Journal of NeuroVirology (1995) 1, 30–39

Cellular localization of human herpesvirus-6 in the brains of children with AIDS encephalopathy

Yoshihiro Saito[1], Leroy R Sharer[2], Stephen Dewhurst[3,5], Benjamin M Blumberg[1,3], Caroline Breese Hall[4] and Leon G Epstein[1,3,4]

Laboratory for Molecular Neurovirology, Departments of [1]Neurology, [3]Microbiology and Immunology, [4]Pediatrics, and [5]Cancer Center, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642; [2]Department of Laboratory Medicine and Pathology, New Jersey Medical School, Newark, NJ 07103, USA

Human herpesvirus-6, the etiologic agent of exanthem subitum, is a ubiquitous virus that infects almost all children by the age of 2 years and that has previously been shown to be neuroinvasive. These characteristics suggest that human herpesvirus-6 may be important in the neuropathogenesis of acquired immune deficiency syndrome (AIDS) in children. To address this hypothesis, we evaluated postmortem pediatric brain tissues for the presence of human herpesvirus-6 infection. Using *in situ* hybridization with a digoxigenin-labeled DNA probe for the large tegument protein gene of human herpesvirus-6, we detected nuclear signals in postmortem brain tissue from 4/5 children with human immunodeficiency virus-1 encephalitis. Human herpesvirus-6 DNA was found in numereous oligodendrocytes of the white matter and less frequently in astrocytes, macrophages, microglia and neurons. The human herpesvirus-6 positive cells detected by *in situ* hybridization were not immunoreactive either for human herpesvirus-6 early nuclear phosphoproteins or for surface glycoproteins associated with productive infection. Only rare human herpesvirus-6 infected cells were found in age-matched control brain tissues. No human herpesvirus-6 infected cells were found in human fetal brain tissue. These data suggest that human herpesvirus-6 is more extensively disseminated in neural cells in the presence of human immunodeficiency infection and immunodeficiency in pediatric AIDS patients, and it may contribute to the pathogenesis of AIDS encephalopathy.

Keywords: human herpesvirus-6 (HHV-6); human immunodeficiency virus type 1 (HIV-1); oligodendrocyte; in situ hybridization; AIDS

Introduction

Human herpesvirus-6 (HHV-6) is a recently discovered herpesvirus that has been isolated from peripheral blood lymphocytes of patients with lymphoproliferative disorders and human immunodeficiency virus type 1 (HIV-1) infection (Salahuddin *et al*, 1986; Tedder *et al*, 1987; Downing *et al*, 1987). It is the causative agent of exanthem subitum (Yamanishi *et al*, 1988) as well as other febrile illnesses in children (Pruksananonda *et al*, 1992), and it has been associated with seizures (Hall *et al*, 1994). Serological studies have shown that HHV-6 is a ubiquitous virus that infects more than 90% of all children before the age of 2 years (Hall *et al*, 1994; Okuno *et al*, 1989; Saxinger *et al*, 1988; Levy *et al*, 1990a). Recently, two distinct variants of HHV-6 have been identified: HHV-6 A and HHV-6 B (Schirmer *et al*, 1991; Ablashi *et al*, 1991; Aubin *et al*, 1991). In the US, HHV-6 B has been frequently isolated in a population of infants with symptomatic primary viral infection (Dewhurst *et al*, 1992, 1993). After the primary infection, there is lifelong viral persistence, possibly in a latent state. Thus, most adults continue to harbor detectable levels of viral DNA in both peripheral blood (Gopal *et al*, 1990; Kondo *et al*, 1991) and the oropharynx, including the salivary glands (Levy *et al*, 1990a; Gopal *et al*, 1990; Fox *et al*, 1990). In immune suppressed individuals, HHV-6 infection can be reacti-

Correspondence: Y Saito
Received 28 September 1994; revised 17 October 1994; accepted 19 October 1994

vated from this latent or low-level productive state, giving rise to active infection with viremia (Okuno *et al*, 1990).

The brain is a common site for persistent viral infections (Johnson, 1982), including herpesviruses, and it is of interest that neurological complications occur frequently during the course of exanthem subitum (Moller, 1956). Evidence for entry of HHV-6 into the central nervous system (CNS) during primary infection includes intrathecal production of anti-HHV-6 antibodies (Ishiguro *et al*, 1990) as well as detection by polymerase chain reaction (PCR) of HHV-6 DNA in cerebrospinal fluid from children (Asano *et al*, 1992; Yoshikawa *et al*, 1992; Caserta *et al*, 1994; Kondo *et al*, 1993) as well as in post-mortem brain tissue (Asano *et al*, 1990). Taken together, these observations suggest that HHV-6 is able to invade the CNS during acute infection and persist. In the light of similarities between HHV-6 and other herpesviruses, including herpes simplex virus type 1 (HSV-1) (Fraser *et al*, 1981; Karlin *et al*, 1994), it is possible that HHV-6 may establish a persistent or latent infection in cells of the CNS.

Recently, Knox and Carrigan have demonstrated productive HHV-6 infection in lung, lymph node, spleen, liver and kidney of young adult patients who died with acquired immune deficiency syndrome (AIDS) (Knox and Carrigan, 1994). Both HIV-1 and HHV-6 are neuroinvasive, and hence HHV-6 may interact with HIV-1 in the CNS, particularly in pediatric subjects who might be expected to have a primary HHV-6 infection before the age of 2 years. In order to investigate the possible role of HHV-6 in the pathogenesis of AIDS encephalopathy, we examined previously characterized brain tissue from pediatric AIDS patients for the presence of HHV-6 infection.

Results

Microscopic findings

Neuropathological findings from the five cases are included in Table 1. No cells bearing nuclear or cytoplasmic inclusions were seen in any of the cases.

In situ *hybridization for HHV-6 of control cells and tissues*

The digoxigenin-labeled HHV-6 DNA probe hybridized strongly to cord blood mononuclear cells (CBMC) that had been infected with HHV-6 (Figure 1a). No hybridization signal was detected in uninfected cord blood cells (Figure 1b). HHV-6 DNA was rarely found in brain sections from the HIV-1 negative, immunocompetent children (Figure 1c), while human fetal brain tissues had no cells containing HHV-6 DNA (Figure 1d). Lack of cross hybridization of this probe with human cytomegalovirus (CMV) was confirmed by performing *in situ* hybridization on a human CMV-infected retinal xenograft (DiLoreto *et al*, 1994), with negative results (Data not shown).

In situ *hybridization for HHV-6 of HIV-1 infected tissues*

Positive hybridization for HHV-6 nucleic acid sequences was obtained in four of the five HIV-1

Table 1 Summary of cases examined

Patient	*Age (yrs)*	*Sex*	*Clinical and neuropathological features*	*PCR HIV*	*ICC p24*	*ISH HHV-6*
1	9	F	Progressive encephalopathy, severe myelopathy Severe HIV-1 encephalomyelitis; vacuolar myelopathy	(+)	3+	3+
2	2	M	Progressive encephalopathy, terminal viral pneumonitis White matter pallor, mineralizations in basal ganglia	(+)	0	2+
3	0.5	M	Probable progressive encephalopathy, with cerebal atrophy on CT Moderately severe HIV-1 encephalitis	(+)	1+	1+
4	1	F	Progressive encephalopathy Moderately severe HIV-1 encephalitis; pontine leukoencephalopathy	(+)	1+	0
5	6	M	Severe progressive encephalopathy Severe HIV-1 encephalomyelitis	(+)	3+	1+

0 = No cells positive, 1+ = 1 to 10 positive cells/20x field, 2+ = to 40 positive cells/20x field, 3+ = Greater than 40 positive cells/20x field

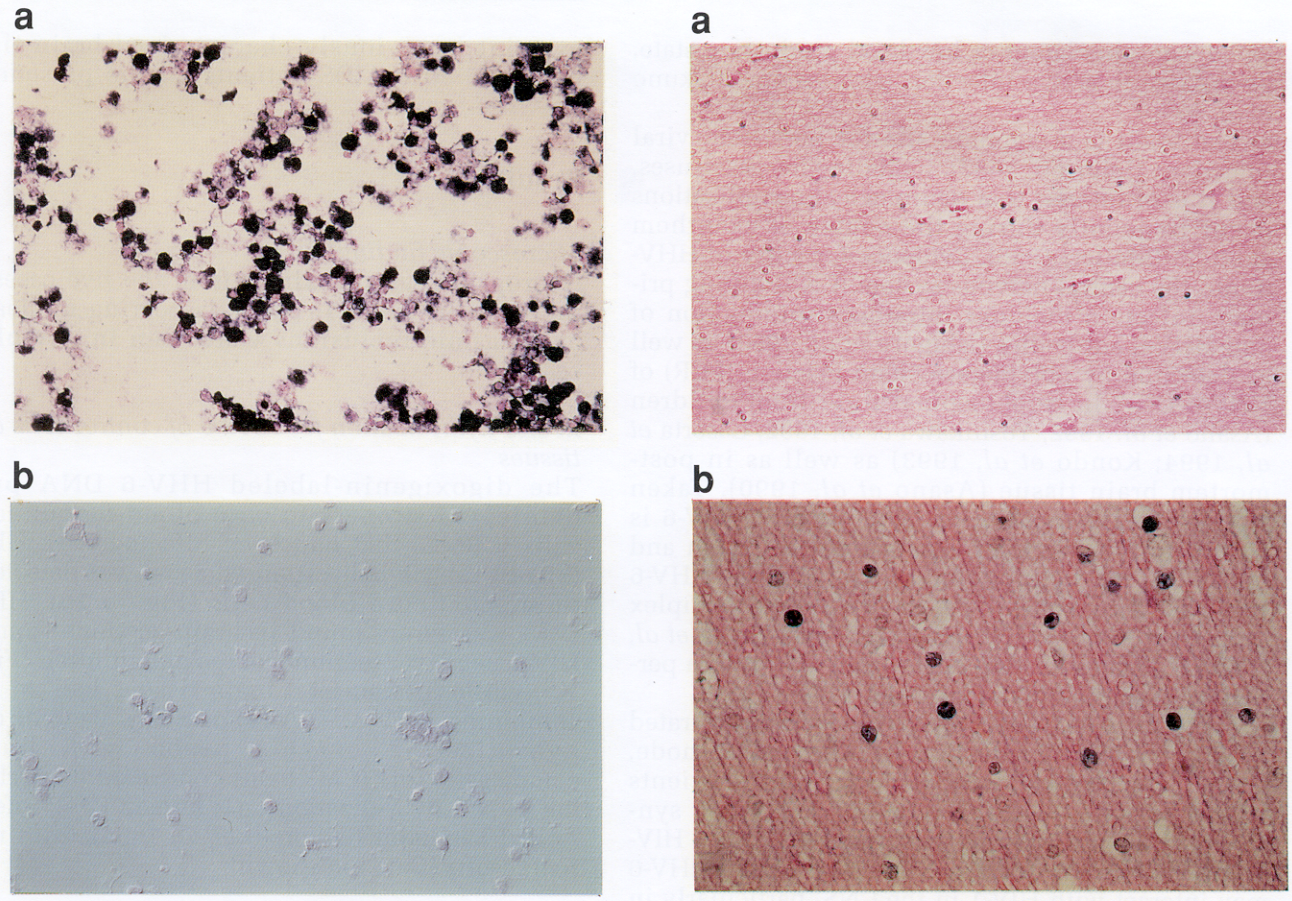

Figure 2 HHV-6 *in situ* hybridization of HIV-1 infected tissue sections. (a) Subcortical white matter from case 2 demonstrating numerous HHV-6 positive nuclei identified by *NBT/X-phos* chromogen. Note also HHV-6 negative nuclei (eosin counterstain, X 120). (b) Subcortical white matter from case 1 demonstrating HHV-6 nuclear staining in cells with morphologic features of oligodendrocytes (eosin counterstained, X 380). Similar positive hybridization for HHV-6 nucleic acid sequences was obtained in cases 3 and 5, with the majority of positive nuclei occurring in the white matter.

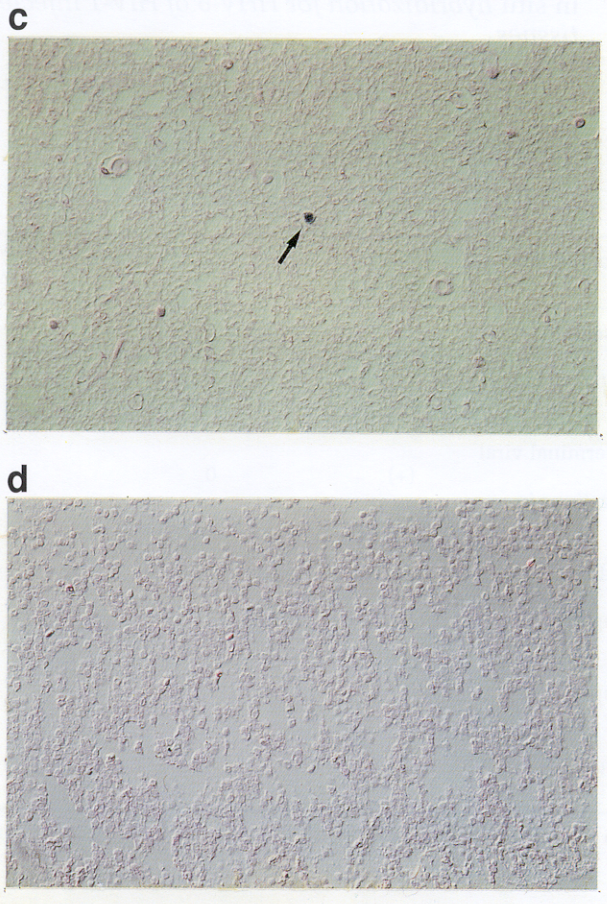

Figure 1 HHV-6 *in situ* hybridization controls. (a) HHV-6 infected cord blood cells show intense blue nuclear signal (X 120). (b) Uninfected cord blood cells have no nuclear staining (X 120). (c) Control brain tissue from 8-year-old girl with acute intracerebral hemorrhage: arrow indicates rare cell containing HHV-6 nucleic acid in nucleus (X 240). (d) 19 week gestation human fetal brain contains no HHV-6 positive nuclei (X 120). (All *in situ* hybridization for HHV-6 with NBT/X-phos, eosin counterstain).

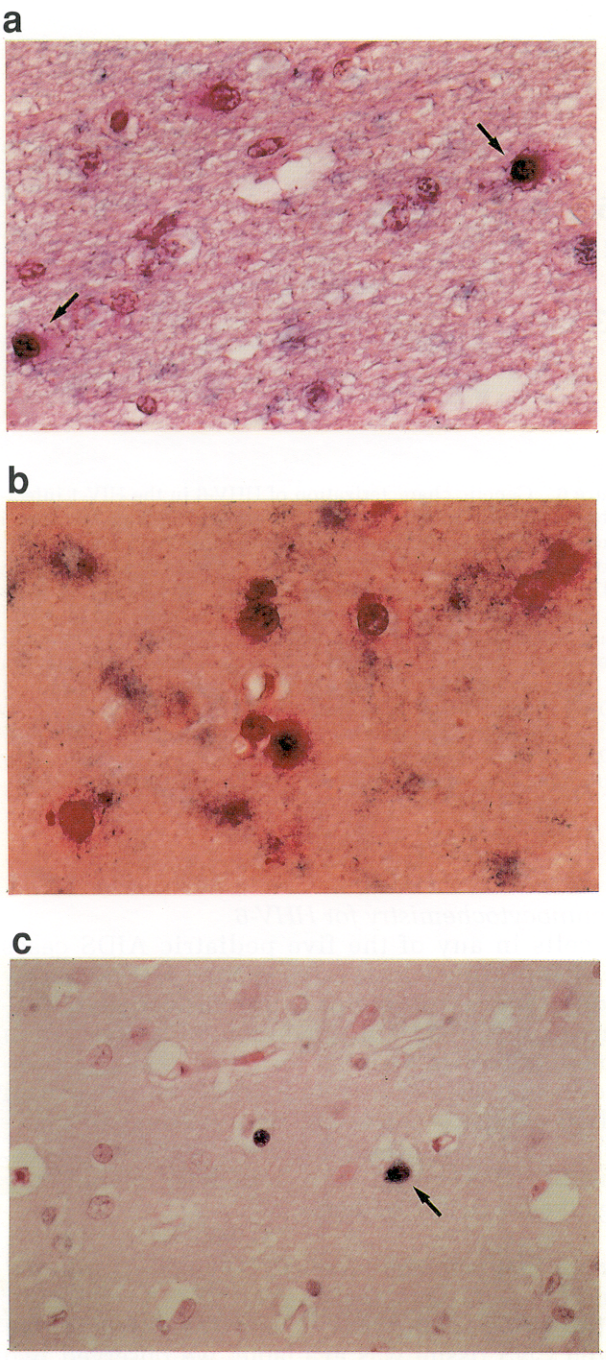

Figure 3 Cellular localization of HHV-6 in neural cells by combined DNA *in situ* hybridization and immunocytochemistry. (a) and (b) Oligodendrocytes containing nuclear signal for HHV-6 DNA in cerebral white matter, case 1 (arrows). (Immunocytochemistry for galactocerebroside with New fuchsin red chromogen and *in situ* hybridization for HHV-6 with *NBT/X-phos* blue chromogen, no counterstain, a; × 420, b; × 450). (c) HHV-6 signal in nucleus of neuron in putamen, case 1 (arrow). The identity of the cell is based on its shape and location. Positive small nucleus of oligodendrocyte is also present (*in situ* hybridization for HHV-6 DNA, *NBT/X-phos* chromogen, eosin counterstain, × 380).

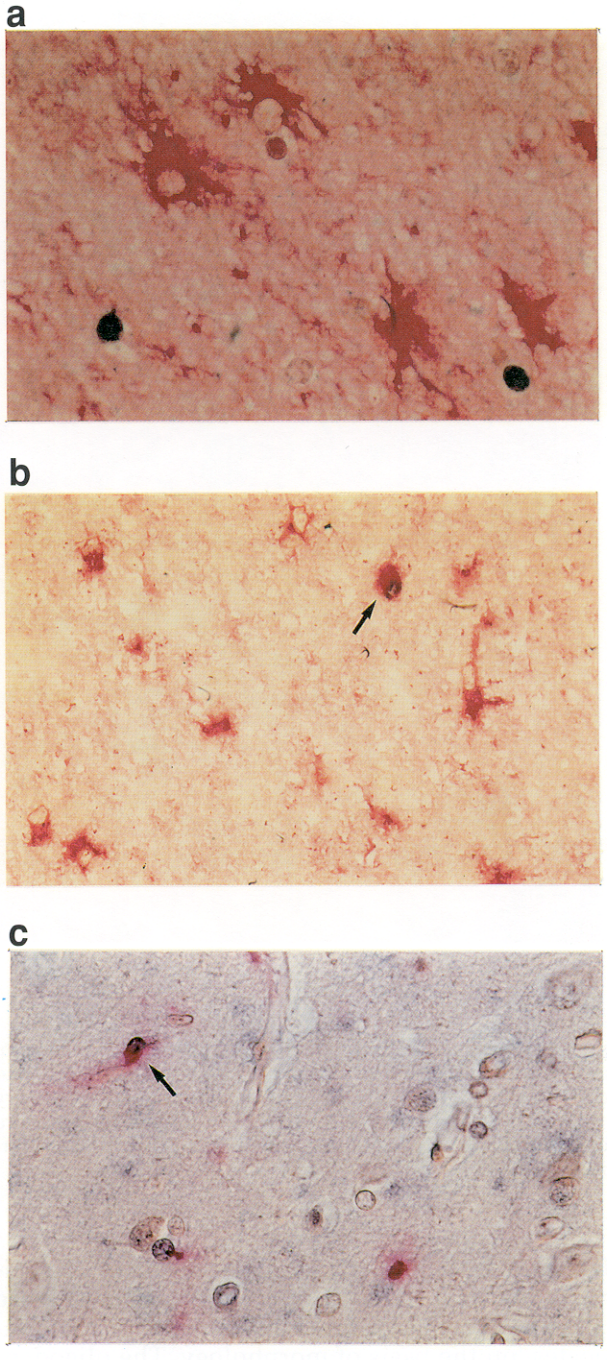

Figure 4 Cellular localization of HHV-6 in neural cells by combined DNA *in situ* hybridization and immunocytochemistry. (a) and (b) White matter, case 2. In most of the regions, astrocytes (in red) are negative for HHV-6 nuclear signal (in blue), although a rare astrocyte is positive (arrow). (Immunocytochemistry for GFAP with New fuchsin and *in situ* hybridization for HHV-6 DNA with *NBT/X-phos*, no counterstain, (a) × 420, (b) × 280 (c) Cerebral cortex, case 1. A few microglial cells have nuclei that are positive for HHV-6 signal (arrow). (Immunocytochemistry for CD68 with New fuchsin and *in situ* hybridization for HHV-6 DNA with *NBT/X-phos*, no counterstain, × 330).

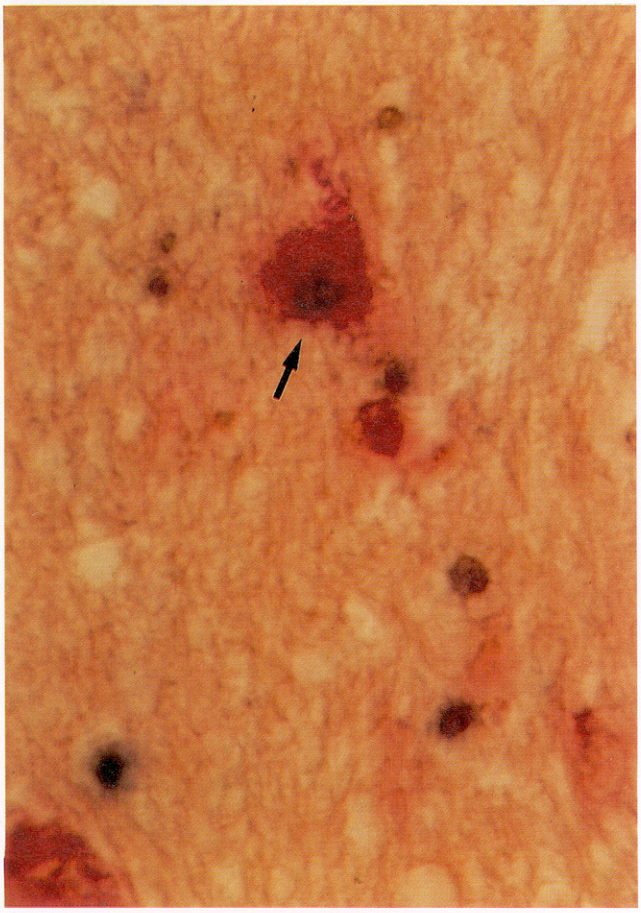

Figure 5 Co-localization of HHV-6 and HIV-1. Basal ganglia, case 1. Macrophage with cytoplasm positive for HIV-1 p24 and nucleus positive for HHV-6 DNA (arrow). (Immunocytochemistry for p24 core protein of HIV-1 with New fuchsin and *in situ* hybridization for HHV-6 DNA with *NBT/X-phos*, no counterstain, × 650).

infected cases (Table 1), with the majority of positive nuclei occurring in the white matter (Figure 2a,b). These cells appeared to be mainly oligodendrocytes, on the basis of morphology. The oligodendrocyte lineage of these cells was confirmed by combined immunocytochemistry for Galactocerebroside and *in situ* hybridization for HHV-6 viral nucleic acids (Figure 3a,b). Positive signal for HHV-6 also occurred, although less frequently, in nuclei of neurons (Figure 3c), astrocytes and macrophages/microglia. Each of these cell types was also confirmed by combined immunocytochemistry and *in situ* hybridization (Figure 4a–c). Coinfection with HHV-6 and HIV-1 could also be demonstrated in a small number of cells in case 1 and 5 by combined immunocytochemistry for HIV p24 and *in situ* hybridization for HHV-6 DNA (Figure 5).

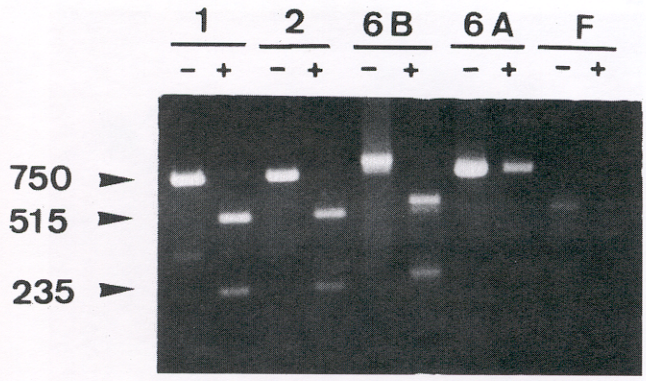

Figure 6 Genetic characterization of HHV-6 in the HIV-1 infected brain tissue. Genotyping of HHV-6 was carried out by using a Hind III restriction site polymorphism within the viral genome. In two patients (cases 1 and 2), after nested PCR amplification and Hind III digestion, two bands of 515bp and 235bp are visible, indicating the presence of a B variant genome. The plus and minus symbols indicate the presence and absence of Hind III digestion, respectively. Sizes of DNA fragments are indicated on the left. Lane 6B is DNA from a HHV-6 B variant control and 6A is DNA from a HHV-6 A variant control. *(Doublet bands represent PCR product from both outer and inner primer sets). Lane F is DNA from fetal brain. (The weak band at ~ 530bp represents an artifact of PCR amplification in the absence of HHV-6 DNA).*

Immunocytochemistry for HHV-6

No cells in any of the five pediatric AIDS cases were immunoreactive for either HHV-6 early nuclear phosphoproteins, viral surface glycoproteins or the 101kDa major immunoreactive virion protein, although staining for HIV-1 p24 was positive in all. HHV-6 infected CBMC expressed all HHV-6 antigens, consistent with productive infection, while uninfected CBMC were negative. Control, HIV-1 negative tissues were completely negative for HHV-6 antigens.

Genetic characterization of HHV-6 in HIV-1 infected brain tissues

In the two patients (case 1 and case 2) in whom frozen CNS tissue was available, we observed two bands of 515bp and 235bp, indicating the presence of the HHV-6 B strain, after nested PCR amplification and Hind III digestion (Figure 6). In the other three cases, DNA was extracted from paraffin embedded tissue but was of insufficient quantity for restriction enzyme analysis. We did not detect HHV-6 DNA by PCR in CNS tissue from the control cases.

Discussion

In this study, we have shown that HHV-6 gene sequences are found considerably more frequently, in cells of the developing CNS, in cases where HIV-

1 infection was present than in controls, a previously unreported finding. Only 10–30% of children born to HIV-1-infected mothers become infected, mostly perinatally, but clinical disease appears in many of these children before the age of 2 years (Scott *et al*, 1989; Rossi *et al*, 1992), and the CNS may be heavily infected (Shaw *et al*, 1985). AIDS encephalopathy is common, and is characterized by neuropathological changes (Sharer *et al*, 1986) and by developmental delays and/or loss of motor milestones and intellectual abilities acquired previously (Blanche *et al*, 1990; Epstein *et al*, 1986). Because HHV-6 infection is acquired postnatally, after the waning of maternal immunity, extensive dissemination of HHV-6 in the CNS may occur in response to inflammatory processes of HIV-1 encephalitis (Genis *et al*, 1992; Gelbard *et al*, 1994) or due to coincident immunodeficiency.

While re-activated CNS infections, particularly JCV and CMV, have been implicated as co-factors in AIDS in adults (Atwood *et al*, 1993), the number of reported opportunistic infections in the CNS of children with HIV-1 infection is substantially less than for adults (Epstein *et al*, 1986; Sharer *et al*, 1986). Indeed, Achim *et al*, (1994) demonstrated by PCR that HHV-6 was not present in the CNS of adult AIDS patients. Although more extensive studies are required to draw definitive conclusions, the presence of HHV-6 gene sequences in four/five pediatric AIDS brains examined (it is possible that case 4 never acquired systemic HHV-6 infection) suggests that HHV-6 is an important opportunistic agent in pediatric AIDS and warrants further study. We speculate that the more extensive dissemination of HHV-6 in the CNS in children than in adults may reflect the combined effects of (1) primary HHV-6 infection as opposed to reactivation, (2) persistent HIV-1 infection of the nervous system and (3) coincident immunodeficiency at the time of HHV-6 infection.

Recently, we and others have demonstrated, in postmortem CNS tissues from children who died with severe HIV encephalopathy, that HIV-1 produces a 'restricted' infection in astrocytes (Saito *et al*, 1994; Tornatore *et al*, 1994) in addition to the well-known productive infection of macrophages/microglia (Sharer, 1992). *In vitro* experiments have suggested that glial cells are susceptible to HHV-6 infection (Tedder *et al*, 1987; Ablashi *et al*, 1987: Levy *et al*, 1990b), but little is known about its cellular tropism in human brain. Recently, Drobyski *et al* (1994) reported a case of fatal encephalitis due to HHV-6 B infection or reactivation in a bone marrow transplant recipient. HHV-6 B was the only virus type identified in two of our five patients and this finding is consistent with previous observations in children with primary HHV-6 infection (Dewhurst *et al*, 1992, 1993). In Drobyski's case, most of the HHV-6-infected cells appeared to be astrocytes, with lesser infection of oligodendrocytes, even though most of the infected cells and tissue damage occurred in the white matter. In contrast, we found HHV-6 DNA in numerous oligodendrocytes of the white matter, but less frequently in astrocytes, macrophages and microglia, and also in neurons. Thus, these data demonstrate that HHV-6 infects a broader spectrum of neural cells than HIV-1 *in vivo*. However, it remains to be determined whether astrocytic or oligodendroglial infection by HHV-6 contributes to white matter pathology in pediatric AIDS patients.

Interestingly, we did not find evidence for expression of HHV-6 antigens with any of the HHV-6 specific antibodies currently available, even in tissues where strong signals were obtained by *in situ* hybridization. This negative finding suggests that HHV-6 in neural cells is latent during late stages of AIDS encephalopathy, although expression of unassayed HHV-6 gene products has not been ruled out. Minimal virus production demonstrated by culture, immunocytochemistry, or electron microscopy only weeks after acute herpes virus encephalitis is not uncommon (Nicoll *et al*, 1991). We speculate that active HHV-6 infection occured at an earlier time, perhaps during primary viral infection, and that this led to viral dissemination in the CNS, with subsequent viral persistence.

We demonstrated that HHV-6 gene sequences and HIV-1 antigens are infrequently co-localized in macrophages, where both these viruses produce active infection, in the CNS of pediatric patients. Co-infection could also occur in microglia, but is less likely in astrocytes or oligodendrocytes, or in neurons where HIV-1 was identified by PCR *in situ* in a single report (Nuovo *et al*, 1994). *In vitro* studies indicate that co-infection leads to activation of HIV-1 long terminal repeat (LTR)-directed gene expression (Horvat *et al*, 1989; Lusso *et al*, 1989; Ensoli *et al*, 1989). Thus, although the frequency of HIV-1/HHV-6 co-infection was low, the possibility exists that HHV-6 proteins may transiently activate cellular or HIV-1 genes, particularly if productive HHV-6 infection of neural cells occurs at the time of primary infection. However, activation of HHV-6 by HIV-1 gene products, such as *tat* (Frankel and Pabo, 1988), is less likely due to the unusual nature of the HHV-6 promoter (Agulnick *et al*, 1994).

In summary, this study provides evidence that HHV-6 is widely disseminated in neural cells in postmortem pediatric AIDS brains, and could thus trigger HIV-1 replication during the course of AIDS encephalopathy in children. Further studies with HHV-6/HIV-1 co-infections in animal model systems (Cvetkovich *et al*, 1992) are needed to establish the temporal relationship of HIV-1 and HHV-6 infections in brain tissue, which is the key to a fuller understanding of the role of HHV-6 in the pathogenesis of AIDS encephalopathy.

Materials and methods

HIV-1 cases
Brain tissue was obtained from five children, ranging in age from 6 months to 9 years, who died with perinatally acquired HIV-1 infection and AIDS. Four of these children had HIV-1 encephalitis, on neuropathological examination. The clinical and pathological findings in these children are shown in Table 1. Tissues were fixed in neutral buffered formalin for up to 4 weeks, and blocks were embedded in paraffin for pathological studies. Regions selected for study included cerebral cortex, cerebral white matter, basal ganglia and pons.

Controls
Tissue controls Sections of formalin fixed brain tissue were obtained from an 8-year-old boy who died with Dandy–Walker malformation, an 8-year-old girl with acute intracerebral hemorrhage, and a 19 week old fetus. These cases were all negative for infection by HIV-1 as determined by paraffin PCR.

Cell controls HHV-6 infected and uninfected CBMC were grown using media and cell culture conditions as previously described (Dewhurst et al, 1992). The cells were centrifuged at 250 X g for 10 min, resuspended in a small volume of PBS, placed on Vectabond-treated slides (Vector Laboratories, Burlingame, CA) and fixed for 20 min in 4% (wt/vol) paraformaldehyde in phosphate buffered saline (PBS).

Preparation and PCR amplification of viral DNAs
Genomic DNA was extracted from either frozen brain tissue or formalin-fixed, paraffin-embedded brain tissue sections using a proteinase K incubation extraction protocol similar to that described by Jackson *et al* (1990). A nested PCR protocol was used to amplify a previously described portion of the large tegument protein gene (LTP) from HHV-6 (Dewhurst *et al*, 1993). Genotyping of HHV-6 was carried out by using a Hind III restriction site polymorphism (Aubin *et al*, 1991; Dewhurst *et al*, 1993), which allows the identification of two distinct viral variants, HHV-6 A and HHV-6 B.

Immunocytochemistry
Avidin-biotin immunocytochemistry was performed as previously described (Saito *et al*, 1994) using ABC kits with an avidin-biotin-alkaline phosphatase complex (Vectastain elite, Vector) with New fuchsin chromogen (DAKO, Carpinteria, CA), which gives a red reaction product. Productive HHV-6 infection was identified by staining with mouse monoclonal antibodies and rabbit hyperimmune serum specific for putative early and late structural proteins of the virus. The following primary antibodies were used: anti-p41 monoclonal antibody 9A5D12 (directed against early nuclear phosphoprotein; 1:1 dilution), monoclonal antibody 6A5D5, directed against viral surface glycoproteins gp116, gp64, gp54 (1:1 dilution) (Balachandran *et al*, 1989), UK 82 rabbit antiserum (directed against surface glycoproteins gp82, gp105; 1:400 dilution) (kindly provided by Dr Bala Chandran, University of Kansas Medical Center); and a monoclonal antibody (C3108 103) (kindly provided by Dr Phillip Pellett, Centers for Disease Control and Prevention) directed against the 101kDa major immunoreactive virion protein of HHV-6 B (1:200 dilution) (Pellett *et al*, 1993).

Preparation of digoxigenin-labeled DNA probe for in situ *hybridization*
A DNA probe was prepared by labeling HHV-6 LTP DNA fragments with digoxigenin 11-dUTP (Boehringer Mannheim, Indianapolis, IN) by PCR (Seibl *et al*, 1990), using previously described primers (Dewhurst *et al*, 1993) and the plasmid pZVH14 containing an 8.7-kb Hind III fragment of HHV-6 GS strain DNA (Josephs *et al*, 1986). The amplification conditions were those used previously (Dewhurst *et al*, 1993), except that dTTP was partially (30%) substituted with dig-11-dUTP.

In situ *hybridization*
Five μm thick brain tissue sections were placed onto Vectabond-coated glass slides and baked at 50°C for 1 h. The sections were dewaxed in three changes of Propar (Anatech, Battle Creek, MI) and rehydrated in graded ethanols. After rehydration in PBS, the sections were treated with proteinase K (2μg ml^{-1}) in 20mM Tris-HCl pH7.5/2mM$CaCl_2$ for 30 min at 37°C. Sections were washed in PBS and then acetylated in 0.25% acetic anhydride/0.1M triethanolamine (pH8.0) for 10 min. The hybridization mixture consisted of digoxigenin-labeled probe (1:200 dilution), 50% formamide, 2XSSC, 50mMTris-HCl pH 7.5, 2XDenhard's solution, 1mM EDTA, 2% dextran sulfate, fragmented salmon-sperm DNA and tRNA. Hybridization mixture(30μl) was applied to the sections and sealed with high pressure vacuum grease under a coverslip. Probe and target DNAs were simultaneously denatured by placing the sections on an 80°C heating block for 10 min. Hybridization was carried out overnight at 40°C in a sealed humidified container. The post-hybridization washes consisted of one wash in 50% formamide/2XSSC, one wash in 2XSSC, and one wash in 0.2XSSC at 40°C for 15min. Hybridized DNA was detected by using an anti-digoxigenin Fab fragment conjugated to alkaline phosphatase (AP) followed by detection of specifically bound AP using nitroblue tetrazolium (NBT) and X-phosphate (X-phos) chromogen (Boehringer Mannheim) that gives a blue tetrazolium pigment, with an eosin counterstain. The same procedure was used on HHV-6 infected and uninfected CBMC except deparaffinization.

Combined immunocytochemistry and in situ *hybridization*

In situ hybridization was combined with immunocytochemistry for simultaneous detection of HHV-6 nucleic acid sequences and either cell specific antigens or HIV-1 antigens. Tissue sections were first immunostained using the alkaline phosphatase reaction with New fuchsin substrate, followed by *in situ* hybridization as described above. The following primary antibodies were used: polyclonal anti-glial fibrillary acidic protein (GFAP) antibody (Dako) as a marker for astrocytes; monoclonal anti-galactocerebroside antibody (Boehringer Mannheim) as a marker for oligodendrocytes; monoclonal anti-CD68 antibody (Dako) as a marker for activated macrophages and microglia; polyclonal anti-PGP 9.5 antibody (UltraClone, Isle of Wight, UK) as a marker for neurons: polyclonal anti p24 antibody (ABT, Cambridge MA) as a marker for productive HIV-1 infection.

Acknowledgments

We thank Dr Robert Gallo for the gift of plasmid pZVH14, we also thank Drs Philip Pellett and Bala Chandran for the gift of primary antibody to HHV-6 (C3108 103 and 9A5D12, 6A5D5, UK82) respectively. We acknowledge Kim McIntyre, Nurcan Ergin, Kirk Dzenko, Bojun Chen, He Wang and especially Harold James, for expert technical assistance. This work was supported by grants 770226-14-PF (YS) from the Pediatric AIDS Foundation, RO1 AI33020-02 (CBH) from the NIAID, KO4 AI01240 (SD) from the NIAID and PO1 NS31492-02 (LGE, BMB, and LRS) from the NINDS. Dr Saito is a Pediatric AIDS Foundation scholar.

References

Ablashi DV, Balachandran N, Josephs SF. Hung CI, Krueger GRF, Kramarsky B, Salahuddin SZ, Gallo RC (1991). Genomic polymorphism, growth properties, and immunologic variations in human herpesvirus-6 isolates. *Virology* **184**: 545–552.

Ablashi DV, Salahuddin SZ, Josephs SF, Imam F, Lusso P, Gallo RC, Hung C, Lemp J, Markham PD (1987). HBLV (or HHV-6) in human cell lines. *Nature* **329**: 207.

Achim CL, Wang R, Miners DK, Wiley CA,(1994). Brain viral burden in HIV infection. *J Neuropathol Exp Neurol* **53**: 284–294.

Agulnick AD, Thompson JR, Ricciardi RP (1994). An ATF/CREB site is the major regulatory element in the human herpesvirus 6 DNA polymerase promoter. *J Virol* **68**: 2970–2977.

Asano Y, Yoshikawa T, Kajita Y, Ogura R, Suga S, Yazaki T, Nakashima T, Yamada A, Kurata T (1992). Fatal encephalitis/encephalopathy in primary human herpesvirus-6 infection. *Arch Dis Child* **67**: 1484–1485.

Asano Y, Yoshikawa T, Suga S, Yazaki T, Kondo K, Yamanishi K (1990). Fatal fulminant hepatitis in an infant with human herpesvirus-6 infection. *Lancet* **335**: 862–863.

Atwood WJ, Berger JR, Kaderman R, Tornatore CS, Major EO (1993). Human immunodeficiency virus type 1 infection of the brain. *Clin Microbiol Rev* **6**: 339–366.

Aubin J-T, Collandre HJ. Candotti D, Ingrand D, Rouzioux C, Burgard M, Richard S, Huraux J-M, Agut H (1991). Several groups among human herpesvirus 6 strains can be distinguished by southern blotting and polymerase chain reaction. *J Clin Microbiol* **29**: 367–372.

Balachandran N, Amelse RE, Zhou WW, Chang CK (1989). Identification of proteins specific for human herpesvirus 6-infected human T cells. *J Virol* **63**: 2835–2840.

Blanche S, Tardieu M, Duliege A-M, Rouzioux C, LeDeist F, Fukunaga K, Caniglia M, Jacomet C, Messiah A, Griscelli C (1990). Longitudinal study of 94 symptomatic infants with perinatally acquired human immunodeficiency virus infection. *Am J Dis Child* **144**: 1210–1215.

Caserta MT, Hall CB, Schnabel K, McIntyre K, Long C, Costanzo M, Dewhurst S, Insel R, Epstein LG (1994). Neuroinvasion and persistence of human herpesvirus-6 in children. *J Infect Dis* **170**: 1586–1589.

Cvetkovich TA, Lazer E, Blumberg BM, Saito Y, Eskin TA, Reichman R, Baram DA, del Cerro C, Gendelman HG, del Cerro M, Estein LG (1992). Human immunodeficiency virus type 1 infection of neural xenografts. *Proc Natl Acad Sci USA* **89**: 5162–5166.

Dewhurst S, Chandran B, McIntyre K, Schnabel K, Hall CB (1992). Phenotypic and genetic polymorphisms among human herpesvirus-6 isolates from North American infants. *Virology* **190**: 490–493.

Dewhurst S, McIntyre K, Schnabel K, Hall CB (1993). Human herpesvirus 6 (HHV-6) variant B accounts for the majority of symptomatic primary HHV-6 infections in a population of US infants. *J Clin Microbiol* **31**: 416–418.

DiLoreto D, Epstein LF, Lazer ES, Britt WJ, del Cerro M (1994). Cytomegalovirus infection of human retinal tissue: An *in vivo* model. *Lab Invest* **71**: 141–148.

Downing RG, Sewankambo N, Serwadda D, Honess R, Crawford D, Jarrett R, Griffin BE (1987). Isolation of human lymphotropic herpesviruses from Uganda. *Lancet* **ii**: 390.

Drobyski WR, Knox KK, Majewski D, Carrigan DR (1994). Fatal encephalitis due to variant B human herpesvirus-6 infection in a bone marrow-transplant recipient. *N Engl J Med* **330**: 1356–1360.

Ensoli B, Lusso P, Schachter F, Josephs SFR, Rappaport J, Negro F, Gallo RC, Wong-Staal F (1989). Human herpes virus-6 increases HIV-1 expression in co-infected T cells via nuclear factors binding to the HIV-1 enhancer. *EMBO J* **8**: 3019–3027.

Epstein LG, Sharer LR, Oleske JM, Connor EM, Goudsmit J, Bagdon L, Robert-Guroff M, Koenigsberger MR (1986), Neurologic manifestations of human immunodeficiency virus infection in children. *Pediatrics* **78**: 678–687.

Fox JD, Briggs M, Ward PA, Tedder RS (1990). Human herpesvirus 6 in salivary glands. *Lancet* **336**: 590–593.

Frankel AD, Pabo CO (1988). Cellular uptake of the tat protein from human immunodeficiency virus. *Cell* **55**: 1189–1193.

Fraser NW, Lawrence WC, Wroblewska Z, Gilden DH,

Koprowski H (1981). Herpes simplex type 1 DNA in human brain tissue. *Proc Natl Acad Sci USA* **78**: 6461–6465.

Gelbard HA, Nottet HSLM, Swindells S, Jett M, Dzenko KA, Genis P, White R, Wang L, Choi Y-B, Zhang D, Lipton SA, Tourtellotte WW, Epstein LG, Gendelman HE (1994). Platelet-activating factor: a candidate human immunodefiency virus type 1-induced neurotoxin. *J Virology* **68**: 4628–4635.

Genis P, Jett M, Bernton EW, Boyle T, Gelbard HA, Dzenko K, Keane RW, Resnick L, Mizrachi Y, Volsky DJ, Epstein LG, Gendelman HE (1992). Cytokines and arachidonic metabolites produced during human immunodeficiency virus (HIV)-infected macrophage-astroglia interactions: Implications for the neuropathogenesis of HIV disease. *J Exp Med* **176**: 1703–1718.

Gopal MR, Thomson BJ, Fox J, Tedder RS, Honess RW (1990). Detection by PCR of HHV-6 and EBV DNA in blood and oropharynx of healthy adults and HIV-seropositives. *Lancet* **335**: 1598–1599.

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

Horvat RT, Wood C, Balachandran N (1989). Transactivation of human immunodeficiency virus promoter by human herpesvirus 6. *J Virology* **63**: 970–973.

Ishiguro N, Yamada S, Takahashi T, Takahashi Y, Togashi T, Okuno T, Yamanishi K (1990). Meningo-encephalitis associated with HHV-6 related exanthem subitum. *Acta Paediatr Scand* **79**: 987–989.

Jackson DP, Lewis FA, Taylor GR, Boylston AW, Quirke P (1990). Tissue extraction of DNA and RNA and analysis by the polymerase chain reaction. *J Clin Pathol* **43**: 499–504.

Johnson RT (1982). *Viral Infections of the Nervous System*. Raven Press: New York, pp 237–43.

Josephs SF, Salahuddin SZ, Ablashi DV, Schachter F, Wong-Staal F, Gallo RC (1986). Genomic analysis of the human B-lymphotropic virus (HBLV). *Science* **234**: 601–603.

Karlin S, Mocarski ES, Schachtel GA (1994). Molecular evolution of herpesviruses: Genomic and protein sequence comparisons. *J Virol* **68**: 1886–1902.

Knox KK, Carrigan DR (1994). Disseminated active HHV-6 infections in patients with AIDS. *Lancet* **343**: 577–578.

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.

Kondo K, Nagafuji H, Hata A, Tomomori C, Yamanishi K (1993). Association of human herpesvirus 6 infection of the central nervous system with recurrence of febrile convulsions. *J Infect Dis* **167**: 1197–1200.

Levy JA, Ferro F, Greenspan D, Lennette ET (1990a). Frequent isolation of HHV-6 from saliva and high seroprevalence of the virus in the population. *Lancet* **335**: 1047–1050.

Levy JA, Ferro F, Lennette ET, Oshiro L, Poulin L (1990b). Characterization of a new strain of HHV-6 (HHV-6 SF) recovered from the saliva of an HIV-infected individual. *Virology* **178**: 113–121.

Lusso P, Ensoli B, Markham PD, Ablashi DV, Salahuddin SZ, Tschachler E, Wong-Staal F, Gall RC (1989). Productive dual infection of human CD4$^+$ T lymphocytes by HIV-1 and HHV-6. *Nature* **337**: 370–373.

Möller KL (1956). Exanthema subitum and febrile convulsions. *Acta Pediatr* **45**: 534–540.

Nicoll JAR, Maitland NJ, Love S (1991). Autopsy neuropathological findings in 'burnt out' herpes simplex encephalitis and use of the polymerase chain reaction to detect viral DNA. *Neuropathol Appl Neurobiol* **17**: 375–382.

Nuovo GJ, Gallery F, MacConnell P, Braun A (1994). *In situ* detection of polymerase chain reaction-amplified HIV-1 nucleic acids and tumor necrosis factor-α RNA in the central nervous system. *Am J Pathol* **144**: 659–666.

Okuno T, Higashi K, Shiraki K, Yamanishi K, Takahashi M, Kokado Y, Ishibashi M, Takahara S, Sonoda T, Tanaka K, Baba K, Yabuuchi H, Kurata T (1990). Human herpesvirus 6 infection in renal transplantation. *Transplantation* **49**: 519–522.

Okuno T, Takahashi K, Balachandra K, Shiraki K, Yamanishi K, Takahashi M, Baba K (1989). Seroepidemiology of human herpesvirus 6 infection in normal children and adults. *J Clin Microbiol* **27**: 651–653.

Pellett PE, Sánchez-Martínez D, Dominguez G, Black JB, Anton E, Greenamoyer C, Dambaugh TR (1993). A strongly immunoreactive virion protein of human herpesvirus 6 variant B strain Z29: Identification and characterization of the gene and mapping of a variant-specific monoclonal antibody reactive epitope. *Virology* **195**: 521–531.

Pruksananonda P, Hall CB, Insel RA, McIntyre K, Pellett PE, Long CE, Schnabel KC, Pincus PH, Stamey FR, Dambaugh TR, Stewart JA (1992). Primary human herpesvirus 6 infection in young children. *N Eng J Med* **326**: 1445–1450.

Rossi P *et al* (1992). Report of a consensus workshop. Maternal factors involved in mother-to-child transmission of HIV-1, Siena, Italy, January 17–18, 1992. *J Acquir Immune Defic Syndr* **5**: 1019–1029.

Saito Y, Sharer LR, Epstein LG, Michaels J, Mintz M, Louder M, Golding K, Cvetkovich TA, Blumberg BM (1994). Overexpression of *nef* as a marker for restricted HIV-1 infection of astrocytes in postmortem pediatric central nervous tissues. *Neurology* **44**: 474–481.

Salahuddin SZ, Ablashi DV, Markham PD, Josephs SF, Sturzenegger S, Kaplan M, Halligan G, Biberfeld P, Wong-Staal F, Kramarsky B, Gallo RC (1986). Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* **234**: 596–600.

Saxinger C, Polesky H, Eby N, Grufferman S, Murphy R, Tegtmeir G, Parekh V, Memon S, Hung C (1988). Antibody reactivity with HBLV(HHV-6) in US populations. *J Virol Methods* **21**: 199–208.

Schirmer EC, Wyatt LS, Yamanishi K, Rodriguez WJ, Frenkel N (1991). Differentiation between two distinct classes of viruses now classified as human herpesvirus 6. *Proc Natl Acad Sci USA* **88**: 5922–5926.

Scott GB, Hutto C, Makuch RW, Mastrucci MT, O'Connor T, Mitchell CD, Trapido EJ, Parks WP (1989). Survival in children with perinatally acquired human immunodeficiency virus type 1 infection. *N Engl J Med* **321**: 1791–1796.

Seibl R, Höltke H-J, Rüger R, Meindl A, Zachau HG, Raßhofer R, Roggendorf M, Wolf H, Arnold N, Wienberg

J, Kessler C (1990). Non-radioactive labeling and detection of nucleic acids: III. Applications of the digoxigenin system. *Biol Chem Hoppe-Seyler* **371**: 939–951.

Sharer LR, Epstein LG, Cho E-S, Joshi VV, Meyenhofer MF, Rankin LF, Petito CK (1986). Pathologic features of AIDS encephalopathy in children: evidence for LAV/HTLV-III infection of brain. *Hum Pathol* **17**: 271–284.

Sharer LR (1992). Pathology of HIV-1 infection of the central nervous system, a review. *J Neuropathol Exp Neurol* **51**: 3–11.

Shaw GM, Harper ME, Hahn BH, Epstein LG, Gajdusek DC, Price RW, Navia BA, Petito CK, O'Hara CJ, Groopman JE, Cho E-S, Oleske JM, Wong-Staal F, Gallo RC (1985). HTLV-III infection in brains of children and adults with AIDS encephalopathy. *Science* **227**: 177–182.

Tedder RS, Briggs M, Cameron CH, Honess R, Robertson D, Whittle H (1987). A novel lymphotropic herpesvirus. *Lancet* **ii**: 390–392.

Tornatore C, Chandra R, Berger JR, Major EO (1994). HIV-1 infection of subcortical astrocytes in the pediatric central nervous system. *Neurology* **44**: 481–487.

Yamanishi K, Okuno T, Shiraki K, Takahashi M, Kondo T, Asano Y, Kurata T (1988). Identification of human herpesvirus-6 as a causal agent for exanthem subitum. *Lancet* **i**: 1065–1067.

Yoshikawa T, Nakashima T, Suga S, Asano Y, Yazaki T, Kimura H, Morishima T, Kondo K, Yamanishi K (1992). Human herpesvirus-6 DNA in cerebrospinal fluid of a child with exanthem subitum and meningo-encephalitis. *Pediatrics* **89**: 888–890.