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The effect of human herpesvirus-6 (HHV-6) on cultured human neural cells: oligodendrocytes and microglia

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Human herpesvirus-6 (HHV-6) is a betaherpesvirus that has been frequently associated with pediatric encephalitis. In 1995 Challoner *et al* reported that HHV-6 variant B (HHV-6B) was linked to multiple sclerosis (MS) due to the presence of viral DNA and antigen in the oligodendrocytes surrounding MS plaques. These findings led us to examine HHV-6B's *in vitro* tropism for primary neural cells. HIV-6B mediated cell-to-cell fusion in cultured adult oligodendroglia. Infection of oligodendrocytes was further confirmed by transmission electron microscopy (EM), which showed the presence of intracellular HHV-6 particles, and by PCR for HHV-6 DNA. However, the release of infectious virus was low or undetectable in multiple experiments. Microglia were also susceptible to infection by HHV-6B, as demonstrated by an antigen capture assay. We did not detect infection of a differentiated neuronal cell line (NT2D). Our findings suggest that HHV-6B infection of oligodendrocytes and/or microglia could potentially play a role in neuropathogenesis.

Keywords: HHV-6; oligodendrocytes; multiple sclerosis; microglia

Introduction

In 1986 Salahuddin and colleagues isolated a novel herpesvirus from the T-lymphocytes of patients with the Acquired Immunodeficiency Syndrome (AIDS) (Salahuddin *et al*, 1986). Subsequent analysis showed that this virus, which contains a linear double-stranded DNA genome approximately 160–170 kb in size, is a herpesvirus, now named human herpesvirus-6 (HHV-6), and is divided into two distinct variant groups, HHV-6A and HHV-6B (reviewed in Inoue *et al*, 1994). Genomic analysis comparing HHV-6 with all other herpesviruses, places HHV-6 within the betaherpesviruses, as it is more homologous to human cytomegalovirus (HCMV), than to either the alphaherpesviruses (herpes simplex viruses 1 and 2 and varicella-zoster virus) or to the gammaherpesviruses (Gompels *et al*, 1995; Moore *et al*, 1996).

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HHV-6 is a ubiquitous virus, as there is serological evidence of prior infection in 90% of children at 1 year of age, and 100% at 3–5 years (Pellet *et al*, 1996). In infants, HHV-6 infection frequently causes exanthem subitum or non-specific febrile illnesses (Yamanishi *et al*, 1988), and HHV-6B also appears to be neurotropic as it is thought to be responsible for some cases of encephalitis and recurrent seizures (Hall *et al*, 1994; Huang *et al*, 1991; Irving *et al*, 1990; Ishiguro *et al*, 1990). HHV-6's neurotropism was further highlighted when HHV-6 DNA was detected in the CSF of infants and children with neurological complications during exanthem subitum (Asano *et al*, 1992; Kondo *et al*, 1993; Suga *et al*, 1993; Yoshikawa *et al*, 1992; Caserta *et al*, 1994) and infectious virus was recovered from the CSF during encephalitis (Moschettini *et al*, 1996). Focal encephalitis in adults has also been reported to be associated with HHV-6 infection (McCullers *et al*, 1995).

Autopsy studies have shown HHV-6 antigen and genomic sequences in the CNS tissue of HIV-infected infants who developed encephalitis during acute systemic HHV-6 infection (Knox and Carrigan, 1995; Saito *et al*, 1995). Furthermore, there are

a number of anecdotal reports associating HHV-6 with myelopathy (Mackenzie *et al*, 1995), subacute leukoencephalopathy resembling multiple sclerosis (Carrigan *et al*, 1996), fatal leukoencephalopathy in immunosuppressed adults (Knox *et al*, 1995b) and fatal encephalitis in a bone-marrow transplant patient (Dorbyski *et al*, 1994). In those cases where the CNS tissue has been examined, the predominant but not exclusive cell types infected are glia, and some reports have emphasized the role of oligodendrocytes in viral replication (Challoner *et al*, 1995; Saito *et al*, 1995). However, the correlation between the presence of HHV-6 in the CNS and neurologic disease is confounded by evidence that HHV-6 genomic sequences may be amplified (with the polymerase chain reaction) from normal brain (Challoner *et al*, 1995; Luppi *et al*, 1994) or cerebrospinal fluid (Liedtke *et al*, 1995). This has limited the value of epidemiological studies.

Evidence for a potential role for HHV-6 in multiple sclerosis (MS) arose from the studies of Challoner *et al* who used representational difference analysis (RDA) to identify sequences that were unique to the brains of MS patients in comparison with peripheral blood lymphocytes obtained from normal controls (Challoner *et al*, 1995). MS brain tissue yielded an HHV-6 sequence, which led them to examine the brains of MS patients and controls with other diseases for the presence of HHV-6 DNA and antigen. As in other studies, HHV-6 DNA was found in control brains, but immunocytochemistry with monoclonal antibodies against two HHV-6 antigens showed that these antigens were specifically present in the nuclei of oligodendrocytes in those brain sections surrounding MS plaques, but not in the oligodendrocytes of control brains. Additionally, Wilborn *et al* (1994), have recently demonstrated that MS patients had significantly higher HHV-6 antibody titers than individuals with other neurological diseases. These studies are tempered by a long history of findings of nonspecific rises in the titers against various viruses in patients with MS (reviewed in Cook *et al*, 1996). Recently, Soldan *et al* demonstrated individuals with relapsing remitting MS had detectable HHV-6 DNA in serum (Soldan *et al*, 1997).

An implicit assumption in these studies is that HHV-6 can infect a wide variety of neural cells, and from the standpoint of MS, specifically oligodendrocytes. Only one group has looked at *in vitro* HHV-6 infection of primary human neural cells (He *et al*, 1996); that study was limited to human fetal astrocytes. Nevertheless, infection of the fetal astrocytes with both HHV-6 A and B strains resulted in viral prodution and cytopathology, chiefly syncytia formation.

Further definition of the cellular tropism of HHV-6 would help determine whether the hypothesis that this virus could play a role in MS pathogenesis is at all practicable, and would clarify the role of glia in its neuro-tropism. To this aim we have attempted to infect primary adult human microglia and oligodendrocytes, and a differentiated neuronal cell line, N-Tera2 (NT2D), which is a surrogate for human neu-rons. Our results indicate that HHV-6 infection can infect and mediate gross cytopathicity of the oligo-dendrocytes and microglia, but not the NT2D cells.

Results

To determine the effect of exposure of different CNS cell types to HHV-6, we cultured oligodendrocytes, microglia, and the NT2D cells in trans-well plates and exposed them to HHV-6B_{Z29}-infected Molt-3 cells for 3–7 days. Seven to 12 days after infection, oligodendrocyte cultures showed signs of overt cytopathology, such as cell loss, decreased size, and dramatically increased cell-to-cell fusion. Figure 1A shows syncytia formation in oligodendrocytes co-cultured with infected Molt-3 cells,

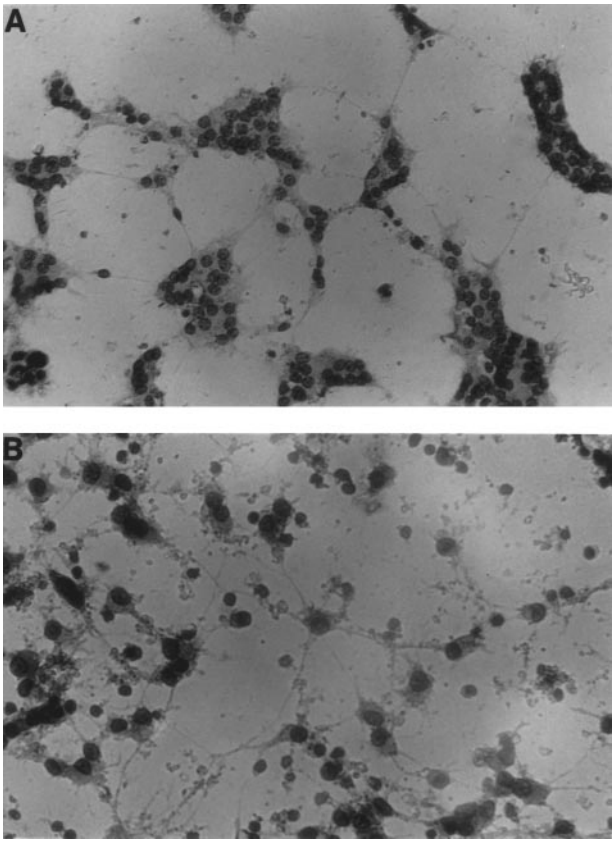

Figure 1 HHV-6 cytopathology in oligodendrocytes. Oligodendrocyte cultures were exposed to HHV-6 infected Molt-3 cells as described in Materials and methods, and observed for cytopathologic change 12 days after infection. (**A**) Oligodendrocytes exposed to infected Molt-3 cells show syncytia of between five and over 20 nuclei. (**B**) Oligodendrocytes exposed to control uninfected-Molt-3 cells show characteristic filamentous processes, but no syncytia formation.

compared with a culture exposed to uninfected Molt-3 cells (Figure 1B) which shows the typical filamentous process morphology of oligodendrocytes.

Although the background level of multinucleated giant cell formation is much greater in uninfected microglia than in oligodendrocytes, there was nevertheless a marked difference between the microglial cultures exposed to HHV-6B$_{Z29}$ (Figure 2A) in comparison with those exposed to uninfected Molt-3 cells (Figure 2B). The HHV-6 infected microglial cultures showed cell clumping and syncytia formation.

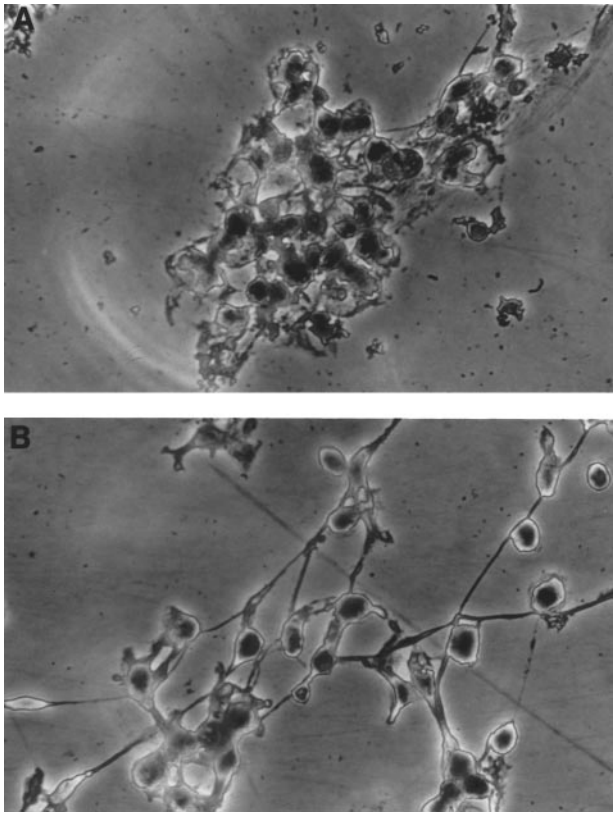

Figure 2 HHV-6 cytopathology in microglial cultures. Microglial cultures were exposed to (**A**) HHV-6 infected Molt-3 cells or (**b**) uninfected Molt-3 cells as described in Materials and methods, and examined for cytopathological change 12 days after removal of the Molt-3 cells. In comparison with the uninfected cells in (**B**), the HHV-6 infected cells demonstrated clumping, syncytia formation above background and cell death.

Table 1 Cytopathology in HHV-6 infected cells

Infection[a]	*Oligodendrocytes*	*Microglia*
1) Mock	0	1
2) Molt-3	0.5	1
3) Molt-3/HHV-6	1.5	3.5

[a]Duplicate cultures were exposed to (1) culture media (Mock), (2) uninfected Molt-3 cells, or (3) HHV-6-infected Molt-3 cells and scored for gross cytopathology (primarily syncytia formation) by two investigators unaware of the culture conditions (0=healthy culture; $1-2 \leqslant 50\%$ of cells fused $3-4 \geqslant 50\%$ of cells fused) and are represented as averages for one experiment. This experiment was repeated $3\times$ for oligodendrocytes and $2\times$ for microglial cultures.

To confirm the specificity of this cytopathology, two individuals scored the Diff-Quik-stained cultures without prior knowledge of the experimental pattern. The infected cultures were easily distinguishable, whereas cells exposed to uninfected Molt-3 cells could not be distinguished from normal cultures (Table 1). We did not detect any evidence of cytopathology in the NT2D cells, but these grow in clumps and subtle changes would have been missed.

To confirm that the syncytia in the oligodendrocyte cultures were composed of oligodendrocytes, the nuclei of the multinucleated cells were stained with Hoescht dye and an antibody against the oligodendrocyte cell surface marker (GalCer). We determined that the fused cells in the oligodendrocyte cultures were all oligodendrocytes [data not shown]. The cells composing the syncytia in the microgial cultures were identified as microglia based on their ability to specifically endocytose diI-Ac-LDL (Strizki *et al*, 1996).

We then determined whether oligodendrocytes, microglia, or NT2D could support full viral replication using several different assays. First, EM conclusively identified viral particles within the cytoplasm of the cultured oligodendrocytes (Figure 3A, arrowheads). The virions ranged in size from 100–200 nm, and were morphologically consistent with previous descriptions of HHV-6 (Biberfeld *et al*, 1987) and with particles in infected Molt-3 cells (Figure 3B). Virus particles were seen within the cytoplasm of cells in the microglial cultures. Although there were no distinctive features indicating that these cells are microglia, the cultures consisted of purified microglia, with generally fewer than 2–4% contaminating astrocytes. The NT2D cells did not produce any viral antigen or infectious virus, and EM was negative for viral particles [data not shown].

Second, to determine whether virus was released, we assayed the cell culture supernatants for HHV-6 antigens (gp116/64/54) utilizing an antigen capture ELISA. Both oligodendrocytes and microglia were positive at days 12 and 20 after infection (Table 2), whereas the NT2D cells were consistently negative [data not shown]. By diluting the supernatants we obtained a rough approximation of the relative levels of HHV-6 antigen in each cell type and the HHV-6B-infected Molt-3 cultures were at least tenfold more productive than the neural cells. To determine if the presence of HHV-6 antigen in the culture supernatant represented infectious virus, we inoculated Molt-3 cells with the infected cell-culture supernatants of neural cultures at various intervals from days 2–14 post-infection. There was

no evidence of productive viral transmission, as determined by the absence of syncytia, which are prominent when these cells are infected by HHV-6.

To assay for HHV-6 DNA in the infected cultures, oligodendrocytes and microglial cultures were exposed to HHV-$6B_{Z29}$ for 4 days, and treated as described in Materials and methods. On day 15 after infection, HHV-6 DNA was amplified using PCR from the DNA isolated from the oligodendrocyte cultures (Figure 4). No HHV-6 DNA was detected in the microglia (Figure 4), although the ELISA results were unquestionably positive. This may reflect an insufficient amount of DNA, as discussed below. SK-N-MC cells, derived from a peripheral neuroblastoma were used as a negative control, and there was no HHV-6 specific amplicon in the PCR reactions of DNA extracted from these cells after exposure to HHV-6 in parallel with the oligodendrocyte infection. As expected, we detected HHV-6 DNA in the cell free supernatant from Molt-3 cells infected with HHV-$6B_{Z29}$ and in J-Jahn cells infected with HHV-$6A_{GS}$ (Figure 4). PCR for β-globin was

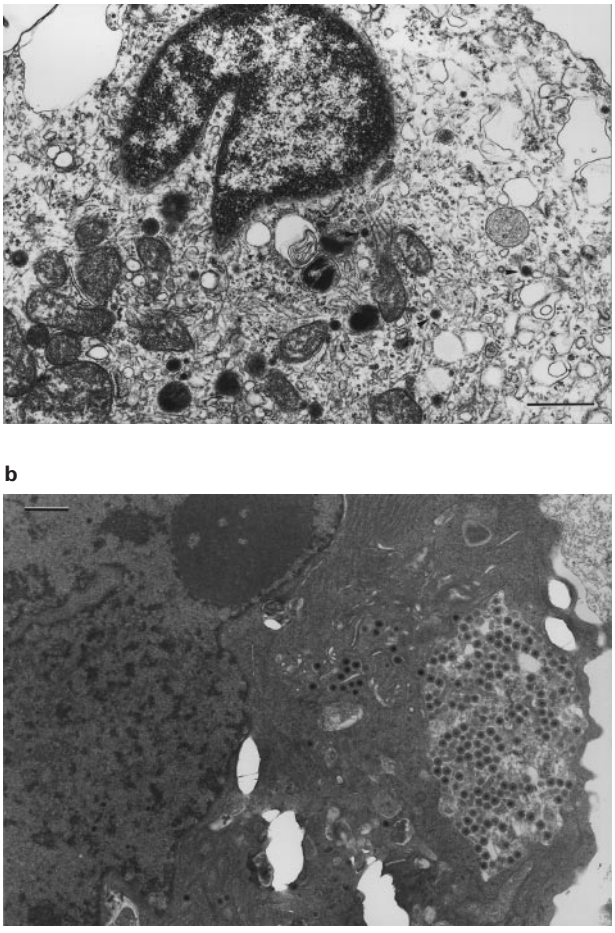

Figure 3 Electron microscopy of HHV-6 infected neural cells. (**A**) Electron microscopy of a cultured oligodendrocyte containing several dense-core viral particles (arrowheads) consistent in size and morphology with HHV-6 virions. Identifications of these cells as oligodendrocytes (in addition to their presence in a purified culture) was based on a combination of positive and negative findings, including nuclear heterochromasia with dense chromatin, a prominent Golgi apparatus, abundant microtubules, paucity of intermediate glial filaments, and the presence of occasional cytoplasmic 'dense bodies' (Peters *et al*, 1970). The bar represents 1 μm. (**B**) Electron micrograph of a Molt-3 cell infected with HHV-6. A continuously infected culture was established, fixed as indicated in Materials and methods and electron micrographs performed to identify viral particles. Note numerous intracytoplasmic particles measuring approximately 200 nm in diameter. The bar represents 1 μm.

Table 2 Production of HHV-6 antigen by oligodendrocytes and microglia

Culture	*Day 12 supernatant*	*Day 20 supernatant*
Oligodendrocytes	0.240[a]	0.135
Uninfected Oligo.	0.099	
Microglia	0.413	0.792
Uninfected Microglia	0.110	0.097

[a]Optical Density at 450 nm. Undiluted supernatants from HHV-6-infected cultures or uninfected microglial cultures were collected and assayed with an antigen capture ELISA that detects the HHV-6 antigens (gp116/64/54). Oligodendrocytes and microglia had detectable levels of HHV-6 antigen at days 12 and 20 post-infection. The undiluted supernatant from HHV-6 infected Molt-3 cultures used as the source for the HHV-6 gave an off-scale reading (>3,000); a 1:10 dilution of this supernatant had absorbance values between 1,000 and 2,000. Positive results were found in 1 of 2 experiments with oligodendrocytes and two of two experiments with microglia.

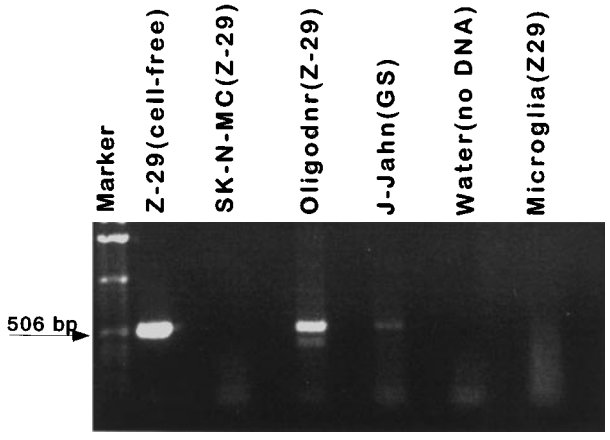

Figure 4 Detection of HHV-6 infection of oligodendrocytes by PCR. Oligodendrocytes and microglial cultures were exposed to HHV-6_{Z29} for 4 days, as described in Materials and methods. On day 15 after infection, PCR was used to amplify HHV-6 DNA from the olgiodendrocyte and microglial cultures. A specific 520 bp fragment was seen in the oligodendrocytes but not in the DNA extracted from the microglia. Negative controls were: SK-N-MC neuroblastoma cells exposed to HHV-6, and a PCR reaction performed with no DNA template. Positive controls were: Cell free supernatant from Molt-3 cells chronically infected with HHV-$6B_{Z29}$, and J-Jahn cells infected with HHV-$6A_{GS}$.

performed in all cells to determine that DNA had been isolated from all of the samples. However, only a weak PCR signal was detected in the DNA isolated from the microglia exposed to HHV-$6B_{Z29}$, which might explain the negative result in these cells.

To compare the cytotoxicity seen with HHV-6 and that produced by HSV-1, we infected oligodendrocyte, microglial and NT2D cultures with cell-free HSV-1_{17+} for 4 h at multiplicities of infection of 0.5 and 0.05 pfu/cell. Microglia (Figure 5B) and HeLa cells demonstrated massive cytopathology at 36 h, while oligodendrocytes (Figure 5A) and NT2D (data not shown) demonstrated only a limited amount of cytopathology 6 days after infection. The cytopathological changes seen with HSV-1 consisted primarily of cell death and dropout, rather than the cell-to-cell fusion observed in the oligodendrocytes and microglia exposed to HHV-6.

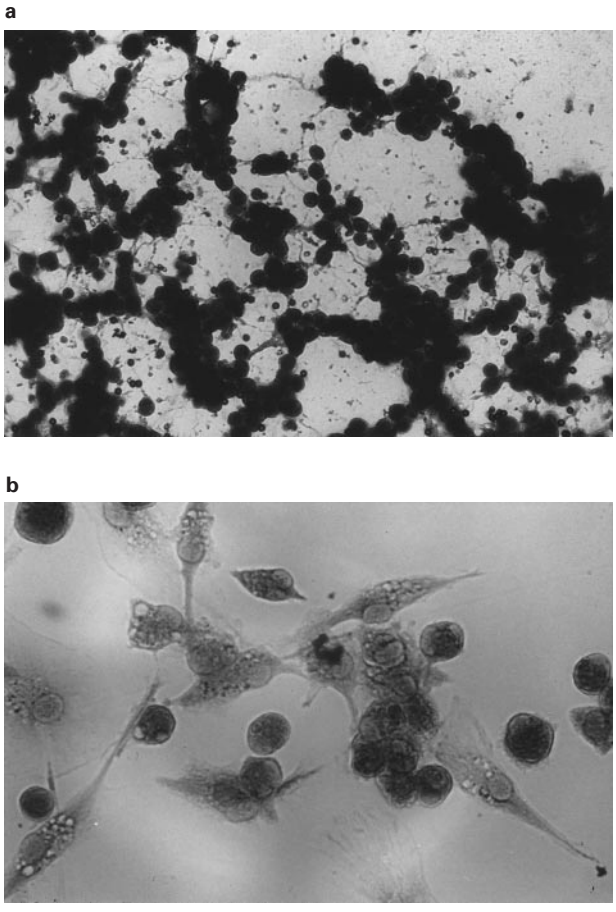

Figure 5 HSV-1 cytopathology in oligodendrocytes and microglia. Oligodendrocyte and microglial cultures were infected with cell-free free HSV-1_{17+} for 4 h at multiplicities of infection of 0.5 and 0.05 p.f.u./cell as described in Materials and methods, and observed for cytopathologic every 12 h after infection. (**A**) Oligodendrocytes infected at 0.5 p.f.u./cell showed limited cytopathology after 6 days in culture. (**B**) Microglia infected at 0.05 p.f.u./cell had massive cytopathology at just 36 h.

We concluded from these findings that oligodendrocytic cytopathology seen in HHV-6 infection was not a characteristic of all herpesviruses.

Discussion

Challoner and colleagues' recent findings of a potential relationship between HHV-6 and MS plaques led us to examine this virus' tropism for oligodendrocytes, microglia, and NT2D cells. We found that HHV-6 can infect and induce cytopathicity in cultured adult oligodendroglia. While we only detected limited viral production with an antigen capture assay, we were able to identify viral particles within oligodendrocytes using EM. The low level of viral production in oligodendrocytes is not surprising, since HHV-6 rarely replicates to a titer of more than 10^{3-4} $TCID_{50}$/ml in permissive cells (Pellett *et al*, 1992). Furthermore, it is unclear whether the small amount of virus produced is infectious, since we were not able to recover infectious HHV-6 from the supernatant of infected oligodendrocytes.

However, despite the apparently low levels of HHV-6 replication in oligodendrocytes and microglia, exposure to virus-infected Molt-3 cells resulted in marked cell-to-cell fusion. It is possible that this extensive cytopathicity was mediated entirely by virus released by the Molt-3 cells, and not by virions produced endogenously by infected oligodendrocytes. Nevertheless, fusion mediated by exogenous viruses, often termed 'fusion-from-without', is widely accepted to be dependent on the presence of appropriate cellular receptors on the target cells (White *et al*, 1981), which is consistent with the susceptibility of oligodendrocytes to infection by HHV-6 shown by PCR. Future studies directed at elucidating the mechanism of this cell fusion are clearly needed.

Previous experiments have demonstrated that HHV-6 replicates in CD4 positive lymphocytes and in a number of other cell types, including cell lines derived from central nervous system tumors (Ablashi *et al*, 1987) and primary human fetal astrocytes (He *et al*, 1996). While HHV-6's cellular receptor has not been identified, a role for CD4 in entry has been ruled out (Lusso *et al*, 1989). Given its general predilection for lymphoid cells, we were not surprised that HHV-6 replicates in microglia, which share many cellular characteristics with monocytes and macrophages (Pellett and Black, 1996).

In comparison with the HHV-6 results, HSV-1, the prototypic neurotropic herpesvirus, infected microglia and to a lesser extent NT2D cells and oligodendrocytes, with distinct cytopathic differences. Specifically, HSV-1 infection resulted primarily in cell death and dropout, rather than the cell-to-cell fusion observed in some of the

cells exposed to HHV-6. This suggests that the exposure of differentiated, cultured oligodendrocytes and microglia to the betaherpesvirus (HHV-6) results in a specific pattern of cytopathology that differs from exposure to the alphaherpesviruses (HSV-1), however an effect due to virus dose could not be ruled out, since it is difficult to extrapolate the multiplicities of infection for these two agents.

Our findings support previous clinical reports indicating that HHV-6 is a neurotropic agent that appears to preferentially infect white matter tissue (Challoner *et al*, 1995; Saito *et al*, 1995; Mackenzie *et al*, 1995; Carrigan *et al*, 1996; Knox and Carrigan, 1995; Drobyski *et al*, 1994) and oligodendrocytes. Carrigan *et al* described a patient who succumbed to a year-long subacute white matter degeneration; the neuropathological examination demonstrated HHV-6 replication in oligodendrocytes and astrocytes in the white matter (Carrigan *et al*, 1996). Similarly, Saito *et al* found HHV-6 predominantly in oligodendrocytes in children with HIV encephalitis (Saito *et al*, 1995). Others have documented a more conventional encephalitis, but with white matter destruction (Drobyski *et al*, 1994).

How could a low-level infection of oligodendrocytes be relevant to a neuropathological process? One could speculate that expression of a subset of viral proteins could result in cell fusion and killing, or attract components of an immune response like cytolytic T-cells. Alternatively, HHV-6 could encode proteins that alter the immune response. For example, the HHV-6 genome encodes several homologs of chemokine receptors and chemokine motifs (Gompels *et al*, 1995), and the recently discovered gammaherpesvirus HHV-8 encodes homologs of macrophage inflammatory protein-1, interferon regulatory factor, and interleukin-6 (Nicholas *et al*, 1997). Disruption of immune homeostasis could result in oligodendroglial destruction via a bystander effect.

Given the long and erratic history of the search for an MS virus, one must be extraordinarily cautious before making any comments about an association between HHV-6 and MS. We expected that HHV-6 infection and cytopathology would be confined to microglial cells, and were somewhat surprised by the finding that oligodendrocytes support a limited infection. Thus, our results support the findings of Challoner *et al*, although none of the data address the subject of causality (Challoner *et al*, 1995). It is possible that oligodendrocytes around MS plaques are induced to replicate a virus that was heretofore latent, through exposure to cytokines and chemokines that are a common feature of the MS lesion (He *et al*, 1996). In such a scenario, HHV-6 might very well just be a 'passenger' virus with no relationship to the pathogenesis of MS (Cook *et al*, 1996). However, further studies in the area are warranted.

Materials and methods

Cells

Human adult oligodendrocytes and microglia were purified from the discarded tissue of temporal lobe resections for chronic epilepsy and cultured as previously described (Albright *et al*, 1996; Yong and Antel, 1992). Briefly, after 16 h of culture, microglia were purified by their selective adherence to plastic, while the oligodendrocytes remained non-adherent in the culture supernatant. To remove contaminating microglia from the oligodendrocytes, 50 μL of magnetic beads (Dynal, Great Plains, NY) were incubated for 2–4 h with the oligodendroycte culture (non-adherent fraction of cells). The microglia phagocytosed the magnetic beads and were removed with a magnet. Ranscht monoclonal antibody (Ranscht *et al*., 1982), which recognizes the oligodendrocyte-specific surface marker galactosylceramide, was coupled to IgG magnetic beads for use in the positive selection of oligodendrocytes (Albright *et al*, 1996). Fifty μL of these beads were incubated with 1 ml of non-adherent brain cells at room temperature for 1 h. Cells that were bound to the beads were separated with a magnet. The purified oligodendrocytes were then plated at $\approx 1 \times 10^6$ cells/well of a 24-well plate, and cultured for 7 days before use. NT2D cells were obtained from Dr David Pleasure (Dept. of Neurology, Children's Hospital of Philadelphia); they had been cultured and differentiated as previously described, and were used 7–10 days after differentiation (Pleasure *et al*, 1992). The purity of both the oligodendrocyte and microglial cultures was checked as previously described (Albright *et al*, 1996). The microglial cultures had fewer than 2–4% contaminating cells (calculated as a percentage of nuclei that were not surrounded by diAc-LDL positive cells).

Viruses

HHV-6$B_{Z\text{-}29}$ was replicated continuously in Molt-3 (CDC) cells to a titer of $1 \times 10^{3-4}$ $TCID_{50}$ doses/ml. Every 7 days, HHV-6B_{Z29}-infected Molt-3 cells were combined 1:4 with uninfected Molt-3 cells and supplemented with fresh RPMI (GIBCO, BRL) with 10% fetal bovine serum (Sigma). J-Jahn cells infected with HHV-6A_{GS} were obtained from Dr Robert Ricciardi and cultured as described for the HHV-6-infected Molt-3's. Herpes simplex virus type 1 (HSV-1) strain 17^+ was obtained from Nigel Fraser (Dept. Microbiology, University of Pennsylvania) and used as a cell-free supernatant.

HHV-6 infection of primary cells

For co-culture experiments, HHV-6-infected Molt-3 cells were placed in 0.4 μM transwell plates (Falcon) and added to cultures of oligodendrocytes, microglia and NT2D cells for 3–4 days, removed, and then the cultures were washed to remove residual virus. For cell-free virus experiments, the

HHV-6-infected Molt-3 cultures were centrifuged at 1400 *g* for 10 min in a tabletop centrifuge to remove cells and cellular debris. When overt cytopathology was observed, cultures were fixed, stained (Diff-Quik, Baxter, Miami, FL), and visualized by phase-contrast microscopy. Neural cultures were infected with HSV-1 at both 0.5 and 0.05 p.f.u./cell for 4 h, monitored for overt cytopathology every 12 h after infection, and then fixed and stained with Diff-Quik.

Electron microscopy
Oligodendrocytes or microglia were co-cultured with HHV-6 infected Molt-3 cells in transwell plates for 3 days, washed, cultured for an additional week, and then the trans-well baskets containing the Molt-3 cells were removed from the culture. Both cell types were then pelleted by centrifugation for 2 min at 800 *g*, the culture supernatant was removed, and the cells were fixed in 2% glutaraldehyde (Electron Microscopy Sciences, Ft, Washington, PA) in phosphate buffered saline (PBS) pH 7.4. EM was performed using a JEOL transmission electron microscope, model JEM100CX, at the Electron Microscopy Core Facility of the Department of Pathology and Laboratory Medicine at the University of Pennsylvania.

HHV-6 antigen ELISA
Supernatants of cells exposed to HHV-$6B_{Z29}$ were assayed for production of HHV-6 antigen with an antigen capture (gp116/64/54) ELISA according to the manufacturers instructions (Advanced Biotechnologies, Inc., Columbia, MD). This assay provides a relative absorbance reading of a colorimetric substrate, dependent on the presence of antigen, but does not provide a specific antigen concentration.

Major capsid protein gene PCR
Oligodendrocytes and microglia were infected as described above for 4 days and total DNA was isolated from the infected neural cultures using the Elu-Quik DNA Purification Kit (Schleicher and Schuell) on day 15 after infection. Infection of a neuroblastoma cell line using identical conditions (SK-N-MC, ATCC) was used as negative control for residual virus. PCR was performed on the isolated DNA, using the primers EX1 and EX2, derived from the major capid protein gene, which resulted in the amplification of a 520 bp fragment (Secchiero *et al*, 1995). Five μL of DNA were added to a 45 μL reaction mix containing $1\times$PCR buffer with 1.5 mM $MgCl_2$ (Perkin-Elmer Cetus, Norwalk, CT), 0.2 mM dNTP, 0.5 μM primers, and 6.25 U of AmpliTaq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT). PCR parameters were as follows: ($92^\circ C\times 1$ min, $50^\circ C\times 1$ min, $72^\circ C\times 1$ min) for 35 cycles followed by $72^\circ C\times 10$ min. PCR products were analyzed by electrophoresis on 2% agarose gels containing ethidium bromide.

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