viral protein complex with CD46. Receptor-mediated endocytosis allows transport of the virus to the nucleus where viral replication can occur.[51] Receptor-mediated endocytosis of HHV-6 may cause downregulation of CD46 upon active HHV-6 infection and render infected cells susceptible to immune-mediated lysis. Excessive complement activation has been proposed as a mechanism of pattern II lesion development and may be important in MS pathogenesis.[1]

OLIGODENDROCYTE PRECURSOR CELLS

The importance of stem-cell differentiation into myelin-producing oligodendrocytes capable of remyelinating lesioned brain tissue is becoming a large area of interest in MS research. Potential pathogenic mechanisms of MS now include those causing a failure of lesions to remyelinate properly.[75] The capacity of precursor cells to differentiate into oligodendrocytes and produce myelin in MS is important in order to understand the extent of remyelination that may occur naturally, or that can be induced by stem cell therapies. *In vitro* studies of HHV-6-infected human glial precursor cells showed that infection induced cell cycle arrest and increased expression of an oligodendrocyte differentiation marker.[76] The authors suggest that premature differentiation of HHV-6-infected precursor cells may limit those recruited to a lesion site and may render them more vulnerable to the inflammatory environment. It is unknown whether HHV 6-infected precursor cells differentiate into functional mature oligodendrocytes, but these findings certainly highlight a new potential mechanism for virus-induced disease. Active HHV-6 infection may impair the recruitment and differentiation of precursors into mature myelin-producing cells and could contribute to the failure of remyelination in MS lesions.

Concluding Remarks

Since the original study detected HHV-6 DNA and antigen in MS lesions,[36] many have investigated whether there is any association between HHV-6 and MS based on serology and detection of viral DNA and antigen. Differences in the conclusions of these studies may support the distinct patterns of pathology of MS lesions proposed by Lassmann e*t al*.[1] and the heterogeneity of this disease. Viral infection may be associated with a subset of MS patients with lesions that follow one of the patterns I–IV. While the associative studies of HHV-6 and MS support the conclusion that subsets of MS patients may have active HHV-6 infection, few reports have proposed mechanisms for virus-induced disease, and association of a virus with a disease certainly does not imply causation. While clinical trials of antiviral agents active against HHV-6 in MS patients would address the issue of causation, *in vitro* studies investigating how HHV-6 infection may alter cell function will be important contributions to the current literature. Understanding how viral infection alters basic neural cell function may identify novel mechanisms of virus-induced disease and will advance our knowledge of neurological disorders and viral infections of the nervous system.

Conflicts of Interest

No conflicts of interest were declared in relation to this article.

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IHMF® Annual Meeting 2005
Focus on the Future

The IHMF® Board is delighted to announce the *12th Annual Meeting of the IHMF®*

Venue: Corinthia Alfa Hotel, Lisbon, Portugal, 28–30 October 2005

IHMF® International Herpes Management Forum

One of the primary aims of the IHMF® is to develop international recommendations and guidelines on the management of herpesvirus infections. The Annual Meeting provides the opportunity for these recommendations to be formulated and debated at an international level. Your input at this meeting is, therefore, important and can have a wide-reaching impact.

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A lively Plenary Debate on the pros and cons of universal varicella immunization

Clinical Case History Sessions and the **Poster Symposium** provide opportunities for you to present your cases and research (submission details below)

Keynote lectures focus on CMV Vaccine Potential, Topical Antiherpes Therapies, Implications of New Data from Ongoing Varicella Vaccine Trials, Managing HSV in HIV-infected Patients, Public Health Issues in HSV Infection and the frequency of HSV shedding

How you can participate:

- Submit your abstract for the Poster Session. Deadline 17 June 2005, via www.IHMF.org or email to jlanigan@hbase.com.
- Submit your Clinical Case History. Deadline 17 June 2005, via www.IHMF.org or email to jlanigan@hbase.com.
- Attend the Annual Meeting and debate the proposed monograph recommendations – make your opinion count.

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Contact the Secretariat at ihmf@hbase.com or visit www.IHMF.org. This website contains detailed information on the meeting, and an on-line registration facility.

Abstract/Clinical Case History Submission

All abstracts and clinical case history outlines should be a maximum of 250 words and submitted in English as word-processed documents to the IHMF® Secretariat by 17 June 2005, either via the website www.ihmf.org or email to jlanigan@hbase.com. These will be reviewed by the IHMF® Board and decisions on acceptance will be communicated by 25 July 2005.

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