Human herpesvirus 6 and *Chlamydia pneumoniae* as etiologic agents in multiple sclerosis – a critical review

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Running title: Infectious agents in MS

Abstract

Multiple sclerosis (MS) is thought by many investigators to have an infectious component, and many microorganisms have been associated with the disease during the last three decades. Recent studies have implicated both human herpes virus 6 (HHV-6) and the obligate intracellular bacterium *Chlamydia pneumoniae* in the etiology of MS. As with earlier studies of other potential agents, however, evidence linking either of these organisms to the disease is equivocal. In this article, we review evidence for and against involvement of HHV-6 and *C. pneumoniae* in MS, as well as evidence concerning auxiliary factors, such as possession of the *APOE* ε4 allele, which may influence the role of these organisms in pathogenesis. Further, we suggest several lines of investigation that should clarify whether either or both pathogens are associated meaningfully with this disease.

Key Words: Human herpesvirus-6 *Chlamydia pneumoniae* multiple sclerosis

Introduction

Multiple sclerosis (MS) is a relatively common demyelinating disease of central nervous system white matter in humans. The disease includes an important autoimmune component, with self-reactive lymphocytes targeting several constituents of the myelin sheath including myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), and others. Inflammation of the white matter is also characteristic in most patients. Although individuals with MS can display wide variations in clinical course, the disease usually occurs in two general forms, remitting-relapsing and chronic progressive, with the former being the more common [1]. Most patients with remitting-relapsing disease eventually progress to the latter form (secondary progressive), with consequent increase in motor dysfunction.

Despite aggressive study in many laboratories over several decades, the etiology of MS remains unknown. However, some epidemiologic evidence suggests involvement of an infectious agent or agents in the process of disease genesis. For example, data from long term studies in the Faroe islands suggest that MS was not present in the island population before about 1940, but that introduction of some infectious agent at that time has since generated pulses of new MS cases at intervals of approximately 13 years [e.g., 2]. In these studies, the agent was argued to be a virus of unspecified type. While these studies remain controversial, good evidence does exist indicating seasonal variation in the incidence of new MS cases, and the disease is known to be most prevalent in

high northern latitudes [3]. Both of these characteristics are, of course, consistent with some sort of infectious involvement in disease genesis.

Involvement of a microbial agent in MS is not only consistent with the epidemiology of MS, it also might explain, at least in part, some aspects of the autoimmune component of the disease. Specifically, elicitation of relevant anti-myelin T cells or autoantibodies *via* a mechanism based on molecular mimicry is a concept that has gained credence during the last two decades [4-6]. Based on both epidemiologic data and the increasingly attractive mimicry hypothesis, many laboratories have searched for relevant viruses and bacteria in cerebrospinal fluid (CSF) and other samples from MS patients. To date, nearly 20 organisms have been associated with the disease. The screening techniques in these many studies varied widely, from serology to polymerase chain reaction (PCR), and overall quality and numbers of controls examined varied as well. To date none of the implicated organisms has been accepted as the causal agent. Indeed, all remain controversial, and some essentially have been discarded because confirmatory evidence from independent laboratories has not been forthcoming.

In the late 1990's, two new organisms were added to the list of possible etiologic agents in MS: human herpes virus 6 (HHV-6) and the intracellular bacterial pathogen *Chlamydia pneumoniae*. Both have elicited significant interest from investigators in the neuroscience research community, and both have generated a good deal of controversy concerning their potential role in disease genesis [e.g., 7-8]. In this article, we review in detail both the positive and negative evidence for involvement of HHV-6 and *C. pneumoniae* in MS, including

the technical means by which that evidence was obtained. On the basis of that information, we suggest directions for future study of these two pathogens, as well as others, in relation to MS that may prove to be useful.

HHV-6 and Multiple Sclerosis

HHV-6 is a member of the β–herpesvirus subfamily of Herpesviridiae. This DNA virus was initially isolated from peripheral blood mononuclear cells (PBMC) of infected AIDS patients, but subsequently numerous isolates were obtained from PBMC of children with undifferentiated acute febrile illness, as well as from healthy adults and immunocompromised patients. HHV-6 is ubiquitous, with a seroprevalence near 100% worldwide [9]. Although viewed as quite T lymphotropic, HHV-6 can infect many different host cell types including monocytes. The surface marker CD46 is apparently part of a co-receptor complex for HHV-6; CD46 is expressed by all nucleated cells, but HHV-6 does not infect all CD46-positive cells [10]. Recently, levels of soluble CD46 were shown to be elevated in serum of MS patients compared to healthy controls. Further, MS patients positive for HHV-6 DNA in their serum had significantly elevated levels of soluble CD46 compared to DNA-negative MS patients [11]. The two variants of the virus, HHV-6A and -6B both can enter the CNS, but some differences have been identified in the *in vitro* cellular tropism of the variants (see below).

In a general sense, HHV-6 is an attractive candidate to play a role in MS. The virus is usually acquired during infancy or early childhood, an observation

consistent with epidemiologic evidence suggesting that childhood exposure to a pathogen is implicated in the disease [2,3]. Moreover, HHV-6 is neurotropic and can persist or remain latent in numerous tissues, including the CNS. Like other herpesviruses, HHV-6 can be reactivated by stress or infection with other microbes. Viral infections and stress also can trigger exacerbations of MS. Thus, in this scenario HHV-6 acquisition in infancy may initiate a persistent/latent CNS infection, and at some later time reactivation of the virus leads to damage to oligodendrocytes, ultimately culminating in MS.

Infection with HHV-6 has been linked to several diseases, including neonatal exanthum subitum (roseola), AIDS-associated encephalomyelitis, and chronic fatigue syndrome [12,13 for review]. The HHV-6B variant reportedly accounts for most HHV-6 infections in infants, including exanthum subitum, whereas the HHV-6A variant has not been associated with human disease [14, 15].

Wilborn *et al.* suggested a potential role for the virus in MS on the basis of finding viral DNA in the CSF of three of 21 MS patients [16]; CSF from 19 patients with facial palsy and 7 with Guillain-Barré syndrome were negative in parallel assays. Further, serum anti-HHV-6 antibody levels were higher in MS than in control patients. A more extensive investigation was conducted by Challoner *et al.* [12]. These workers employed subtractive hybridization followed by a nested PCR assay to show that HHV-6 DNA was present in 78% of MS brain specimens. However, that DNA was also identified in 74% of controls with other neurological diseases or non-neurological diseases; thus, the presence of

HHV-6 was not specific for MS. Using immunocytochemistry, these workers observed nuclear staining of oligodendrocytes in MS, but not control, brain specimens. In the former, HHV-6-staining of oligodendrocytes was more prevalent in plaque regions (56% positive) than in normal-appearing white matter (15% positive). Cytoplasmic staining of neurons was also observed, and the frequency and intensity of staining was highest in plaque regions. However, neuronal staining was also observed in some patients with Parkinson disease and stroke, as well as in some control brains. The authors suggested that HHV-6 activation within oligodendrocytes may precede the immunologic injury that accompanies MS plaque formation [12].

These findings were extended by Soldan *et al.*, whose group studied antibody responses to HHV-6 p41/38 (the early antigen of HHV-6 found in MS plaques) in a large group of patients with relapsing-remitting MS, chronic progressive MS, other neurologic diseases, other autoimmune diseases, and normal controls [13]. They found a highly significant increase in the anti-HHV-6 IgM response in patients with relapsing-remitting disease compared with the other groups. No increase in anti-HHV-6 IgG levels was found in any group. Moreover, HHV-6 DNA was found in the serum of 30% of relapsing-remitting patients by PCR. The presence of HHV-6 DNA and antibodies of the IgM isotype is consistent with reactivation of the virus during the course of the disease in that subset of patients. In a later study [14], this group showed that HHV-6 DNA was differentially present in MS patient specimens, depending upon sample source. Serum and urine were 23-26% positive by PCR for DNA encoding the HHV-6

major capsid protein or the large tegument protein only in the MS group; normal donor sera and urine all were PCR-negative for such coding sequences. In contrast, similarly high percentages of saliva and peripheral blood lymphocyte samples were PCR-positive for the same genes in both the MS and control groups. Interestingly, no control donors were HHV-6A positive by PCR. MS serum and urine samples were PCR-positive predominantly for HHV-6A, but HHV-6B predominated in saliva and PBLs [14]. The authors suggest that reactivation of latent HHV-6A in a subset of MS patients may result in the release of virus into serum and urine that can be detected by PCR [14].

Tejada-Simon et al. [15] recently confirmed that IgM antibodies specific for the 101-kDa HHV-6 virion protein were present a significantly higher proportion of MS patients compared to control subjects (82% of patients vs. 38% of controls). In contrast, IgG responses were decreased in the patient group. The precursor frequency of 101-kDa-specific T cells was decreased in MS patients, and T cell lines secreted Th1 proinflammatory cytokines (IFN-γ and TNF-α), whereas the cell lines from control subjects produced IL4, IL-10 and TNF-α, but not IFN-γ. Thus, the decreased IgG responses in the MS patients might reflect deficient Th2 cell help for B cells [15].

Further support for an association between active HHV-6 infection and MS was reported by Knox *et al.* [17]. By immunohistochemical means, these investigators found HHV-6 in CNS tissue in 8/11 MS patients and two patients with HHV-6 leukoencephalitis, but not in 8 individuals with other neurologic diseases or in 7 normal control subjects. The infected cells included microglia,

lymphocytes within the perivascular cuffs, and small cells within the region undergoing demyelination; the latter had the appearance of oligodendrocytes. These investigators also evaluated blood samples from MS patients for HHV-6 using a rapid cell culture assay in which patient leukocytes were co-cultivated with fibroblasts; the fibroblasts were stained with fluorochrome-labeled antibodies specific for the major immediate-early HHV-6 protein. The results revealed that MS patients with evidence of HHV-6 viremia were significantly younger and had disease of significantly shorter duration than patients without viremia [17]. The authors concluded that a strong association exists between active or reactivated infection with HHV-6 and MS.

Other studies have failed to support an association between HHV-6 and MS. For example, Enbom *et al.* detected anti-HHV-6 IgM antibodies in only 1/55 MS patients, but found antibody of the IgG isotype in 15 of these patients [18]. This is in contrast to the findings of Soldan *et al.* [14]. The Enbom group also failed to detect differences in lymphoproliferative responses to HHV-6A or –6B between MS patients and healthy control subjects [18]. These authors concluded that their results did not support an etiologic relationship between HHV-6 and MS, although they did not exclude the possibility that it may be important in a subset of patients.

A number of possible explanations may be relevant to such conflicting results. HHV-6 is ubiquitous, and a majority of adults have antibodies to this virus [see 13]. Thus, the failure to observe differences in IgG levels or lymphoproliferative responses between MS or other patients and normal controls

[18] might merely reflect memory responses to a previously encountered virus. In contrast, Knox *et al.* [17] reported viremia in 8/11 MS patients, but not in controls, which is indicative of active infection. Thus, it probably would be important to follow these patients to determine whether viremia correlates with clinical status over time. Further, the finding of anti-HHV-6 antibodies of the IgM but not IgG isotype suggests a primary immune response and thus is consistent with recent encounter with the virus [14]. Importantly, some investigators have not specified the type of MS with which their patients are afflicted, either remitting-relapsing or progressive, which makes data comparison extremely difficult among the various reports [e.g., 18; see also below]. This point becomes especially critical in view of a recent histopathologic analysis of MS patient samples which indicated that different forms of the disease are discernable at that level [19; see also below].

Demyelination and axonal degeneration are characteristic features of MS [20-22]. Although these pathological changes could occur as a direct result of HHV-6 infection of the CNS, there is considerable evidence to indicate that autoimmunity against CNS components is prominent in MS [e.g., 23-24]. It has been proposed that antigenic cross reactivity or molecular mimicry between an exogenous infectious agent and a "self" myelin antigen (e.g., MBP, PLP, *etc*.) plays a role in the development of MS [4-6]. Experimental support for this hypothesis can be found in Theiler's murine encephalomyelitis, where a variant viral strain engineered to contain an encephalitogenic PLP epitope has been shown to induce a remitting-relapsing disease similar to MS [25]. Further, in a different system of experimental autoimmune encephalomyelitis, we

demonstrated that a unique *C. pneumoniae* peptide which is partially homologous to an encephalitogenic MBP epitope effectively elicits MS-like disease in rats [26]. Thus, at this point available evidence does not support a firm conclusion as to whether HHV-6 is or is not overtly involved in the genesis or exacerbation of MS. As reviewed next, this is the case also for data concerning a role for *C. pneumoniae* in the disease.

C. pneumoniae and Multiple Sclerosis

Chlamydia pneumoniae is an obligate intracellular bacterial pathogen of the respiratory tract that causes community-acquired pneumonia. It was described as a unique species in 1989 and is thought to be solely a human pathogen, *i.e.,* no animal reservoir for the organism is known. Like all *Chlamydiae*, *C. pneumoniae* infects mucosal surfaces, in this case the oral and nasal mucosa, and dissemination of the organism from its site of primary infection has been well documented [27 for general review]; the vehicle of dissemination appears to be the monocyte/macrophage. Interestingly, since its description as a respiratory pathogen, *C. pneumoniae* has been associated with a surprisingly diverse panel of chronic human diseases, including atherosclerosis, temporal arteritis, Alzheimer's disease, and others, in addition to MS. All these associations remain enormously controversial, although the potential role of this organism in atherogenesis appears to be gaining credence [28]. Epidemiologic studies indicate that *C. pneumoniae*, like HHV-6, is

essentially ubiquitous, and that the prevalence of infection increases with increasing age.

The initial report of a CNS association for *C. pneumoniae* was published in a serologic study of patients with encephalitis and CNS infection by the Helsinki Study Group [29]. The first association between the organism and MS appeared in a quite striking case report two years later from a group at Vanderbilt University [30]. In that report, an MS patient with high EDSS score was shown to have *C. pneumoniae* in his CSF, and neurologic improvement closely paralleled antibiotic treatment. The Vanderbilt group subsequently published a larger study of *C. pneumoniae* in MS patients *vs.* individuals with other neurologic disorders [31]. In that study they employed culture, PCR, and other methods to examine CSF for presence of the organism in 17 patients with remitting-relapsing disease, 20 with chronic progressive disease, and 27 with unrelated neurologic disorders. Samples from 8/17 remitting-relapsing patients, and 16/20 progressive MS patients, were culture-positive for *C. pneumoniae*; samples from only 3 patients with other neurologic disorders were similarly positive. PCR-positivity among the MS patients was reported to be extremely high: all 17 patients with remitting-relapsing disease and 19/20 individuals with progressive disease showed full-length 1200 bp amplicons from the *C. pneumoniae* major outer membrane protein gene (*ompA*), but only 5/27 patients with other neurologic disorders were similarly PCR-positive. Interestingly, the PCR assay employed in this study was not nested. Congruence between culture- and PCR-positivity was reasonably good in these sample groups. ELISA-based assessments in this study indicated

a higher level of anti-*C. pneumoniae* antibodies in CSF from MS patients than in controls with unrelated neurologic disorders.

Other laboratories have failed to confirm these high rates of culture- and/or PCR-positivity in samples from MS patients. For example, in one study of CSF from 32 MS patients, only 9.3% were PCR-positive for the organism; none of the similar samples from 30 patients with other neurologic disorders was positive in parallel assays [32]. Other laboratories have found similarly low rates of PCR-, immunohistochemical-, and/or culture-positivity for *C. pneumoniae* in MS patient samples [e.g., 33], and in a number of studies no positive samples at all were identified using culture and/or PCR [e.g., 34]. One recent study reported DNA from the organism in CSF from 12/58 MS patients and 20/47 patients with other neurologic disorders [35]; interestingly, CSF samples from 67 normal control individuals were universally PCR-negative for *C. pneumoniae* in this report. The conclusion from this latter study was therefore that the organism is relatively common in CSF from patients with neurologic disorders, but that the organism cannot be specifically associated with MS. Thus, while some *C. pneumoniae*-positive MS patients have been identified by laboratories other than that at Vanderbilt, the general assertion that this organism is associated with MS remains in doubt.

The wildly inconsistent reports of PCR- and/or culture-positivity for *C. pneumoniae* in samples from MS or other patients raises a number of important issues concerning how any association between a microorganism and a particular clinical entity is established. As pointed out by a number of

investigators involved [e.g., 34], no “standard” PCR screening system targeting chromosomal DNA from *C. pneumoniae* in CNS or other samples has been generally agreed upon; thus, each laboratory develops and employs its own assay system(s), and these can vary rather widely in sensitivity, and to some extent specificity as well, making it difficult to compare screening results published by different groups. Similarly, methods (and skill levels) vary from laboratory to laboratory with regard to chlamydial culture, and no agreement has been reached in the research community as to how many sequential passages are required to be negative for *C. pneumoniae* before the clinical sample under assessment is considered negative for the organism. Other aspects of how screening is actually done can also contribute to confusion. For example, in a few reports relevant to the *Chlamydia*-MS controversy no mention is made of whether samples from remitting-relapsing or progressive MS patients were studied, and little or no information is presented concerning clinical aspects of the cases (e.g., disease duration, EDSS score, *etc.*). Given the heterogeneity of this disease, as well as recent neuropathologic information (see below), sample selection is likely to be a critical issue in any such analysis. Moreover, some studies have reported PCR, culture, or other data from CSF samples, while others have focused on tissue samples from the CNS. In our view, all these issues severely equivocate any firm conclusions as to whether CNS infection with *C. pneumoniae* is, in fact, related to development or exacerbation of MS.

A characteristic feature of MS is intrathecal immunoglobulin (Ig) production, characterized by the presence of oligoclonal Ig bands in the CSF.

These have been speculated to represent antibody specific for the etiologic agent of MS, since oligoclonal Ig also occurs in patients with measles-induced subacute sclerosing panencephalitis, a disease in which the bands can be absorbed out of the CSF with measles virus. Over the years, many unsuccessful attempts have been made to establish the specificity of the oligoclonal bands in MS CSF. Rather, it was determined that polyclonal immune responses to many viruses are common in MS patients, but that they do not correspond to the CSF oligoclonal bands. Recently, Sriram's group reported that oligoclonal bands could be partially or completely absorbed from the CSF of 14 of 17 MS patients with elementary body antigens of *C. pneumoniae* [36]. In an independent study, Derfuss et al. [37] found that 11 of 46 patients with definite MS displayed intrathecal IgG responses to *C. pneumoniae*, compared to 3 of 61 patients with other neurological diseases. However, using a sensitive isoelectric focusing-western blot procedure, the major CSF-specific oligoclonal bands did not recognize *C. pneumoniae*. These authors concluded that the response to *C. pneumoniae* is part of the polyspecific intrathecal Ig production, as has been reported for other pathogens [37].

One further set of observations is relevant to the issue of whether *C. pneumoniae* plays a role in MS. Work from many groups has shown that possession of the ε4 allele type at the *APOE* locus is a risk factor for earlier onset and rapid progression in sporadic Alzheimer's disease. In work unrelated to MS, one of us reported data suggesting some as yet unknown relationship between possession of the ε4 allele and the pathobiology of *C. pneumoniae* [38]. If this is

indeed the case, and if *C. pneumoniae* is somehow associated with MS, then among other possibilities i) infected MS patients, or perhaps a subset of them, should show a higher overall prevalence of ε4 than do individuals with other or no neurologic disorders; or ii) infected MS patients who possess the allele should show earlier onset of symptoms and/or faster progression to disability; or iii) a combination of these. Recent observations support possibility ii. In a study of 204 MS patients, prevalence of the ε4 allele was about equivalent to that of the general population, suggesting that possession of the allele is not a risk factor for development of MS [39]. However, ε4-bearing MS patients showed somewhat earlier onset of disease and more rapid progression to disability than did patients lacking the allele; interestingly, patients with progressive disease outnumbered those with remitting-relapsing disease in the ε4-bearing group. Another study of 374 patients also showed no difference in prevalence of this allele in MS patients *vs.* non-MS controls, but the ε4-bearing patients studied progressed more rapidly than did ε4-lacking patients [40]. In neither study were patients screened for *C. pneumoniae.* We note that several groups reported failure to confirm these observations. The *APOE* gene products perform important functions in the nervous system and elsewhere, and the increasingly firm association between possession of the ε4 allele and earlier onset/rapid progression of MS symptoms does not, of course, confirm a role for *C. pneumoniae* in genesis of the disease. However, the association is consistent with such a role and is suggestive that further studies in this context might be enlightening.

Infectious Agents and Neuropathology in Multiple Sclerosis

As indicated briefly at the beginning of this article, MS is an extremely heterogeneous disease. Given such wide variation among patients in clinical course, responses to treatment, *etc.*, well-controlled, large-scale, comparative neuropathologic studies of MS patient materials have been surprisingly rare. A recent report regarding CNS pathology in a large sample set of MS patient materials has provided an important initial basis for understanding the heterogeneity in many aspects of MS. In a collaborative study involving centers in the US, Germany, and Austria, neuropathology was defined comparatively in biopsy and autopsy materials from 83 patients with firm diagnoses of MS [19]. All patient samples studied included one or more lesions in active stages of demyelination, and patients from whom samples were chosen included individuals with detailed clinical histories and well-documented remitting-relapsing or progressive disease. Importantly, this landmark study determined that among the patient materials studied, four distinct patterns of demyelination were present. Two of these patterns (designated I and II) showed similar features and were consistent with a T cell- or T cell plus antibody-mediated process of demyelination. Demyelination in both these patterns centered around inflamed small veins. The primary feature distinguishing pathology in pattern I from that in pattern II was the presence in the latter of depositions of Ig (largely IgG) and the complement C9neo antigen at sites of demyelination, neither of which were present in samples showing pattern I. Pattern III lesions included a clear inflammatory infiltrate made up primarily of T lymphocytes, with some

macrophages and activated microglia. Demyelination in pattern III was not focused at venules, and all samples showing this pattern displayed oligodendrocyte death and loss of myelin-associated glycoprotein (MAG). Pattern IV samples showed oligodendrocyte death in areas of demyelination, but no evidence of apoptosis was identified; patient samples showing pattern IV displayed no marked loss of MAG or other myelin-related proteins (e.g., PLP, MBP). Among the samples studied, pattern II proved to be the most common, followed by III, I, and IV in decreasing order of prevalence. Patterns II and III were identified with frequency in patients with acute MS, while pattern III was rare in individuals with established disease. Within any given patient the pattern of neuropathology was consistent, and regardless of pattern all patients from whom samples were studied developed clinically definable MS.

From these observations the authors concluded that patterns I and II are consistent with demyelination *via* autoimmune-mediated mechanisms, while patterns III and IV are more consistent with demyelination *via* toxin-, virus- (or perhaps other microbially-) mediated mechanisms. Although more study will be required to confirm and extent these findings [see 41], all these data suggest that MS may have more a heterogeneous etiology than previously thought, *i.e.*, clinically evident MS may be a common end point for a number of different starting points. Development of the disease in this scenario would depend on exposure to a specific initiator at an appropriate time or age, with the genesis of subsequent neuropathology a function of genetic background of the patient.

The neuropathologic observations briefly summarized here do not address, and therefore cannot be used to support, a role for HHV-6, *C. pneumoniae*, or any other organism in the etiology of MS, but they are at least consistent with such a role. For example, in the context of this review autoimmune-derived demyelination of patterns I or II might develop in some subset of patients subsequent to infection with HHV-6 or *Chlamydia pneumoniae* *via* molecular mimicry, while demyelination of pattern III or IV in other patients might derive more directly from the infection *via* virally-elicited or bacterial toxin-induced oligodendrocyte death. All these observations indicate what we consider to be a potentially important line of inquiry in relation to any role for these two organisms (or others) in eliciting the disease, *i.e.*, that screening of MS patient materials must be done with both knowledge of the neuropathologic pattern(s) of the patients from whom samples were obtained and detailed clinical histories of those patients. If MS does indeed have a diverse set of initiating factors including one or more micro-organisms, and if the pattern of neuropathogenesis culminating in the disease derives from exposure to those diverse factors in the context of the specific genetic background of the patient, then one would not expect CSF or tissue samples from any set of randomly chosen MS patients to show universal, or even necessarily a high proportion of, positivity for any one initiating factor, including a particular micro-organism; indeed, there is no *a priori* reason to assume that the virus, bacteria, or other initiating factor(s) should endure in the CNS past the initiation phase and throughout the disease course in every patient. Rather, one would expect precisely what has been reported for

both HHV-6 and *C. pneumoniae* in relation to MS: variable positive/negative results depending on the distribution of those samples among the four patterns of neuropathology described above, the stage and clinical characteristics of disease in each patient from which samples were procured, and so on. Results would also vary with the assay system used for screening and other technical factors relating to the analyses.

Summary

Epidemiologic data support the contention that some infectious agent or agent(s) is/are involved in MS, although it is not clear at this point whether that involvement is at the level of etiology or disease exacerbation. Given the quantity and quality of data available at the time of this writing, we consider it premature to conclude that either HHV-6 or *C. pneumoniae* does/does not play a role in the causation or exacerbation of neuropathogenesis in this disease. However, in our view enough preliminary evidence does exist in relation to each organism to support further study. We suggest that future screening of MS patient materials targeting these two organisms or others be done on samples for which detailed neuropathologic pattern characteristics have been defined carefully, and for which patient clinical histories are known in some depth. Knowledge of the patients' *APOE* genotype should also be obtained. Further, it will be important to study well-characterized patient materials from individuals early in the process of disease development and from those with established disease. We suggest that, while it will be difficult to reach agreement among investigators on a common

PCR-based or other screening assay system, such a system or group of systems would facilitate comparative analyses between/among laboratories. Given enough samples screened from both acute and chronic MS patients, and given enough detailed information concerning the clinical and genetic characteristics of those patients and their disease, it should be possible to reach reasonable conclusions as to whether HHV-6, *Chlamydia pneumoniae*, or both are involved somehow in disease genesis, and which subset(s) of MS patient(s) are affected. Such information is critically important, since it will influence profoundly the design of treatment regimens for those patients. Moreover, if future studies do indeed confirm a role for either or both organisms in some subset of MS patients, then the next major topic for basic research in this area certainly will be elucidation of the host genetic factors, in addition to possession the *APOE* ε4 allele, which predispose individuals to disease development, early onset, or rapid progression.

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