Journal of NeuroVirology, 13: 462–467, 2007

ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280701447059

informa
healthcare

Short Communication

The prevalence of human herpesvirus 6 in human sensory ganglia and its co-occurrence with alpha-herpesviruses

Katharina Hüfner,[1] Viktor Arbusow,[1] Susanne Himmelein,[1] Tobias Derfuss,[1,2] Inga Sinicina,[3] Michael Strupp,[1] Thomas Brandt,[1] and Diethilde Theil[1]

[1]*Department of Neurology, Klinikum Grosshadern, Ludwig-Maximilians University, Munich, Germany;* [2]*Institute for Clinical Neuroimmunology, Klinikum Grosshadern, Ludwig-Maximilians University, Munich, Germany; and* [3]*Department of Legal Medicine, Ludwig-Maximilians University, Munich, Germany*

Human herpesvirus 6 (HHV-6) persists in the central nervous system, but its prevalence in the peripheral nervous system, a preferred latency site for herpesviruses, has not been studied. Using nested polymerase chain reaction (PCR), the authors determined the distribution of HHV-6 in human sensory ganglia. HHV-6 was present in 30% of trigeminal, 40% of geniculate, 25% of vestibular, and 55% of dorsal root ganglia. It co-occurred with alpha-herpesviruses (herpes simplex virus type 1 or varicella-zoster virus) in 91% of the ganglia. As HHV-6 positivity did not depend on the presence of inflammatory cells, known to harbor the virus, HHV-6 probably resides in the ganglia themselves. *Journal of NeuroVirology* (2007) **13,** 462–467.

Keywords: HHV-6; HSV-1; human; immune cells; peripheral nervous system; VZV

Introduction

Members of the Herpesviridae family have the ability to persist for the life of their infected host. When reactivated they may cause a wide spectrum of clinical manifestations, ranging from asymptomatic viral shedding to fatal disease. Human sensory nerve ganglia harbor latent alpha-herpesviruses such as herpes simplex virus type 1 (HSV-1) and varicella-zoster virus (VZV). If HSV-1 is reactivated from trigeminal ganglia (TG), the distressing, yet relatively benign, disease *herpes orolabialis* can result, but if reactivated from other sensory ganglia more serious diseases, such as vestibular neuritis, sudden hearing loss, and Bell's palsy may develop (Theil *et al*, 2001). If VZV is reactivated in the TG or dorsal root ganglia (DRG), it can lead to *herpes zoster*, which can involve postherpetic neuralgia as a serious sequela. If reactivated in the geniculate ganglia (GG), VZV can cause *herpes zoster oticus*, characterized by ear pain and a vesicular rash around and within the ear canal. Other symptoms include vertigo, hearing loss, and tinnitus. When a palsy of facial and vestibulocochlear nerves co-occurs the condition is called *Ramsay Hunt syndrome* (Sweeney and Gilden, 2001).

It was recently shown that a beta-herpesvirus, human herpes virus 6 (HHV-6), can persist in the central nervous system (CNS), where it can be detected at various sites in normal brain tissue (Chan *et al*, 2001), as well as in brain tissue from multiple sclerosis lesions (Challoner *et al*, 1995), from patients with progressive multifocal leucoencephalopathy (Mock *et al*, 1999), and in various brain tumors (Cuomo *et al*, 2001). HHV-6 is also thought to persist in other tissues such as the salivary glands and tonsils. It probably establishes latency in lymphocytes, monocytes, and their progenitor cells (Kondo *et al*, 1991). No studies are yet available on the prevalence of HHV-6 in

Address correspondence to Diethilde Theil, DVM, Department of Neurology, Klinikum Grosshadern, Marchioninistr. 23, 81377 Munich, Germany. E-mail: diethilde.theil@med.uni-muenchen.de

This work was supported by grants from the Deutsche Forschungsgemeinschaft to D.T. and T.D. (TH 894) and from the Münchner Medizinische Wochenschrift to K.H. The authors would like to thank Judy Benson and Katie Ogston for carefully copyediting the manuscript.

Received 2 April 2007; accepted 23 April 2007.

the peripheral nervous system (PNS), specifically the sensory ganglia, a notorious site of latency for some human herpesviruses (VZV and HSV-1).

Because previous studies from our group had found abundant immune cell infiltration in TG latently infected with HSV-1 (Theil *et al*, 2003) and few scattered T-cells in some DRG (Hüfner *et al*, 2006), we suspected that such lymphocytes and monocytes could also harbor HHV-6. The aims of our study were (1) to determine the prevalence and distribution of HHV-6 in different human sensory cranial nerve ganglia and DRG; (2) to evaluate its co-occurrence with alpha-herpesviruses; and (3) to ascertain whether the presence of HHV-6 correlates with the immune cell infiltration in the TG and DRG.

Results

Distribution of HHV-6 in human TG, GG, VG, and DRG

The left and right TG from five individuals were examined for the presence of HHV-6 by nested polymerase chain reaction (PCR). Three of 10 (30%) TG were found to be positive for HHV-6 (Figures 1A, 2A). Another set of 20 GG and vestibular ganglia (VG) from an additional 10 individuals were tested using the same technique. Eight of the 20 GG (40%) and five of the 20 VG (25%) were found to be positive (Figures 1B, C, 2A). In a set of 11 DRG from six individuals, HHV-6 was found in six ganglia (55%) (Figures 1D, 2A). Sequencing of the HHV-6 PCR product revealed HHV-6 type A in 18 of the HHV-6–positive ganglia; only four harbored variant B.

Distribution of HSV-1 and VZV in the TG, GG, VG, and DRG

HSV-1 nested PCR was positive in all tested TG (100%), in 11/20 of the GG (55%), in 11/20 of the VG (55%), and in 2/11 of the DRG (18%). VZV was found in all TG (100%), in 6/20 of the GG (30%), in 2/20 of the VG (10%), and in 7/11 of the DRG (64%). In many cases both left and right ganglia were either negative or positive for a respective virus (Figures 1A–D, 2A).

Co-occurrence of alpha- and beta-herpesviruses

A co-occurrence of all three viruses was found in seven of the 22 HHV-6–positive ganglia (32%); an additional infection with either HSV-1 or VZV was present in 13 of the HHV-6–positive ganglia (59%), resulting in 20 of the 22 HHV-6–positive ganglia being infected with one or more additional herpesviruses (91%). Only two ganglia were solely positive for HHV-6 (9%) (Figure 2B). Chi-square statistics showed that the infection of a sensory ganglion with HHV-6 was associated with infection with another herpesvirus (either HSV-1 or VZV; $P < .01$). The occurrence of HSV-1 and VZV in the ganglia was not statistically linked ($P > .01$). HHV-6 was most frequently present in the DRG, whereas HSV-1 and VZV were mostly found in the TG. Overall no statistically significant difference in frequencies of HHV-6 was found between the tested ganglia (Kruskal-Wallis analysis of variance [ANOVA]; $P > .01$). HSV-1 and VZV did, however, differ significantly in their distribution between the tested ganglia ($P < .01$ and $P < .00001$, respectively).

Prevalence of HHV-6 and immune cell infiltrates in the sensory ganglia

HHV-6 has been shown to be present in peripheral blood mononuclear cells (PBMCs) during active infection. It may also become latent in these cells (Kondo *et al*, 1991). We therefore evaluated human TG and DRG to test for a possible correlation between the presence of HHV-6 and an abundance of immune cell infiltrates in the respective ganglia (Table 1, Figure 1E, G). TG contained between 17.3 and 52 CD3 + T-cells per field of view (×400); the DRG contained much fewer T-cells (0 to 7.3). Larger numbers of T-cells were present in the TG than in the DRG (3- to almost 10-fold), whereas more of the DRG were positive for HHV-6. T-cell infiltrates did not vary significantly between HHV-6–positive or –negative ganglia (Mann-Whitney U test; $P > .01$). The same held true for VZV-positive or -negative ganglia ($P > .01$). HSV-1–positive ganglia, however, contained significantly more T-cells than those ganglia negative for HSV-1 ($P < .00001$). Only single, scattered T-cells were found in the GG and VG (Figure 1F).

Table 1 Number of CD3-positive T-cells in TG and DRG

Case	*T-cells*
TG 1R	18
TG 1L	26.3
TG 2R	44.3
TG 2L	37.3
TG 3R	52
TG 3L	33
TG 4R	18.3
TG 4L	23
TG 5R	17.3
TG 5L	27.6
DRG 1Th4	7.3
DRG 1Th5	1.6
DRG 2Th3	0
DRG 3Th1L	9
DRG 3Th3R	3
DRG 3Th4R	3.3
DRG 4Th4L	0
DRG 4Th5L	1.3
DRG 4Th6L	2.3
DRG 5Th5R	6.6
DRG 6Th2R	2.3

Note. One to three sections from the respective ganglia were stained with a primary antibody against a pan T-cell marker. The number of CD3-positive cells was evaluated in three randomly selected fields of view at a magnification of (×400) and then averaged.

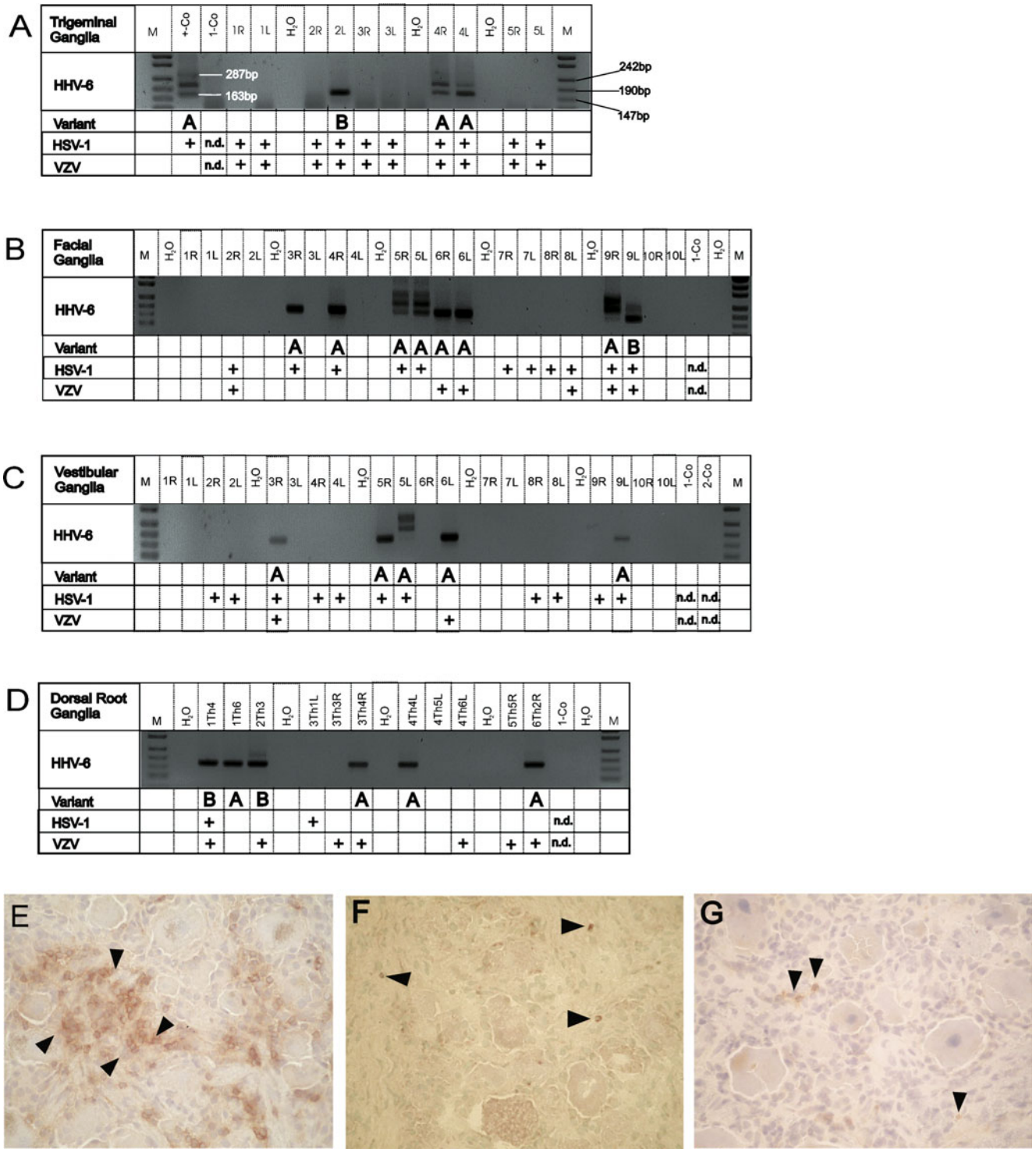

Figure 1 Overview of the HHV-6, HSV-1, and VZV nested PCRs performed on TG, GG, VG, and DRG (**A–D**). For HHV-6 the electrophoresis gel with the controls and the variant (A or B) is shown. The HHV-6 product represents a 163-bp fragment of the larger tegument protein. In some of the samples double or triple bands are seen. This is due to the fact that a visible amplicon was generated with the first set of primers (287 bp). The middle band is most likely a hybrid of the amplicon from the first and second round. For subtyping, either the 163-bp or 287-bp fragment was gel purified and sequenced with the corresponding primers. The results of the HSV-1 and VZV PCRs are indicated schematically below the corresponding samples. Samples marked + were positive in the respective PCR, negative results are indicated by a blank. 1-Co: PBMCs; 2-Co: medial longitudinal fascicle (MLF); +-Co: GG 5L; M: pUC Mix Marker 8 (Fermentas, St. Leon-Rot, Germany); n.d.: not done. Representative CD3 immunohistochemistry micrographs of the TG, GG, and DRG (**E–G**). Abundant T-cell infiltration (*arrowheads*, brown staining with diaminobenzidine) is seen in the TG (**E**), but only a few T-cells (*arrowheads*) are found in the GG (**F**) and DRG (**G**) ×400; counterstaining H&E or methylgreen.

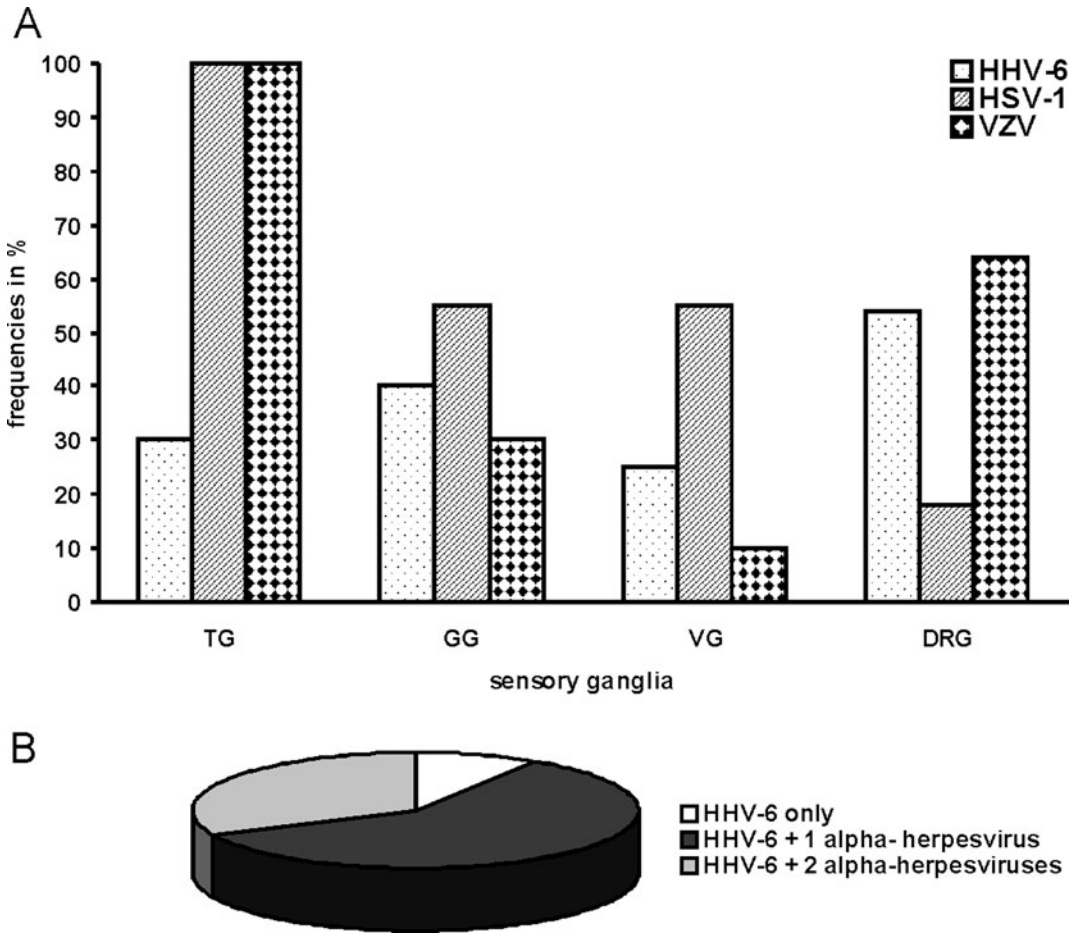

Figure 2 Graphical overview of the detection frequencies for HHV-6, HSV-1, and VZV in human sensory ganglia. (**A**) Frequencies of HHV-6, HSV-1, and VZV in human TG, GG, VG, and DRG. The numbers represent the percent (%) of all ganglia analyzed for a specific anatomical location. (**B**) The number of ganglia in percent (%) infected with HHV-6 only or infected with HHV-6 and one or two alpha-herpesviruses.

Discussion

This study presents evidence that HHV-6 is prevalent in the PNS. All the evaluated human sensory ganglia, e.g., TG, GG, VG, and DRG, contain HHV-6 with frequencies previously reported in the CNS (overall frequency reported here 36%; for an overview see De Bolle *et al*, 2005). In previous studies HHV-6 DNA has been detected in 42.9% of brain samples from frontal cortex; however, these samples could have included blood cells, possibly containing HHV-6 DNA (Chan *et al*, 1999). When more than one brain sample per patient were analysed, 24.3% of the samples were found to be positive for HHV-6 DNA, resulting in 85% of the patients having one or more samples which tested positive for HHV-6 (Chan *et al*, 2001). Similar frequencies were found in a different study where 37.3% of samples from brain tumors and 32.2% of normal brain tissue were found positive for HHV-6 DNA (Cuomo *et al*, 2001). In a further study HHV-6–specific sequences were identified in six of nine brain samples from immunocompetent subjects, in four of seven brain samples from acquired immunodeficiency syndrome (AIDS) patients, but only in six of the 37 primary brain tumor biopsies examined (Luppi *et al*, 1995). HHV-6 DNA could also be amplified from surgical brain resections from four of eight patients with mesial temporal lobe epilepsy, whereas this was not possible from control patients undergoing surgery for neocortical epilepsy (Donati *et al*, 2003).

Because it has been shown that HHV-6 can persist in human PBMCs, we were interested to see if the immune cells found in the TG and DRG correlated with the presence of HHV-6 in the TG and DRG (Hüfner *et al*, 2006; Theil *et al*, 2003). This was, however, not the case, suggesting that the HHV-6 DNA is actually present in the glial or neural cells previously shown to contain HHV-6 *in vitro* and *in vivo* (Ahlqvist *et al*, 2005; Challoner *et al*, 1995).

In most cases (91%) HHV-6 was not the only virus present in the ganglia: all but two showed coinfection with either HSV-1 or VZV. It is possible that HHV-6 is the first of the herpesviruses to infect the

ganglia, because infection occurs quite early, usually within the first year of life (Hall *et al*, 1994). In contrast, HSV-1 (Smith and Robinson, 2002) and VZV (Arvin, 1996) infections usually occur later. Latent HHV-6 could create an environment in the ganglia, which facilitates the invasion of other viruses. In point of fact, it has been demonstrated that HHV-6 can interact with a series of other viruses during active infection and reactivation. In post-transplant patients cytomegalovirus (CMV) disease was associated with the detection of HHV-6 DNA (Mendez *et al*, 2001). Polyoma JC virus (JCV) might also cooperate with HHV-6 as a causative agent of progressive multifocal leucoencephalopathy (PML) (Mock *et al*, 1999). So far, there are no studies on the interaction between HSV-1, VZV, and HHV-6; however, because the site of latency for these viruses is the same, it is possible that there is some interference among them during infection, latency, or reactivation.

It is still a subject of debate whether HHV-6A or HHV-6B shows a greater neurotropism. Although mostly variant B was detected in normal brain tissue (Chan *et al*, 1999, 2001), in mesial temporal lobe epilepsy surgical brain resections (Donati *et al*, 2003), and in the white matter surrounding multiple sclerosis (MS) plaques (Challoner *et al*, 1995); variant A has been reported to be more prevalent in the CNS by others (Cuomo *et al*, 2001) and to be more neuroinvasive among children with primary HHV-6 infection (Hall *et al*, 1998). We found that HHV-6A preferentially infected the sensory ganglia.

In conclusion, we have shown that human sensory ganglia become infected with HHV-6. It remains to be shown whether the co-infection with HSV-1 and VZV is merely a coincidence or if HHV-6 can influence the pattern of infection, latency, and reactivation of HSV-1 and VZV, as has been demonstrated for other viruses.

Materials and methods

Tissue samples

The use of autopsy samples for the present study was approved by the Ethics Committee of the Medical Faculty of the Ludwig-Maximilians University of Munich. GG and VG were obtained from the temporal bones of the same individual. TG and DRG (thoracic segment, different levels) were taken from different individuals. The ganglia were removed 6 to 24 h after death. The age of the subjects included in the study varied between 4 months and 67 years. The cause of death of the subjects was mainly related to trauma. The subjects had neither lesions suggestive of an active orolabial herpes or zoster infection nor a history of cranial nerve disorders. The ganglia were collected over a period of 6 years. Ganglia were embedded in Tissue Tek compound (Sakura, Zoeterwoude, The Netherlands) and stored at -70°C until use.

DNA extraction and PCR

For DNA preparation the tissue was homogenized and DNA was extracted using QIAamp DNA micro Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA was eluted with distilled water, and the concentration was determined by spectrophotometry. The DNA was extracted in different rounds, and several weeks to years passed between these DNA extraction rounds.

To confirm the presence of amplifiable DNA, a nonpolymorphic mitochondrial fragment of 530 bp was amplified in a separate reaction for each sample (Theil *et al*, 2004). Nested PCRs for HHV-6, HSV-1, and VZV fragments were performed. All manipulations were done using aerosol-free pipette tips. PCR pipetting of the first and second round were done in separate laboratories. A minimum of 200 ng of DNA was used in each PCR reaction. The sequence of the primers, product size, and cycling conditions for HSV-1, VZV, and HHV-6 PCRs have been described previously (Aurelius *et al*, 1991; Chan *et al*, 2001; Read and Kurtz, 1999). HSV-1 PCR detects the glycoprotein D, whereas VZV primers amplify gene 29 and HHV-6 primers are designed to amplify the larger tegument protein. For determining the variant of HHV-6, the PCR product was sequenced for all positive samples. DNA samples from human TG found previously to be positive for HSV-1, VZV, and HHV-6 were used as positive controls. Water controls were included every fifth sample. DNA from human brain tissue (a sample taken from the medial longitudinal fascicle, which had tested negative for HHV-6 in a previous work (Theil *et al*, 2004)) and human peripheral blood mononuclear cells (PBMCs) from a healthy donor were used as negative controls.

Immunohistochemistry (IHC)

Frozen sections of 10-μm thickness were made and mounted on positively charged slides (SuperFrost/Plus; Menzel, Braunschweig, Germany). Immunohistochemical stainings were done with a primary antibody against a pan T-cell marker using rabbit anti-human CD3 (1:1000; DAKO, Hamburg, Germany) (Theil *et al*, 2003). The prevalence of CD3 antigen was evaluated in one to three sections from the TG and the DRG from each individual and in two GG and VG, as previously described (Theil *et al*, 2003).

Statistical analysis

Nonparametric statistical analysis was performed on viral frequencies and T-cell numbers, results exceeding a threshold of $P < .01$ were considered significant.

References

Ahlqvist J, Fotheringham J, Akhyani N, Yao K, Fogdell-Hahn A, Jacobson S (2005). Differential tropism of human herpesvirus 6 (HHV-6) variants and induction of latency by HHV-6A in oligodendrocytes. *J NeuroVirol* **11:** 384–394.

Arvin AM (1996). Varicella-zoster virus. *Clin Microbiol Rev*, **9:** 361–381.

Aurelius E, Johansson B, Skoldenberg B, Staland A, Forsgren M (1991). Rapid diagnosis of herpes simplex encephalitis by nested polymerase chain reaction assay of cerebrospinal fluid. *Lancet*, **337:** 189–192.

Challoner PB, Smith KT, Parker JD, MacLeod DL, Coulter SN, Rose TM, Schultz ER, Bennett JL, Garber RL, Chang M (1995). Plaque-associated expression of human herpesvirus 6 in multiple sclerosis. *Proc Natl Acad Sci U S A*, **92:** 7440–7444.

Chan PK, Ng HK, Hui M, Cheng AF (2001). Prevalence and distribution of human herpesvirus 6 variants A and B in adult human brain. *J Med Virol*, **64:** 42–46.

Chan PK, Ng HK, Hui M, Ip M, Cheung JL, Cheng AF (1999). Presence of human herpesviruses 6, 7, and 8 DNA sequences in normal brain tissue. *J Med Virol*, **59:** 491–495.

Cuomo L, Trivedi P, Cardillo MR, Gagliardi FM, Vecchione A, Caruso R, Calogero A, Frati L, Faggioni A, Ragona G (2001). Human herpesvirus 6 infection in neoplastic and normal brain tissue. *J Med Virol*, **63:** 45–51.

De Bolle L, Naesens L, De Clercq E (2005). Update on human herpesvirus 6 biology, clinical features, and therapy. *Clin Microbiol Rev*, **18:** 217–245.

Donati D, Akhyani N, Fogdell-Hahn A, Cermelli C, Cassiani-Ingoni R, Vortmeyer A, Heiss JD, Cogen P, Gaillard WD, Sato S, Theodore WH, Jacobson S (2003). Detection of human herpesvirus-6 in mesial temporal lobe epilepsy surgical brain resections. *Neurology*, **61:** 1405–1411.

Hall CB, Caserta MT, Schnabel KC, Long C, Epstein LG, Insel RA, Dewhurst S (1998). Persistence of human herpesvirus 6 according to site and variant: possible greater neurotropism of variant A. *Clin Infect Dis*, **26:** 132–137.

Hall CB, Long CE, Schnabel KC, Caserta MT, McIntyre KM, Costanzo MA, Knott A, Dewhurst S, Insel RA, Epstein LG (1994). Human herpesvirus-6 infection in children. A prospective study of complications and reactivation. *N Engl J Med*, **331:** 432–438.

Hüfner K, Derfuss T, Herberger S, Sunami K, Russell S, Sinicina I, Arbusow V, Strupp M, Brandt T, Theil D (2006). Latency of alpha-herpes viruses is accompanied by a chronic inflammation in human trigeminal ganglia but not in dorsal root ganglia. *J Neuropathol Exp Neurol*, **65:** 1022–1030.

Kondo K, Kondo T, Okuno T, Takahashi M, Yamanishi K (1991). Latent human herpesvirus 6 infection of human monocytes/macrophages. *J Gen Virol*, **72:** 1401–1408.

Luppi M, Barozzi P, Maiorana A, Marasca R, Trovato R, Fano R, Ceccherini-Nelli L, Torelli G (1995). Human herpesvirus-6: a survey of presence and distribution of genomic sequences in normal brain and neuroglial tumors. *J Med Virol*, **47:** 105–111.

Mendez JC, Dockrell DH, Espy MJ, Smith TF, Wilson JA, Harmsen WS, Ilstrup D, Paya CV (2001). Human beta-herpesvirus interactions in solid organ transplant recipients. *J Infect Dis*, **183:** 179–184.

Mock DJ, Powers JM, Goodman AD, Blumenthal SR, Ergin N, Baker JV, Mattson DH, Assouline JG, Bergey EJ, Chen B, Epstein LG, Blumberg BM (1999). Association of human herpesvirus 6 with the demyelinative lesions of progressive multifocal leukoencephalopathy. *J NeuroVirol*, **5:** 363–373.

Read SJ, Kurtz JB (1999). Laboratory diagnosis of common viral infections of the central nervous system by using a single multiplex PCR screening assay. *J Clin Microbiol*, **37:** 1352–1355.

Smith JS, Robinson, NJ (2002). Age-specific prevalence of infection with herpes simplex virus types 2 and 1: a global review. *J Infect Dis*, **186(Suppl 1):** S3–S28.

Sweeney CJ, Gilden DH (2001). Ramsay Hunt syndrome. *J Neurol Neurosurg Psychiatry*, **71:** 149–154.

Theil D, Arbusow V, Derfuss T, Strupp M, Pfeiffer M, Mascolo A, Brandt T (2001). Prevalence of HSV-1 LAT in human trigeminal, geniculate, and vestibular ganglia and its implication for cranial nerve syndromes. *Brain Pathol*, **11:** 408–413.

Theil D, Derfuss T, Paripovic I, Herberger S, Meinl E, Schueler O, Strupp M, Arbusow V, Brandt T (2003). Latent herpesvirus infection in human trigeminal ganglia causes chronic immune response. *Am J Pathol*, **163:** 2179–2184.

Theil D, Horn AK, Derfuss T, Strupp M, Arbusow V, Brandt T (2004). Prevalence and distribution of HSV-1, VZV, and HHV-6 in human cranial nerve nuclei III, IV, VI, VII, and XII. *J Med Virol*, **74:** 102–106.