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Review

Human herpesvirus-6: neurologic implications of a newly-described viral pathogen

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Discovered only 12 years ago, human herpesvirus-6 (HHV-6) has been associated with central nervous system (CNS) findings such as febrile seizures, encephalitis, meningitis, and possibly multiple sclerosis. These manifestations have been reported in both immunocompetent and immunocompromised individuals. The applications of such sophisticated laboratory tools as polymerase chain reaction, *in situ* hybridization, immunohistochemical staining, and representational difference analysis have expanded knowledge of the spectrum of CNS disease attributable to HHV-6 while delineating pathogenic mechanisms of both primary HHV-6 infection and reactivation from latency. This article reviews existing knowledge of the CNS manifestations of HHV-6, focusing on both clinical aspects of HHV-6 infection and its pathogenesis on neurologic diseases.

Keywords: human herpesvirus-6; HHV-6; seizure; encephalitis; viral meningitis; multiple sclerosis

Introduction

In 1986, a novel virus was isolated from six patients with lymphoproliferative syndromes, two of whom were also infected with the human immunodeficiency virus (HIV) (Salahuddin *et al*, 1986). Molecular and structural characterization indicated that it was a herpesvirus, though serologic and genomic analyses confirmed it to be distinct from all previously identified human herpesviruses (Josephs *et al*, 1986). Due to the initial belief that the new virus selectively infected freshly isolated human B cells, the virus was given the name human B-lymphotropic virus (HBLV) (Salahuddin *et al*, 1986). Subsequent investigation revealed a broader cell tropism, with notable T-cell lymphotropism (Lusso *et al*, 1988). For this reason, current nomenclature refers to this virus as human herpesvirus-6 (HHV-6).

With the discovery of HHV-6 in AIDS patients, initial investigation of possible diseases resulting from HHV-6 infection focused on its role as a possible pathogen or co-factor in HIV-infected persons. As several researchers investigated and characterized HHV-6 in HIV-infected adults, Yamanishi *et al*, (1988) pursued its association with disease in immunocompetent children. In 1988, Yamanishi discovered that HHV-6 caused the very common childhood disease exanthem subitum (roseola infantum). Indeed, HHV-6 proved to be the most ubiquitous of all human herpesviruses (Farr *et al*, 1990). Neurologic manifestations of exanthem subitum have been described since the beginning of this century. Over the past decade, investigations have confirmed and expanded the clinical associations of HHV-6 to include such neurologic manifestations as febrile seizures, encephalitis, meningitis, and possibly multiple sclerosis.

This article will review the current knowledge of the biology and pathology of HHV-6 infection. Proven and suspected associations with neurologic diseases and sequelae will be discussed.

Virology

Human herpesvirus-6 is a member of the *Herpesviridae* family. Genomic analysis places HHV-6 among the β-herpesviruses, along with cytomegalovirus

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(CMV) and human herpesvirus-7 (HHV-7). On the basis of DNA restriction analysis, *in vitro* tropism studies, and antigenic relationships defined by reactivities of monoclonal antibodies, HHV-6 can be separated into two variants, designated variant A (HHV-6A) and variant B (HHV-6B) (Ablashi *et al*, 1993). Characteristic HHV-6A strains include GS (the original strain) and U 1102 (isolated from a Ugandan AIDS patient) (Salahuddin *et al*, 1986; Downing *et al*, 1987). The prototypic HHV-6B strain is Z 29, isolated from a Zairian AIDS patient (Lopez *et al*, 1988). Variant B strains can be further characterized as belonging to one of two groups, designated group 1 and group 2. The intravariant nucleotide sequence homology ranges from 97–100%, while intervariant homology ranges from 94–96% (Aubin *et al*, 1991; Teo *et al*, 1991; Aubin *et al*, 1993; Gompels *et al*, 1993; Chou and Marousek, 1994).

Though the first isolate of HH-6 (GS) was a variant A strain, only HHV-6B strains have been definitively proven to cause disease (exanthem subitum in childhood, as described below). At the current time, it is unclear if HHV-6A causes any disease. Variant A strains of HHV-6 are mainly isolated from AIDS patients or persons with lymphoproliferative disorders, while HHV-6B strains are primarily recovered from patients with exanthem subitum. Investigations into the clinical consequences of HHV-6A and -6B infections are further hampered by the lack of reliable methods for distinguishing between antibody responses to HHV-6A and HHV-6B.

In vitro biologic properties

Human herpesvirus-6 exhibits predominantly CD4 T-lymphocyte tropism (Lusso *et al*, 1988; Takahashi *et al*, 1989). The HHV-6 receptor has not been identified, though it has been definitively proven to not be the CD4 molecule (Lusso *et al*, 1989). Natural killer cells can also be infected with HHV-6 (Lusso *et al*, 1993). Efficient HHV-6 replication in primary cell culture requires both prior mitogen activation of primary T cells, as provided by phytohemagglutinin (PHA) (Salahuddin *et al*, 1986; Lopez *et al*, 1988), and full progression of the cell cycle, as demonstrated by the requirement for interleukin-2 (IL-2) (Black *et al*, 1989; Frenkel *et al*, 1990). Both HHV-6A and HHV-6B strains have been adapted by serial passage to replicate in continuous cell lines. However, strains of HHV-6B replicate less readily in continuous cell lines than do those of HHV-6A (Black *et al*, 1989). Established human cell lines that support HHV-6A replication include those of T cell (including the T-lymphoblastoid cell line HSB-2), B cell, megakaryocyte, and glial cell lineages, as well as transformed cervical epithelial cells (Pellett and Black, 1996). Human herpesvirus-6B strains replicate well in the Molt-3 T cell line and the T cell lymphoma line MT-4 (Black *et al*, 1989; Ablashi *et al*, 1991).

In vitro evidence exists supporting the possible neurotropism of HHV-6. Human herpesvirus-6 is tropic for glioblastoma cell lines (Ablashi *et al*, 1988) and for embryonic glia (Tedder *et al*, 1987). The SF strain of HHV-6B replicates at low levels persistently in the neuroblastoma cell line SK-N-MC (Levy *et al*, 1990a). Furthermore, both HHV-6A and HHV-6B variants infect primary human fetal astrocytes in cell culture (He *et al*, 1996). However, no findings of HHV-6 infection of primary oligodendrocytes or primary neurons *in vitro* have been reported in the literature to date.

Epidemiology

Incidence and prevalence of infection

Seroprevalence studies of HHV-6 infection have demonstrated remarkable reproducibility from widely separated regions of the globe. With rare exceptions (Yadav *et al*, 1990), the prevalence of antibodies to HHV-6 is high among populations throughout the world. Epidemiologic studies in normal children have shown that the vast majority of primary HHV-6 infections occur within the first year of life (Yoshikawa *et al*, 1989; Farr *et al*, 1990). Human herpesvirus-6 IgG can be detected in more than 90% of neonates (Knowles and Gardner, 1988; Leach *et al*, 1992), reflecting both the high seroprevalence of HHV-6 among adults (Brown *et al*, 1988b; Ranger *et al*, 1991; Leach *et al*, 1992) and the active transport of HHV-6 IgG across the placenta (Yoshikawa *et al*, 1989). The prevalence of HHV-6 IgG drops significantly by 4–6 months of life as maternal antibodies decline, then increases through the third year of life and remains high into adulthood (Yoshikawa *et al*, 1989; Farr *et al*, 1990). More than 90% of immunocompetent children become infected with HHV-6 by 12 months of life (Leach *et al*, 1992), and virtually 100% acquire infection by 3 years of age (Brown *et al*, 1988b).

Recent data suggest that HHV-6 variants A and B may differ with respect to their geographical distributions (Kasolo *et al*, 1997). In the United States, variant B strains predominate in children (Dewhurst *et al*, 1993), whereas variant A strains have mostly been isolated from adult AIDS patients from Africa (Downing *et al*, 1987; Tedder *et al*, 1987). However, a recent study reported that HHV-6 variant A accounted for 44% of all HHV-6 isolates obtained from Zambian infants experiencing their first febrile episode, a finding dramatically different from the United States' profile of less than 3% (Kasolo *et al*, 1997). Additional investigations are needed to further define these possible geographic differences throughout the world.

Transmission

Though the mode(s) of transmission of HHV-6 has yet to be definitively proven, most children probably acquire infection through contact with the secretions of adult caretakers shedding the virus in saliva (Fox *et al*, 1990; Gopal *et al*, 1990; Harnett *et al*, 1990; Krueger *et al*, 1990; Levy *et al*, 1990b). Reports of isolation of HHV-6 from the saliva of healthy adults (Harnett *et al*, 1990; Levy *et al*, 1990b) and patients infected with HIV (Levy *et al*, 1990b) document salivary shedding in more than 85% of persons. Human herpesvirus-6 DNA can be detected by polymerase chain reaction (PCR) in saliva or PBMCs of 90% of healthy individuals (Cone *et al*, 1993). Using *in situ* hybridization and immunohistochemical staining, HHV-6 DNA and HHV-6 protein expression have been demonstrated in tissue from submandibular glands (Fox *et al*, 1990), parotid glands (Fox *et al*, 1990), salivary glands (Krueger *et al*, 1990), and bronchial glands (Krueger *et al*, 1990). Breast milk is unlikely to be an important source of early HHV-6 infection (Takahashi *et al*, 1988).

Pathogenesis

Analysis of pathologic specimens confirms that HHV-6 can infect a wide variety of cell and tissue types. During acute infection, viral replication occurs within lymphocytes, macrophages, histiocytes, endothelial cells and epithelial cells, with $CD4^+$ T lymphocytes being the predominant target cell type in the blood. The cell target of HHV-6 within the oropharynx during primary infection remains under investigation.

Direct evidence of persistence of HHV-6 infection is provided in comparisons of serial isolates obtained from an immunocompromised bone marrow transplant (BMT) recipient (Yoshikawa *et al*, 1992a). Specifically, restriction endonuclease analyses of pre- and post-BMT HHV-6 isolated from a child with leukemia were identical, suggesting persistence of HHV-6 infection or reactivation from latency. Additionally, HHV-6 DNA can be demonstrated by PCR in as many as 90% of PBMC specimens from healthy adults (Jarrett *et al*, 1990; Cone *et al*, 1993; Cuende *et al*, 1994), a frequency much higher than that detected by viral culture from normal adults. The relatively frequent detection of HHV-6 antigens in normal salivary glands (Fox *et al*, 1990; Krueger *et al*, 1990), lymph node tissue (Levine *et al*, 1992) and neurons and glial cells of the brain (Challoner *et al*, 1995) also suggest that the virus can persistently infect cells, or can establish latency and subsequently reactivate. Evidence for persistence of HHV-6 within the CNS is presented below (Kondo *et al*, 1993; Caserta *et al*, 1994).

Clinical manifestations of HHV-6

Primary infection in immunocompetent patients

Children The only disease for which HHV-6 has been shown definitively to be the causative agent is exanthem subitum (Yamanishi *et al*, 1988; Ueda *et al*, 1989; Yoshiyama *et al*, 1990). A common disease of childhood, exanthem subitum was first described in 1910 by Zahorsky, who termed the illness roseola infantum (Berenberg *et al*, 1949). In 1921, Veeder helped confirm this syndrome as a specific pathologic entity and suggested the name exanthem subitum (Berenberg *et al*, 1949). Illness is characterized by a constant or intermittent, high fever to 104–105°F for 3–5 days in a patient who appears relatively well. The patient may have mild catarrhal inflammation of the pharyngeal mucosa and otitis media. Coincident with or immediately following the return of the temperature to normal, a rose pink macular rash appears predominantly on the neck and trunk, although it may involve the proximal extremities, postaural regions and face. The rash is not pruritic, does not desquamate and fades after 24–48 h.

As early as 1950, a possible viral etiology of exanthem subitum was proposed (Kempe *et al*, 1950; Hellstrom and Vahlquist, 1951). It was not until 1988, however, that Yamanishi and colleagues definitively proved the viral pathogenesis of exanthem subitum by successfully isolating HHV-6 from the peripheral blood lymphocytes of four infants in the febrile phase of exanthem subitum and by documenting seroconversion to HHV-6 in these patients (Yamanishi *et al*, 1988). Subsequent studies have confirmed exanthem subitum as a manifestation of primary HHV-6 infection (Ueda *et al*, 1989; Yoshiyama *et al*, 1990). Variant B of HHV-6 is responsible for essentially all exanthem subitum-associated HHV-6 infections (Dewhurst *et al*, 1992; Dewhurst *et al*, 1993).

At least half of first episodes of fever during infancy are due to primary HHV-6 infection (Okada *et al*, 1993; Asano *et al*, 1994). Approximately 60% of primary HHV-6 infections in Japan are associated with a rash and result in the clinical diagnosis of exanthem subitum (Kusuhara *et al*, 1992), while only 9–40% of children in the United States with primary HHV-6 infection have an illness clinically compatible with exanthem subitum (Pruksananonda *et al*, 1992; Segondy *et al*, 1992; Portolani *et al*, 1993; Hall *et al*, 1994). The reasons for this geographic discrepancy are not fully understood. Atypical presentations of primary HHV-6 infection include exanthem subitum without fever (Asano *et al*, 1989a), exanthem subitum without rash (Suga *et al*, 1989; Pruksananonda *et al*, 1992; Segondy *et al*, 1992; Portolani *et al*, 1993) and exanthem subitum at extremely young ages (Kawaguchi *et al*, 1992).

Two recent large reports have prospectively evaluated primary HMV-6 infection in infants and young children (Pruksananonda *et al*, 1992; Hall *et*

al, 1994). In the first, 243 children 2 years of age or younger who had acute febrile illness were evaluated for HHV-6 infection (Pruksananonda *et al*, 1992). In 34 (14%) of the 243 subjects, HHV-6 was cultured from the peripheral blood obtained at the time of the initial visit. Children with HHV-6 viremia were irritable and had high fevers and inflammation of the tympanic membranes. Only three (9%) of the 34 patients with HHV-6 viremia presented with or subsequently developed disease clinically compatible with exanthem subitum. Subjects who were HHV-6 culture-positive had significantly lower average white blood cell counts at presentation when compared with HHV-6 culture-negative patients (8.9×10^9 versus 13.2×10^9 per liter, $P < 0.001$). All but one of the strains of HHV-6 cultured were variant B, with the single aberrant culture yielding virus that contained a mixture of A and B variant genotypes (Dewhurst *et al*, 1993).

In the second large prospective study, 2587 children under 3 years of age presenting to the emergency department with acute illnesses, both febrile and afebrile, were evaluated for HHV-6 infection by PBMC culture, serologic testing and PCR (Hall *et al*, 1994). This investigation found HHV-6B to be a major cause of emergency room (ER) visits, febrile seizures and hospitalizations. Specifically, 160 (9.7%) of the 1653 infants and young children with acute febrile illnesses had primary HHV-6 infection. While ranging in age from 2 weeks to 25 months, 23% of these patients were under 6 months of age. Human herpesvirus-6 infections accounted for 20% of all visits to the emergency department for febrile illnesses among children 6 to 12 months of age. Of the 160 infants and young children with acute HHV-6 infections, 21 (13%) required hospitalization.

Adults With virtually all children acquiring HHV-6 early in childhood, primary HHV-6 infection in adults is rarely documented or reported. When it occurs, however, such clinical conditions as lymphadenopathy (Niederman *et al*, 1988; Stettner-Gloning *et al*, 1992), heterophile-negative mononucleosis (Niederman *et al*, 1988; Steeper *et al*, 1990; Akashi *et al*, 1993) and hepatitis (Dubedat and Kappagoda, 1989; Sobue *et al*, 1991) can result. Human herpesvirus-6 also has been implicated in histiocytic necrotizing lymphadenitis (Kikuchi's disease) (Niederman *et al*, 1988) and sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease) (Levine *et al*, 1992), though its precise role in these conditions, if any, remains to be determined.

Central nervous system (CNS) involvement

Acute CNS disease Central nervous system manifestations of exanthem subitum were recognized long before the viral etiology was definitively proven to be HHV-6. Symptoms and signs of patients in these early reports included bulging fontanels, irritability, febrile seizures, meningoencephalitis and residual encephalopathy (Berenberg *et al*, 1949; Posson, 1949; Moller, 1956; Burnstine and Paine, 1959). Following the identification of HHV-6 as the causative agent of exanthem subitum, many additional instances of CNS involvement in primary HHV-6 infection have been reported (Ishiguro *et al*, 1990; Asano *et al*, 1991a; Huang *et al*, 1991; Asano *et al*, 1992; Segondy *et al*, 1992; Yoshikawa *et al*, 1992b; Kondo *et al*, 1993; Suga *et al*, 1993; Caserta *et al*, 1994; Hall *et al*, 1994; Ward and Gray, 1994; McCullers *et al*, 1995). Association of such neurologic findings with HHV-6 is suggested by the concomitant identification of virus by culture from PBMCs, by seroconversion, or, less commonly, by detection of the viral genome in CSF by PCR (Ishiguro *et al*, 1990; Asano *et al*, 1992; Yamanishi *et al*, 1992; Yoshikawa *et al*, 1992b; Caserta *et al*, 1994; Ward and Gray, 1994).

Association of HHV-6 with seizures

In the large series of 2587 infants and young children discussed above, the principal complication of primary HHV-6 infection was seizures (Hall *et al*, 1994). Of the 160 HHV-6-positive patients, 21 (13%) had seizures. In comparison, 9% of HHV-6-negative children under 24 months of age with febrile illnesses experienced seizures. While differences in these two comparative groups are not statistically significant (P=0.18), analysis of subpopulations of HHV-6-positive and HHV-6-negative patients did reveal significant findings: eight (36%) of 22 HHV-6-positive infants 12–15 months of age had seizures, as compared to 17 (13%) of 131 HHV-6-negative infants of the same age (P=0.001; odds ratio, 0.26; 95 percent confidence interval, 0.09 to 0.8) (Hall *et al*, 1994).

Of the 21 HHV-6-positive patients with seizures, all were febrile at the time of the convulsion. Nine (43%) of the 21 experienced seizures late in the febrile course (on days 2–9), and in three children the seizures were prolonged and recurred within three days. Overall, HHV-6 accounted for 31% of febrile seizures in children under two years of age (Hall *et al*, 1994).

The complete lack of an inflammatory response in the cerebrospinal fluid (CSF) was notable in all HHV-6-positive patients, both with and without seizures, on whom a lumbar puncture was performed. All patients on whom nontraumatic lumbar punctures were obtained had normal cerebrospinal fluid (CSF) indices, including white blood cell counts, glucose and protein. Human herpesvirus-6 DNA was detected by PCR in seven of 29 samples tested, including two of seven samples from children with seizures. No CSF sample grew HHV-6 in viral culture (Hall *et al*, 1994).

In another report, the role of HHV-6 in febrile seizures was evaluated in 42 children (Barone *et al*, 1995). All had first-time febrile convulsions. A total of 11 (26%) of the 42 children had virologic or serologic evidence of acute HHV-6 infection, a finding that is comparable to the 31% discussed above.

Detection of HHV-6 DNA in the CSF of children with repeated febrile seizures and after primary infection suggests that viral persistence and reactivation within the CNS can occur (Kondo *et al*, 1993; Caserta *et al*, 1994). In a study of 172 children under 3 years of age, the neuroinvasiveness of HHV-6 (defined as the presence of viral DNA in the CSF) was documented in seven (23%) of 30 children with acute HHV-6 infection documented by viremia and seroconversion and in 65 (46%) of the 142 children with past HHV-6 infection (Caserta *et al*, 1994). These data suggest that the CNS may be a reservoir of latent or persistent HHV-6 infection. Another report documented HHV-6 DNA in the CSF of seven (70%) of 10 children with exanthem subitum (Yamanishi *et al*, 1992). However, in this study patients with exanthem subitum were chosen for inclusion based upon the presence of such neurologic symptoms as febrile convulsions, vomiting, or bulging of the anterior fontanel during the febrile phase of illness, thus introducing the possibility of overstating the actual frequency by sampling bias. Nevertheless, one can conclude that neuroinvasion with primary HHV-6 infection occurs frequently, with the risk ranging from 23% (Caserta *et al*, 1994) to 70% (Yamanishi *et al*, 1992).

The presence of the HHV-6 DNA in the central nervous system (CNS) at the time of the initial HHV-6 infection has been reported to increase the risk of recurrence of febrile convulsions (Kondo *et al*, 1993). Other reports, however, have found that children whose first febrile seizure is caused by primary HHV-6 infection do not demonstrate an increased risk for recurrent seizures when compared with children whose first febrile seizures are from other etiologies (Jee *et al*, 1998). Additional investigations into the neurotropic potential of HHV-6 during primary infection and the possible consequences of such neuroinvasion are required to address this discrepancy.

Association of HHV-6 with meningitis/encephalitis

Human herpesvirus-6 has been implicated as a cause of meningitis and encephalitis in children (Ishiguro *et al*, 1990; Huang *et al*, 1991; Asano *et al*, 1992; Yoshikawa *et al*, 1992b; Knox *et al*, 1995; McCullers *et al*, 1995; Yanagihara *et al*, 1995) and adults (Sloots *et al*, 1993; Drobyski *et al*, 1994; McCullers *et al*, 1995). Most descriptions in the literature are case reports. In one large series, though, the variant B of HHV-6 was associated with focal encephalitis (McCullers *et al*, 1995). In this report, CSF from 138 patients with clinical or laboratory evidence of encephalitis was analyzed retrospectively by PCR. Human herpesvirus-6 DNA was detected in the CSF of nine (7%) of the 138 patients and no other proven etiology for disease existed for any of the patients evaluated. No significant differences could be demonstrated in clinical presentation, laboratory findings, or neurodiagnostic imaging results between the nine patients with confirmed HHV-6 infection and the 129 patients without evidence of HHV-6 infection. Cerebrospinal fluid indices from the two groups were similar (HHV-6-positive patients: average WBC count of 81/mm^3, average protein concentration of 57 mg/dL; HHV-6-negative patients: average WBC count of 184 cells/mm^3, average protein concentration of 86 mg/dL). Five of the nine patients were under 18 years of age. Four of the nine patients had abnormal neuroimaging studies, with two demonstrating focal lesions (parietal lobe and fronto-temporal area). Electroencephalograms were performed in eight of the nine patients and were abnormal in seven of them. Four of the nine patients recovered fully; three had mild to moderate neurologic impairment; one patient had a persistent, severe, focal seizure disorder; and one patient died of complications of his neurologic disease (McCullers *et al*, 1995).

In another large investigation of 50 patients with meningitis or encephalitis, 10 (20%) had increased intrathecal anti-HHV-6 early antigen IgM or IgG (Patnaik and Peter, 1995). None of the 50 control patients had intrathecal HHV-6 antibody. For the ten HHV-6-positive patients, none had elevations of intrathecal antibody levels to 26 other viruses, including herpes simplex virus, Epstein-Barr virus, CMV, varicella-zoster virus, arboviruses, echoviruses, measles virus and mumps virus (Patnaik and Peter, 1995).

Human herpesvirus-6 has been demonstrated by immunohistochemical staining of postmortem brain tissue from a child with HIV who dies of fulminant encephalitis (Knox *et al*, 1995) and at autopsy of an adult bone marrow transplant patient with encephalitis (Yanagihara *et al*, 1995). In the former case, HHV-6-infected cells were demonstrated in the gray matter, mostly in astrocytes but also in oligodendrocytes and neurons (Knox *et al*, 1995). In the latter case, HHV-6-infected cells were demonstrated in neurons of affected gray matter and in astrocytes from areas of white matter lesions, where areas of demyelination were observed (Yanagihara *et al*, 1995). Figure 1 presents a single photon emission computed tomography (SPECT) from the latter patient, showing a hypoperfused area of the left hemisphere (Yanagihara *et al*, 1995).

Human herpesvirus-6 was present in high amounts in multifocal demyelinating white matter lesions of an immunocompetent young woman with fulminant encephalomyelitis (Novoa *et al*, 1997). Human herpesvirus-6 was identified by PCR and

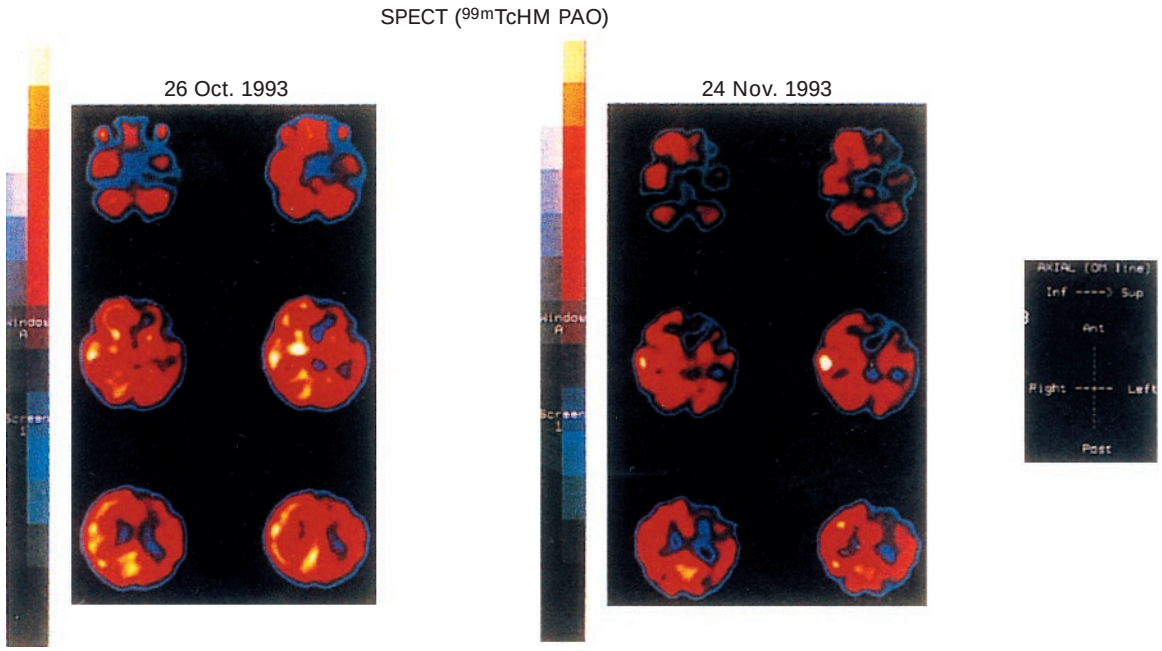

Figure 1. ^{99M}Tc-HM-PAO SPECT in a patient infected with human herpesvirus-6, showing hyperperfused area of the left hemisphere. From Yanagihara *et al*, 1995.

immunohistochemistry of both brain biopsy (performed 3 weeks into the course of illness) and brain tissue at autopsy. Ultrastructural study of brain tissue revealed viral particles within oligodendrocytes.

Finally, immunohistochemical staining of brain tissue samples from an immunocompetent 27-year-old woman who died of a subacute demyelinating leukoencephalitis demonstrated widespread active infection with HHV-6 (Carrigan *et al*, 1996). In all of the postmortem specimens evaluated, the HHV-6-infected cells were located within or immediately adjacent to areas of active demyelination and no infected cells were observed in tissue samples free of pathologic changes or in old, highly gliotic plaques. Prior to the retrospective postmortem analysis for HHV-6, the woman had been clinically and histopathologically diagnosed as having acute multiple sclerosis. Discussion of additional evidence suggesting that HHV-6 may play a role in the pathophysiology of multiple sclerosis is presented below.

Detection of HHV-6 in absence of acute CNS disease

Reports vary on the frequency with which HHV-6 can be detected in the CNS of immunocompetent and immunocompromised patients who do not have acute encephalitis or primary HHV-6 infection. Most studies have detected HHV-6 DNA in the brains of such patients with high frequency. In one report, HHV-6 DNA was detected by PCR in 11 (85%) of 13 brain tissue specimens obtained within 18 h of death from 13 adult immunocompetent patients without clinical signs of viral disease (Luppi *et al*, 1994). Variant analysis was performed in five of the 11 PCR-positive patients and all were determined to be HHV-6 variant A (Luppi *et al*, 1994).

Additional studies have evaluated possible HHV-6 involvement in the CNS of persons with advanced HIV infection (Achim *et al*, 1994; Knox and Carrigan, 1995; Luppi *et al*, 1995; Saito *et al*, 1995). In a study of postmortem pediatric brain tissues from children with progressive AIDS encephalopathy, HHV-6 nucleic acids were detected using *in situ* hybridization in four (80%) of five patients (Saito *et al*, 1995). Human herpesvirus-6 DNA was detected primarily in oligodendrocytes of the white matter and less frequently in astrocytes, macrophages, microglia and neurons. The HHV-6-positive cells did not show evidence of productive HHV-6 infection, as evidenced by a lack of detection of HHV-6 protein products (Saito *et al*, 1995).

In an investigation of six adults who died of AIDS, four had demyelinating lesions in brain tissue on postmortem examination (Knox and Carrigan, 1995). Immunohistochemical staining demonstrated HHV-6-infected cells in areas of demyelination but not in histologically normal regions of the brains, suggesting active HHV-6 infection in these areas. Three of the four patients had only a few foci of demyelination, while the fourth had diffuse demyelination. The neuropatho-

logic findings were similar to those from the bone marrow transplant recipient described above (Yanagihara *et al*, 1995).

In a third study, investigators performed PCR for HHV-6 using brain tissue specimens obtained at necropsy from immunocompetent adult subjects and patients who died of AIDS (Luppi *et al*, 1995). Human herpesvirus-6-specific sequences were identified in six (67%) of nine brain samples from immunocompetent subjects and in four (57%) of seven brain samples from AIDS patients. The HHV-6 DNA was detected both in gray matter (frontal cortex and basal ganglia) and in periventricular white matter.

While at least one report documents a much lower frequency of HHV-6 detection in the brain of patients dying of advanced HIV infection (Achim *et al*, 1994), findings from these studies, taken as a whole, affirm the suggestion that HHV-6 is neurotropic and that it establishes latency within the CNS. Additional investigation is required, however, to fully elucidate what role, if any, HHV-6 plays in AIDS encephalopathy.

Human herpesvirus-6 can also cause CNS disease at the level of the spinal cord. The case of an elderly woman with chronic myelopathy with progressive spastic paraparesis has been reported (MacKenzie *et al*, 1995). At autopsy, widespread demyelination, axonal loss, chronic inflammation and gliosis were demonstrated. Immunohistochemical staining documented the presence of HHV-6 antigens predominantly in astrocytes in regions of white matter degeneration. Human herpesvirus-6 DNA was detected in the abnormal spinal cord tissues as well, suggesting an association between HHV-6 and chronic myelopathy (MacKenzie *et al*, 1995).

Multiple sclerosis

Over the years, many microbiologic agents have been proposed as possibly being associated multiple sclerosis (MS). Under scientific scrutiny, each subsequently has been discredited as a causal agent. Thus, recent reports that have suggested that HHV-6 may play a role in the pathogenesis of MS must be approached with caution. Nevertheless, preliminary findings are intriguing, as described below.

Patients with MS have been shown to have higher titers of HHV-6 serum antibody as compared to control patients (Sola *et al*, 1993; Wilborn *et al*, 1994). In a recent report, HHV-6 IgM responses were detected in patients with relapsing-remitting MS (RRMS), as compared with patients with chronic progressive MS (CPMS), patients with other neurologic disease (OND), patients with other autoimmune disease (OID) and normal controls (Soldan *et al*, 1997). Furthermore, HHV-6 DNA was detected in serum of 15 (30%) of 50 patients with MS, but in 0 (0%) of 47 non-MS patients (18 healthy controls, 19 patients with OND and ten patients with OID) ($P<0.0001$) (Soldan *et al*, 1997). Detection of HHV-6 DNA in cell-free plasma specimens suggests the presence of active HHV-6 replications *in vivo* (Huang *et al*, 1992; Secchiero *et al*, 1995).

Human herpesvirus-6 DNA has been detected in the CSF of MS patients (Luppi *et al*, 1994; Liedtke *et al*, 1995). In one patient with MS who acutely developed inflammation of the CNS and was diagnosed with encephalitis, HHV-6 DNA was detected in the CSF by PCR during the acute episode, whereas it had not been present in CSF collected prior to the encephalitis (Merelli *et al*, 1996).

As noted above, HHV-6 appears to be present in a majority of adult brains (Luppi *et al*, 1994), with HHV-6 antigen detectable in neurons and glial cells (Challoner *et al*, 1995). However, in a study of the brains of MS patients, HHV-6 antigen was also found in nuclei of oligodendrocytes associated with the MS plaques (Challoner *et al*, 1995). This investigation utilized representational difference analysis (RDA) to detect a DNA sequence in brain tissue of MS patients that was shown to be 99.4% identical to the major DNA binding protein gene of HHV-6. While examination of 86 brain specimens from both MS patients and controls demonstrated that HHV-6 was present in >70% of specimens, nuclear staining of oligodendrocytes with two monoclonal antibodies against HHV-6 was observed in MS cases only but not in controls. Furthermore, in MS cases this staining was observed around plaques more frequently than in uninvolved white matter. Prominent cytoplasmic staining of neurons occurred in gray matter around plaques in MS patients, although neurons expressing HHV-6 were also found in certain controls (Challoner *et al*, 1995).

Taken together, the results of these studies are titillating. However, other reports have failed to detect serologic (Nielsen *et al*, 1997) or virologic (Sanders *et al*, 1996; Martin *et al*, 1997; Merelli *et al*, 1997) evidence of an association between HHV-6 and MS. As such, definitive proof of a role for HHV-6 in the pathogenesis of MS awaits further study.

Infection in immunocompromised patients

Reactivation of HHV-6 from latency can occur between 2 weeks and 3 months following organ transplantation. The precise frequency of such episodes of viral reactivation is contingent upon several things, including the type of transplant and, likely, the degree of immunosuppression. Human herpesvirus-6 has been implicated as a cause of disease in renal transplant recipients (Asano *et al*, 1989b; Morris *et al*, 1989; Gudnason *et al*, 1991; Yoshikawa *et al*, 1992c), bone marrow transplant recipients (Asano *et al*, 1991b; Yoshikawa *et al*, 1991; Knox and Carrigan, 1992; Rosenfeld *et al*, 1995; Knox and Carrigan, 1996b) and liver transplant recipients (Ward *et al*, 1989; Sutherland *et al*, 1991; Singh *et al*, 1995). Neurologic disorders

attributable to HHV-6 have been reported in bone marrow (Yanagihara *et al*, 1995) and liver transplant patients (Ward *et al*, 1989; Sutherland *et al*, 1991; Singh *et al*, 1995) and include seizures, encephalopathy and encephalitis.

Investigations of HHV-6 in HIV infected patients have yielded conflicting results. Some studies have suggested a role for HHV-6 co-infection in the progression of HIV disease (Chen *et al*, 1992; Corbellino *et al*, 1993; Fairfax *et al*, 1994; Knox and Carrigan, 1994; Blázquez *et al*, 1995; Clark *et al*, 1996; Knox and Carrigan, 1996a), while others have found no correlation between HHV-6 infection and the course of HIV infection (Brown *et al*, 1988a; Fox *et al*, 1988; Spira *et al*, 1990; Essers *et al*, 1991; Gautheret *et al*, 1995). Possible CNS involvement by HHV-6 in HIV infected persons is discussed above.

Conclusion

The rapid pace of discovery following the identification of HHV-6 just over a decade ago is quite remarkable. The neuroinvasive potential of HHV-6 has been demonstrated in both immunocompetent and immunocompromised individuals. Furthermore, HHV-6 has been associated clinically with a wide array of neurologic manifestations in these patients. The knowledge amassed to date detailing the CNS manifestations of HHV-6 largely has been built on small case reports and retrospective studies, which have prepared the groundwork for larger prospective investigations. Controlled investigations of antiviral agents with activity against HHV-6 are now warranted to define which patients will benefit from antiviral administration, both as active and suppressive therapy. In the conduct of these studies, additional knowledge of the natural history of HHV-6 in the CNS will be acquired.

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