Plaque-associated expression of human herpesvirus 6 in multiple sclerosis (representational difference analysis/neurological disease/virus)

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ABSTRACT  Representational difference analysis was used to search for pathogens in multiple sclerosis brains. We detected a 341-nucleotide fragment that was 99.4% identical to the major DNA binding protein gene of human herpesvirus 6 (HHV-6). Examination of 86 brain specimens by PCR demonstrated that HHV-6 was present in >70% of MS cases and controls and is thus a commensal virus of the human brain. By DNA sequencing, 36/37 viruses from MS cases and controls were typed as HHV-6 variant B group 2. Other herpesviruses, retroviruses, and measles viruses were detected infrequently or not at all. HHV-6 expression was examined by immunocytochemistry with monoclonal antibodies against HHV-6 virion protein 101K and DNA binding protein p41. Nuclear staining of oligodendrocytes was observed in MS cases but not in controls, and in MS cases it was observed around plaques more frequently than in uninvolved white matter. MS cases showed prominent cytoplasmic staining of neurons in gray matter adjacent to plaques, although neurons expressing HHV-6 were also found in certain controls. Since destruction of oligodendrocytes is a hallmark of MS, these studies suggest an association of HHV-6 with the etiology or pathogenesis of MS.

Multiple sclerosis (MS) is a disease of young adults that is characterized clinically by a variable relapsing and remitting course and pathologically by the progressive accumulation of plaques of demyelination within the white matter of the central nervous system. In normal white matter, the axons of neurons are surrounded by myelin sheaths, made from the cell membranes of oligodendrocytes. In MS plaques, the myelin sheaths are destroyed, leaving the naked axons intact but impaired in their conduction of action potentials. The currently held view is that an autoimmune inflammatory reaction against components of myelin results in destruction of oligodendrocytes. The demyelinating lesions in MS are found throughout the central nervous system, with a predilection for the periventricular white matter, optic nerve, brainstem, spinal cord, and cerebellum, resulting in a disease that is pleomorphic in its clinical presentation.

In spite of the substantial evidence that autoimmune-mediated demyelination plays a major role in the progression of MS, epidemiologic studies suggest that an infectious agent may also be involved (1). Prior reports have suggested that viral infection of cells within the central nervous system may initiate the events leading to focal demyelination (2), and a number of viruses have been implicated in the pathogenesis of MS (3). Despite extensive investigation, however, none of these associations has been confirmed (4), and the issue of viral involvement in the pathogenesis of MS remains unresolved.

To search for an MS-associated pathogen, we used representational difference analysis (RDA) (5). In RDA, successive rounds of subtractive hybridization and PCR amplification enriched for DNA sequences that were present in a DNA preparation from diseased tissue (MS brain) but absent from control DNA (nondiseased cases). The power of RDA is that the enrichment of non-human sequences in diseased tissue is unbiased and that non-human DNA can be amplified without prior knowledge of the identity of a pathogen within the DNA of the diseased tissue sample. We describe here the results of these studies and the unexpected finding that human herpesvirus 6 (HHV-6) variant B (6, 7) is expressed to an unusual degree in the oligodendrocytes of MS patients.

MATERIALS AND METHODS

Tissue Samples. One hundred thirty frozen brain specimens were obtained from the National Neurological Specimen Bank, Los Angeles (8), including 77 MS patients, 18 patients who died of other neurological diseases [9 with Alzheimer disease, 5 with amyotrophic lateral sclerosis (ALS), and 4 with Parkinson disease], and 35 patients who died of nonneurological diseases. Additional brain specimens from 14 adults who died of traumatic causes were obtained from the National Disease Research Interchange, Philadelphia.

Formalin-fixed, paraffin-embedded brain tissues from 11 Seattle MS patients and 13 controls were also obtained. They were routinely placed into 10% neutral buffered formalin within 12 hr of death, fixed for 2 wk prior to dissection, placed into cassettes, and fixed for an additional 24 hr prior to further histologic processing. Two Los Angeles MS samples were processed as described (8).

In addition to the neurological diseases listed above, controls included brain samples from AIDS/varicella–zoster virus (VZV) encephalitis, epilepsy, two cerebrovascular accident (CVA) cases, two patients who died of nonneurological causes, and four 28- to 38-wk stillbirths.

RDA. Tester samples were dissected from cryopreserved MS brain tissue. To avoid cross-contamination, all procedures were performed in a biosafety cabinet with new instruments and gloves for each sample. Driver samples were obtained from the peripheral blood leukocytes (PBLs) of healthy donors by differential centrifugation over Histopaque-1077 (Sigma). DNA from all samples was prepared by incubation at 60°C for 4–12 hr in digestion buffer (100 mM NaCl/10 mM Tris-HCl, 0.5 mM MgCl2/1 mM EDTA, 0.1% sodium dodecyl sulfate) for 1 hr followed by ethanol precipitation.

Abbreviations: MS, multiple sclerosis; RDA, representational difference analysis; HHV-6, human herpesvirus 6; CMV, cytomegalovirus; VZV, varicella–zoster virus; EBV, Epstein–Barr virus; HSV, herpes simplex virus; MBDP, major DNA binding protein; ALS, amyotrophic lateral sclerosis; CVA, cerebrovascular accident; PBL, peripheral blood leukocyte; ICC, immunocytochemistry; mAb, monoclonal antibody.

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variable glycoprotein with four cycles of mM EDTA, pH 8.0, at a concentration of 200 ng/μl. The driver consisted of a pool of equimolar amounts of DNA from 24 unrelated, healthy donors and was used separately against tester DNA from the brains of 5 unrelated MS patients. RDA experiments were based on HindIII digestion as described (5), with four cycles of hybridization and amplification.

HHV-6 Amplification by PCR. A nested PCR assay was used to detect HHV-6 in brain samples. Reactions were performed in 50 μl total volume with 100 μM dNTPs, 0.1 μM oligonucleotides, 67 mM Tris-HCl (pH 8.8), 4 mM MgCl2, 16 mM (NH4)2SO4, 10 mM 2-mercaptoethanol, and 100 μg of bovine serum albumin per ml. One microgram of brain DNA was used as template for primary amplifications of 35 cycles of 1 min each at 95°C, 54°C, and 72°C. Five microliters of the primary reaction products was used as template for secondary amplification reactions of 35 cycles of 30 sec each at 95°C, 54°C, and 72°C. The following primer pairs, targeting the major DNA binding protein (MDBP) gene, were used for prevalence studies: primary amplification, 5'-CTATCCCTCATACCTCACCTCACGC-3', 5'-GCCGAGTTAGTTGTAGA-3'; secondary amplification, 5'-TGAAGACCTGGCCCTTGACC-3', 5'-TGGTCAGGGCAGTTGCTTC-3'. HHV-6 variants A, B group 1, and B group 2 were distinguished by amplifying and sequencing a segment of the immediate early region (9) with the following primer pairs: primary amplification, 5'-GCCTGAGTACAGATCTG-3', 5'-GTTGACCTGCTGTTGTGGTGTA-3'; secondary amplification, 5'-GCCGCTGATACTT-3', 5'-CATTGTATTCTTCTACCTC-3'.

To quantitate HHV-6 we used a semiquantitative, nested PCR assay targeted to the BHLF2 variable glycoprotein gene (ref. 10; GenBank locus no. HV6IDDNA), which detected <10 copies of HHV-6 DNA per μg of DNA. DNA samples from white matter and cortex were 2-fold serially diluted in sterile water and adjusted to a total of 1 μg with HHV-6-negative human DNA from PBLs. The following primer pairs were used: primary amplification, 5'-GGAGTACAGACAACGTTC-3', 5'-ACGGAGATGTAACAAACATGGAC-3'; secondary amplification, 5'-AAGAACCCACAAATCTACC-3', 5'-TGGTTTTCGTTGGGTTCATC-3'. Primary PCR amplification consisted of 40 cycles of 1 min each at 95°C, 51°C, and 72°C, followed by 10 min at 72°C. One-tenth of the primary PCR was then amplified with internal primers (nested) for 5 cycles of 1 min each at 95°C, 51°C, and 72°C, and 30 further cycles of 30 sec each at 95°C, 51°C, and 72°C, followed by 10 min at 72°C. The reaction products (10 μl) were analyzed by electrophoresis on a 2% agarose gel, and the titer was expressed as the reciprocal of the highest dilution at which the 67-bp nested amplification product was detected. In each experiment, serial dilutions of DNA from cloned HHV-6 variable glycoprotein gene and HHV-6-negative PBLs were included as positive and negative controls. All assays were run in duplicate.

DNA Sequencing. PCR products were purified with Microcon 100 microconcentrators (Amicon) and sequenced using an Applied Biosystems ABI 373A automated sequencer.

Immunocytochemistry (ICC). Sections from formalin-fixed, paraffin-embedded tissue were stained with an avidin-biotin complex immunodetection system in a TechMate instrument (BiorTech Solutions, Santa Barbara, CA). Four-micron sections mounted on positively charged slides were deparaffinized with xylene and rehydrated, immersed for 10 min in 3% hydrogen peroxide/methanol to quench endogenous peroxidase, and microwaved for 10 min in citrate buffer (11). After slow cooling, the sections were incubated successively with primary antibody, biotin-labeled goat anti-mouse or anti-rabbit immunoglobulin, streptavidin-biotin peroxidase complex, and 3,3'-diaminobenzidine tetrahydrochloride chromogen. Tissues were counterstained with Mayer’s hematoxylin.

Mouse IgG1 monoclonal antibody (mAb) to HHV-6B 101K virion protein (12) was obtained from P. Pellett (Centers for Disease Control, Atlanta) and from Chemicon International and used at a dilution of 1:200. Mouse IgG2a mAb C5 to DNA binding protein p41 (13) was used at a dilution of 1:50 (Biodesign International, Kennebunkport, ME). The specificities of these antibodies were confirmed by testing isotype-matched control mouse mAbs against human IgG (IgG1, X031; IgG2a, X493; IgG2b, X944; Dako) in selected HHV-6-positive cases. Control and test antibodies were used at the same IgG concentrations.

Antibodies to other herpesviruses included the following: anti-cytomegalovirus (CMV) mAbs DDG9 and C2H2 (Dako) to early and immediate early antigens; anti-Epstein–Barr virus (EBV) mAbs CS1, CS2, CS3, and CS4 to epitopes of latent membrane protein LMP-1 (NovoCastra, Newcastle upon Tyne, U.K.); and rabbit polyclonal anti-herpes simplex virus 1 (HSV-1) antibody B114 (Dako). Other antibodies included mouse anti-human macrophage marker Ham-56 (Enzo Diagnostics), mouse anti-glial fibrillary acidic protein to visualize astrocytes (Zymed), mouse anti-human leukocyte common antigen (IgG1 clone PD7/26, CD45RB; Dako) to identify B and T lymphocytes, and rabbit anti-human myelin basic protein to discriminate myelinated white matter from demyelinated plaques (Dako). All antibodies were used as recommended by the manufacturers. Histochemical staining of myelin with luxol fast blue was used to identify regions of demyelination.

Quantitation of HHV-6 staining in neurons and oligodendroglial cells was performed by microscopic examination of sections stained with 101K antibody. The percentage of HHV-6-positive neurons in the gray matter and oligodendroglial cells in the white matter was determined by counting the percentage of stained cells at 40X magnification. We define a “field” analyzed as a contiguous region of the slide corresponding to a minimum of 2000 oligodendrocytes or 400 neurons. In sections containing <2000 oligodendrocytes or <400 neurons, all of the cells on the section were counted. Neurons were evaluated as to their intensity and frequency of staining. Cytoplasmic 101K staining in neurons was scored on a scale, 1+ represented a level of staining indistinguishable from background, and 4+ represented the most intense deposition of chromogen. Nuclear staining of oligodendrocytes was scored as either positive or negative. Because of the clearly identifiable nature of the disease, the slides could not be blinded as to disease diagnosis at the time of histopathologic analysis.

Statistical Analysis. The Fisher–Irwin exact test (two-tailed) was used to determine the statistical significance of 2 × 2 contingency tables (14).

RESULTS

RDA. DNA preparations from five MS brains were analyzed independently by RDA (5) after digestion with HindIII. MS DNA was used as tester and DNA from PBLs of healthy donors was used as driver. After four rounds of RDA, the products were cloned and analyzed by gel electrophoresis (Fig. 1) and DNA sequencing. Of >70 DNA fragments sequenced, a 341-bp fragment from one case, MS-1, was found to be virtually identical (99.4%) to the MDPB gene of the Z29 isolate of HHV-6 variant B. The DNA sequences differed in only two positions, neither of which changed the encoded amino acid sequence, though one introduced a HindIII restriction site. The remaining RDA products are believed to be human sequences containing polymorphic restriction sites.
To characterize the virus in case MS-1, we determined the complete sequences of the MDBP (3399 nt), phosphotransferase (1692 nt), DNA polymerase (3039 nt), and glycoprotein B genes (2493 nt). The encoded amino acid sequences were all at least 99% identical to corresponding HHV-6 variant B group 1 sequences (strain Z29); overall, 3532/3537 (99.9%) of the amino acid residues in case MS-1 were identical to Z29. This demonstrated that significant portions of the HHV-6 genome were present. We subtyped the virus by analyzing the glycoprotein H gene and the immediate early region, which showed that case MS-1 contained HHV-6 variant B group 2 (9, 10).

Detection of HHV-6 DNA in MS and Control Brains by PCR. The prevalence of HHV-6 in MS and control brains was measured by using a nested PCR assay targeting the MDBP gene, with primers that detected 10 molecules of HHV-6A or HHV-6B DNA per µg of total DNA. HHV-6 was found in 25/32 MS specimens (78%) and 40/54 controls (74%), demonstrating that the virus was highly prevalent in MS and control brains. DNA sequence analysis of the immediate early gene (9) showed that 13/13 MS samples and 23/24 control isolates were HHV-6 variant B group 2, and one was a variant B group 1 virus (data not shown).

To determine approximate titers of HHV-6 DNA in brain, samples from frozen white matter and cortex were tested in a semiquantitative PCR assay, in which samples were serially 2-fold diluted prior to amplification. The highest titer in 51 controls was 1/16. Of the 49 MS cases tested, 47 had viral titers comparable to those of controls, but two cases, MS-1 and MS-2, had markedly higher titers of 1/262,000 and 1/65,000. HHV-6 was also detected in the cerebrospinal fluid of case MS-1.

Immunocytochemical Localization of HHV-6 Antigens. To determine the localization of HHV-6 in the brain, we used ICC with mAbs to virion protein 101K (12) and DNA binding protein p41 (13) (Figs. 2–4). Both HHV-6 mAbs produced identical staining patterns and identified similar fields of positive cells (Fig. 4 A and C). No staining was observed with reagent controls. Antibodies to human macrophage Ham-56, glial fibrillary acidic protein (an astrocyte marker), human leukocyte common antigen, and human myelin basic protein were used to assist in the interpretation of staining patterns (data not shown).

Specimens from 19 MS cases, 26 cases with other neurological diseases and 15 patients with nonneurological diseases were examined. Significant differences, qualitative and quantitative, in the staining of oligodendrocytes and neurons were observed between MS and non-MS control cases, including other neurological diseases. The analyses of white matter and gray matter are described separately below.
In the white matter, HHV-6 nuclear staining of oligodendrocytes was observed in 12/15 MS cases (Fig. 2, Table 1). In contrast, nuclear staining of oligodendrocytes was not detected in 41 non-MS cases or in 4 stillborn fetal brains. The difference in staining of oligodendrocytes in MS vs. control cases (12/15 vs. 0/45) was statistically significant ($P < 0.0001$).

To establish quantitative estimates of the percentage of nuclear-stained oligodendrocytes within MS cases, we examined a total of 128 fields of white matter, each containing at least 2000 oligodendrocytes, from plaque and nonplaque regions (Table 1). We observed HHV-6 staining in oligodendrocytes from 27/48 (56%) plaques. In the same brain specimens, in areas of histologically normal-appearing white matter that were distant from plaques, fewer fields (10/80, 15%) containing stained oligodendrocytes were observed. The finding of more frequent nuclear staining of oligodendrocytes in regions containing plaques compared to normal white matter (27/48 vs. 10/80) was statistically significant ($P < 0.0001$).

Within individual MS cases, plaques were heterogeneous with respect to oligodendrocyte staining; in a typical case, for example, one plaque contained no stained oligodendrocytes, while two other plaques contained 10% and 40% stained cells. Positive foci of oligodendrocytes that were distant from plaques were found in four MS cases and contained 2–40% stained oligodendrocytes, notably in the absence of demyelination and inflammation.

A total of 225 fields of gray matter from 16 MS and 39 control cases was examined for HHV-6 101K antigen (Fig. 4). In MS cases, cytoplasmic staining of neurons was observed; fluorescence microscopy and staining with periodic acid/Schiff stain showed that this staining was unrelated to lipofuscin. Neurons adjacent to plaques had a higher frequency and intensity of staining than those in uninvolved areas; 17/45 (38%) fields from regions containing plaques showed strong neuronal staining (3+ to 4+) vs. 9/61 (15%) fields from nonplaque regions. In contrast to MS, neuronal staining was weak (1+ to 2+) in young adults who died of trauma, Alzheimer disease, ALS, epilepsy, nonneurological deaths, and stillborn fetuses. However, neuronal staining was not entirely specific for MS, and staining (3+) was seen in 2/4 cases of Parkinson disease and 2/2 CVA cases. Interestingly, one MS case had a region with a recent cerebrovascular infarct, which showed very weak staining (1+) of adjacent, surviving neurons, although the neurons surrounding the plaques in this case were heavily stained.

In contrast to oligodendrocytes and neurons, several other cell types stained for HHV-6 but showed no association with MS. In MS cases and controls, HHV-6 antigens were detected in the cytoplasm of astrocytes and macrophages, particularly in the subependymal and subial regions, and in the cytoplasm of oligodendrocytes, in ependymal cells, in the epithelial cells of the choroid plexus, and occasionally in endothelial cells and in smooth muscle cells of blood vessels.

In control cases showing active inflammation (CVA, VZV encephalitis), HHV-6 was particularly prominent in the macrophage component of the inflammatory infiltrate, but not so in lymphocytes. In such regions of active inflammation, we did not observe nuclear staining of oligodendrocytes.

**Other Viruses.** We screened for other herpesviruses by nested PCR. We detected HSV-1 in 2/25 MS cases and 2/42 controls and VZV in 0/17 MS cases and 2/24 controls. Neither CMV nor human herpesvirus 7 was detected in 25 MS cases or 14 controls. In particular, the two MS cases with unusually high titers of HHV-6 were negative for the other four herpesviruses. They were also found to be negative for HSV-1, CMV, and EBV by ICC.

We tested for other viruses by PCR, including measles virus, adenoviruses, polyomaviruses (JC, BK, simian virus 40), human immunodeficiency viruses 1 and 2, and human T-lymphotropic viruses I and II, and none of these was detected in MS cases. Thus, in contrast to HHV-6, many other pathogenic viruses, including herpesviruses, were not generally detected in the brains of MS patients.

**DISCUSSION**

HHV-6, a member of the *Herpesviridae*, is the causative agent of exanthem subitum (roseola), a common febrile illness of infants. It is known to have tropism for T lymphocytes and macrophages (15). Most infants are infected with HHV-6, and it can be detected in the majority of adults (16–21). Although cases of fatal encephalitis caused by HHV-6 have been reported in AIDS patients and in an immunosuppressed bone marrow transplant patient (22–24), the virus has yet to be associated with a major chronic disease.

We have obtained evidence that HHV-6 is a common commensal virus of the brain, consistent with a previous report (21), and that it is expressed in neurons and glial cells. Most notably, we observed expression of HHV-6 antigens in the nuclei of oligodendrocytes in MS cases, but not in various controls. Moreover, in MS patients, nuclear-stained oligodendrocytes were most commonly associated with plaques, demonstrating that this virus is specifically associated with the characteristic pathological lesions of MS.

In four MS cases, we observed foci of oligodendrocytes showing nuclear positivity for HHV-6 101K, in the absence of any histological evidence of demyelination or inflammation. The presence of such foci in MS cases suggests that HHV-6 expression is not a consequence of immunologic attack by infiltrating lymphocytes or macrophages. A possible interpretation of this observation is that viral activation within oligodendrocytes precedes the immunologic injury that accompanies plaque formation.

The presence of a HindIII restriction site polymorphism in the MDBP gene fragment contributed to its detection by RDA, since we found by PCR that low titers of HHV-6 DNA were also present in the pooled donor. Interestingly, this polymorphism occurred in the two highest-titered MS cases, suggesting that there may be subtypes of HHV-6 variant B group 2 viruses that differ in their biological behavior. Whether such subtypes associate with MS is unclear.

Several previous studies are consistent with an association between HHV-6 and MS. HHV-6 is acquired during infancy or early childhood (16, 18–20), which is compatible with the epidemiology of MS (1, 25, 26). HHV-6 is neuropathogenic, in that primary HHV-6 infections in infants may have neurologic sequelae (16, 27–29). After roseola infection in infancy, the virus persists in the central nervous system (19). HHV-6 causes

| Table 1. Expression of HHV-6 in nuclei of oligodendrocytes |
|-----------------|---------|-------|---------|---------|
| Diagnosis       | Patients | Fields |
|-----------------|---------|-------|---------|---------|
| MS              | n       | Age*  | Pos.    | n       | Pos.    |
| Plaque          | 15      | 54    | 12      | 48      | 27      |
| Nonplaque       | 17      | 54    | 4       | 80      | 10      |
| ALS             | 7       | 64    | 0       | 20      | 0       |
| Alzheimer       | 3       | 71    | 0       | 8       | 0       |
| Disease         | 8       | 73    | 0       | 18      | 0       |
| CVA (stroke)    | 2       | 54    | 0       | 7       | 0       |
| AIDS encephalitis| 2    | 39    | 0       | 20      | 0       |
| Epilepsy        | 1       | 11    | 0       | 2       | 0       |
| Nonneurological | 18      | 65    | 0       | 21      | 0       |

Oligodendrocyte nuclear staining in MS cases and controls, analyzed by ICC with mAb to HHV-6 101K. Fields containing at least 2000 oligodendrocytes were evaluated from multiple histologic sections. Results are expressed as the number of fields examined and the number of fields containing at least 1% nuclear-stained oligodendrocytes. Pos., positive.

*Average age in years.
enveloped viruses in immunosuppressed individuals (22-24). Moreover, HHV-6 has been identified in plaques of demyelination in an HHV-6-infected bone marrow transplant recipient (23) and in AIDS patients (24), indicating that it can be associated with demyelination even in the absence of an intact immune system. Higher levels of anti-HHV-6 antibodies have been found in MS patients than in controls (30, 31), and HHV-6 has been detected by PCR in the cerebrospinal fluid of some MS patients (31). Finally, two drugs with demonstrated beneficial effects in MS, β interferon and azathioprine, which have been considered to function by an immunosuppressive mechanism, have been shown to have anti-HHV-6 activity in vitro (decreased infection of cord blood cells as measured by indirect immunofluorescence with a polyclonal human anti-HHV-6 serum; K. Folger, personal communication).

A substantial body of evidence indicates that autoimmune mechanisms contribute to the pathology of MS, demyelination being mediated by macrophages and subsets of T lymphocytes that are sensitized to components of myelin. Within this context, HHV-6 infection early in life may establish a persistent infection in the central nervous system, with subsequent virus activation leading to cytopathic and/or immunological damage to oligodendrocytes. Alternatively, HHV-6 activation may be secondary to immune-mediated injury. In our studies, however, oligodendroglial staining was not observed in other neurological diseases where there was a strong inflammatory component (CVA and encephalitis), and foci of HHV-6 positive oligodendrocytes were observed in regions of white matter in the absence of inflammation and demyelination, both of which argue against the latter hypothesis.

A number of neurotropic viruses produce demyelination in animal models, and some parallels with MS may be drawn. Semliki forest virus is an instructive example. In weanling mice this virus persistently infects neurons and oligodendrocytes (32), leading to focal demyelination (33). However, no loss of oligodendrocytes is observed in persistently infected athymic mice, and demyelination has been shown to be mediated by T cells (34). Canine distemper virus infects astrocytes and oligodendrocytes, leading to multifocal demyelination (35). Théiler murine encephalomyelitis virus infects oligodendrocytes and astrocytes, leading to chronic, relapsing demyelination (36). There is thus ample evidence that exposure to viruses early in life can lead to persistent infection of the central nervous system and demyelination throughout life.

Numerous viruses have been proposed as playing a role in the etiology and pathogenesis of MS. Since none of these reports has been confirmed, any new candidate MS-associated virus must of necessity be viewed with suspicion. The lessons to be learned from these former claims have been summarized previously (4) and have been addressed in our study. In particular, >100 cases from three geographical locations (Los Angeles, Seattle, and Philadelphia) were studied, HHV-6 had never been studied in our laboratory previously, the methods used, PCR and ICC, are well understood, and the antibody reagents were of known specificity.

Although our observations demonstrate an association between HHV-6 and MS, they are insufficient to establish a causal link. Direct demonstration that HHV-6 causes demyelination in an animal model or a successful clinical trial of an antiviral drug in MS would provide further evidence for a pathogenic role of HHV-6 in this disease.