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Human Herpesvirus 6 DNA Levels in Cerebrospinal Fluid Due to Primary Infection Differ from Those Due to Chromosomal Viral Integration and Have Implications for Diagnosis of Encephalitis▽

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The prevalence and concentration of human herpesvirus 6 (HHV-6) DNA in the cerebrospinal fluid (CSF) of the immunocompetent in primary infection was compared with that in viral chromosomal integration. Samples from 510 individuals with suspected encephalitis, 200 young children and 310 older children and/or adults, and 12 other patients were tested. HHV-6 DNA concentration (log_{10} copies/ml) was measured in CSF, serum, and whole blood using PCR. Serum HHV-6 immunoglobulin G antibody was measured by indirect immunofluorescence. Primary infection was defined by antibody seroconversion and/or a low concentration of HHV-6 DNA (<3.0 log_{10} copies/ml) in a seronegative serum. Chromosomal integration was defined by a high concentration of viral DNA in serum (≥3.5 log_{10} copies/ml) or whole blood (≥6.0 log_{10} copies/ml). The prevalences of CSF HHV-6 DNA in primary infection and chromosomal integration were 2.5% and 2.0%, respectively, in the young children (<2 years) and 0% and 1.3%, respectively, in the older children and/or adults. The mean concentration of CSF HHV-6 DNA in 9 children with primary infection (2.4 log_{10} copies/ml) was significantly lower than that of 21 patients with viral chromosomal integration (4.0 log_{10} copies/ml). Only HHV-6B DNA was found in primary infection, whereas in viral integration, 4 patients had HHV-6A and 17 patients HHV-6B. Apart from primary infection, chromosomal integration is the most likely cause of HHV-6 DNA in the CSF of the immunocompetent. Our results show that any diagnosis of HHV-6 encephalitis or other type of active central nervous system infection should not be made without first excluding chromosomal HHV-6 integration by measuring DNA load in CSF, serum, and/or whole blood.

Not long after its discovery in 1986 (32), human herpesvirus 6 (HHV-6) was associated with neurological disease when primary infection was described in a liver transplant patient with grand mal fits (44). At that time, primary infection, although usually silent, was known sometimes to cause exanthem subitum (50). However, in the next decade, there were further reports of primary infection and neurological disease, especially in young immunocompetent children with fever and seizures (19, 23, 35, 43). Additionally, occasional cases of encephalitis were diagnosed in young children with primary infection (for a review, see reference 51). Most recently, primary HHV-6 infection has been shown to be an important cause of the neurological emergency of status epilepticus with fever in children up to 2 years old (41).

Primary HHV-6 infection almost invariably occurs in the first 2 years of life (19, 45, 52), but rare cases of encephalitis, presumed due to HHV-6 reactivation, have been reported in immunocompetent older children and adults (28). In the absence of primary infection, the key finding linking HHV-6 to these cases was the detection of viral DNA in cerebrospinal fluid (CSF) by PCR, leading to the assumption that HHV-6 was replicating in the central nervous system (CNS). However, this interpretation has been questioned in view of the phenomenon of HHV-6 chromosomal integration (8, 46).

HHV-6 is the only human herpesvirus found integrated into host chromosomes (10, 37, 38). The occasional individual with such integration is easily identifiable, since every leukocyte inevitably contains viral sequences and there are thus characteristically persistent high levels of HHV-6 DNA in both serum and whole blood (8, 46). Since normal CSF, i.e., in the absence of inflammation, contains a few leukocytes, it is only to be expected that, in cases of HHV-6 integration, viral DNA will be present in CSF even though there is no viral replication. This is in sharp contrast to the usual scenario in immunocompetent persons in whom, after primary infection, HHV-6 persists throughout life and is only found in the infrequent leukocyte (7), and hence, viral DNA is not normally found in CSF.

Thus, when assessing the significance of viral DNA in CSF and its relationship to neuropathogenesis, chromosomal HHV-6 integration must be distinguished from primary infection in young children and from putative virus reactivation in older individuals. In this context, it is also important to differ-

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TABLE 1. Frequency of HHV-6 DNA in the CSF of immunocompetent children with primary infection

Patient no. (sex)[a]	Age at onset of illness	Presence of HHV-6 DNA in acute-phase serum (variant)	CSF		Clinical details
			No. of days after onset of illness that sample was taken	Presence of HHV-6 DNA (variant, $\log_{10}$ HHV-6 DNA copies/ml)	
P1 (F)[b]	4 mo	+ (B)	0[c]	−	Fever, rash, possible encephalitis/meningitis
P2 (M)[b,d]	9 mo	+ (B)	0	−	Fever, convulsions
P3 (F)[d]	10 mo	−	1	+ (B, 1.6)	Fever, convulsions
P4 (F)[d]	11 mo	+ (B)	0	−	Fever, rash, convulsions
P5 (M)[b,d]	11 mo	+ (B)	3	−	Fever, rash, convulsions
			5	+ (B, 3.6)	Fever, rash, convulsions
P6 (M)[b,d]	1 yr	+ (B)	1	+ (B, 2.6)	Fever, convulsions
P7 (F)[b,d]	1 yr	+ (B)	1	−	Fever, rash, convulsions
P8 (M)[d]	1 yr, 3 mo	−	1	+ (NT, NT)	Fever, rash, convulsions
P9 (M)[b,d]	1 yr, 8 mo	+ (B)	1	+ (B, 1.6)	Fever, encephalopathy

[a] Further details for patients have been reported elsewhere (41, 47). M, male; F, female.
[b] Primary infection defined by low concentration HHV-6 DNA, i.e., <3.0 $\log_{10}$ HHV-6 DNA copies/ml in an acute seronegative serum.
[c] Day of onset of illness unknown so day of first sample defined as 0.
[d] Primary infection defined by seroconversion to HHV-6 IgG between acute- and convalescent-phase sera.

entiate HHV-6 variant A (HHV-6A) from HHV-6 variant B (HHV-6B), as either virus may be chromosomally integrated (8, 46) but only HHV-6B is found in primary infection in young children (14). We now report on the prevalence and concentration of HHV-6A and B DNA in the CSF of immunocompetent patients with suspected encephalitis and compare the findings for primary infection with those for viral chromosomal integration. The implications for diagnosis of HHV-6 encephalitis are discussed.

MATERIALS AND METHODS

Patients. (i) Group 1, routine diagnosis patients. There were 510 immunocompetent patients of all ages with neurological illness whose serum and CSF samples had been referred between October 1998 and September 2003 for routine diagnosis of HHV-6 infection. This group includes children aged 2 months to 3 years reported to a British Isles-wide survey between October 1998 and September 2001 because of suspected encephalitis (41).

(ii) Group 2, referred HHV-6 patients. There were 12 immunocompetent patients found to have viral DNA in their CSF with presumed HHV-6 encephalitis. Samples of whole blood and/or serum and CSF from these patients had been sent to us for further investigation between October 1998 and September 2005.

The samples from both groups of patients had originated from all over the British Isles, and informed consent was obtained in the usual way by the referring clinician in accordance with good clinical practice.

Semiquantitative PCR for HHV-6. Nucleic acid (both RNA and DNA) was extracted from serum or CSF using the QIAmp RNA mini kit (QIAGEN Ltd., Crawley, United Kingdom) and the extract tested for HHV-6 DNA using a nested PCR (22). As previously described (47), the amount of viral DNA in positive specimens was estimated semiquantitatively by PCR using serial 10-fold dilutions. The result was expressed as $\log_{10}$ copies/ml. This method was used on samples up to 2003, after which it was superseded by the quantitative PCR described below.

Quantitative PCR for HHV-6. DNA was extracted from whole blood, serum, or CSF using the QIAmp DNA mini kit (QIAGEN Ltd., Crawley, United Kingdom). Five microliters of nucleic acid extract was tested for HHV-6 DNA. A *Taq*Man PCR was performed using an ABI 7700 sequence detector (Applied Biosystems). Amplification primers were U67F, 5′-GGCTAGAACGTATTTG CTGCAGA-3′, and U67R, 5′-AATGTACGTCCCCGAAATGG-3′, and the *Taq*Man probe was U67P, 5′-(FAM)CGTTTCGCGGACTCAAGATCAACAA GTT(TAMRA)-3′. A 73-bp sequence of the U67 open reading frame was amplified (8). The lower limit of detection was 5 copies/reaction mixture. Control standards were known copy numbers of plasmid cloned amplicon. All samples were tested in duplicate, and the mean was used to calculate the HHV-6 DNA concentration, which was expressed as $\log_{10}$ copies/ml of original sample. The mean difference between replicates was 8%.

Determination of HHV-6 DNA copies/cell. Five microliters of DNA extract from whole blood, serum, or CSF was subjected to quantitative HHV-6 PCR, and the result was compared with the result of quantitative PCR for human β-globin (25). The HHV-6 PCR (8) amplifies DNA from part of the HHV-6 U67 gene, of which there is only one copy per virus genome (16). Since there are 2 copies of β-globin/cell, the number of viral DNA copies/cell is $2 \times$ HHV-6 DNA copies/β-globin DNA copies (46).

Variant typing of HHV-6. Restriction enzyme analysis of PCR products was used to distinguish HHV-6A from HHV-6B, as previously described (22).

Definitions. (i) Primary HHV-6 infection. Validated tests for HHV-6 antibody avidity (42, 45, 48) and DNA (47) were used. As described elsewhere (41, 47), the diagnosis of primary HHV-6 infection was based on seroconversion to HHV-6 immunoglobulin G between acute- and convalescent-phase sera and/or PCR to detect low level HHV-6 DNA, i.e., concentration of <3.0 $\log_{10}$ copies/ml in an acute seronegative serum.

(ii) High-level serum HHV-6 DNA. A serum HHV-6 DNA concentration of >3.5 $\log_{10}$ copies/ml was defined as high based on the 95% confidence limits for the mean level found in individuals with persistent high levels of HHV-6 DNA (24, 46, 47).

(iii) High-level whole-blood HHV-6 DNA. An HHV-6 DNA concentration of >6.0 $\log_{10}$ copies/ml in whole blood was defined as high (8, 46).

(iv) HHV-6 chromosomal integration. In HHV-6 chromosomal integration, there is ≥ 1 copy of HHV-6 DNA/leukocyte and, consequently, a high viral DNA concentration in whole blood. Similarly, in serum there is ≥ 1 copy of HHV-6 DNA/lysed leukocyte, and the level of HHV-6 DNA is high, although about 50-fold lower than that of whole blood. These high levels define viral chromosomal integration and are not seen in other circumstances (46).

Statistics. The exact confidence limits for proportions were calculated by using the method of Armitage and Berry (3). The *t* distribution was used to calculate the 95% confidence limits for a sample mean. The significance of the difference between two sample means was estimated using the paired *t* test and the Mann Whitney two-sample rank sum test.

RESULTS

HHV-6 DNA in the CSF and serum of immunocompetent children with primary infection. Table 1 shows the results of testing CSF and acute-phase serum from 9 patients in group 1 (routine diagnosis patients P1 to P9) with primary infection (see "Definitions" in Materials and Methods). All were young children, <2 years old, and all presentations were similar, involving fever and convulsions sometimes accompanied by rash.

TABLE 2. Frequency of HHV-6 DNA in the CSF of immunocompetent patients with high-level viral DNA in serum and/or whole blood[a]

Patient no. (sex)	Age at onset of illness	High-level[b] HHV-6 DNA			CSF		Clinical details
		Presence in serum (variant)	Presence in whole blood (variant)	No. of samples tested[c] (no. of days between 1st and last samples)	No. of days after onset of illness that sample was taken	Presence of HHV-6 DNA (variant, $\log_{10}$ HHV-6 DNA copies/ml)	
HL1 (M)[d]	0 mo	+ (B)	NT	3 (186)	5	+ (B, NT)	Cerebral abnormalities at birth, suspected sepsis/encephalitis[e]
HL2 (F)[f]	0 mo	+[g] (B)	+[g] (B)	6 (174)	5	+ (NT, 4.1)	Neonatal convulsions
HL3 (F)[f]	0 mo	NT	+[g] (B[h])	2 (232)	3	+ (NT, 4.6)	Tachypneic, meconium aspiration
HL4 (M)	0 mo	+ (B)	NT	1 (0)	0	+ (NT, NT)	Neonatal convulsions, intractable epilepsy[e]
HL5 (F)	0 mo	+[g] (B)	+[g] (B)	2 (0)	21	+[g] (B, 3.7)	Neonatal convulsions
HL6 (F)[d]	0 mo	+ (A)	NT	2 (306)	107	+ (NT, NT)	Intrauterine growth retardation, afebrile convulsions, developmental delay
					371	+ (A, 4.0)	
HL7 (NK)	4 mo	+ (B)	NT	1 (0)	0[i]	+ (B, 4.6)	Bulging fontanel, pyrexia, suspected meningococcal meningitis
HL8 (M)[d]	4 mo	+ (A)	NT	6 (184)	0	+ (NT, NT)	HSV-1 encephalitis
HL9 (M)	10 mo	+ (B)	NT	1 (0)	122	+ (B, 3.3)	Fever, convulsions
HL10 (F)	11 mo	+ (B)	NT	1 (0)	0	+ (B, 2.6)	Fever, convulsions
HL11 (M)	1 yr, 1 mo	+ (B)	NT	1 (0)	2	−	Fever, rash, and convulsions
HL12 (NK)	1 yr, 1 mo	+ (B)	NT	1 (0)	0[i]	+ (B, 2.9)	Pyrexia, rash
HL13 (F)	1 yr, 3 mo	+ (B)	NT	1 (0)	0[i]	+ (NT, 5.2)	Encephalitis
HL14 (M)[d]	1 yr, 11 mo	+ (B)	NT	10 (452)	1	+ (B, 5.6)	Acute demyelinating encephalitis
					16	+ (B, 3.0)	
HL15 (F)[d]	2 yr, 9 mo	+ (A)	NT	3 (9)	180	+ (A, NT)	Chronic/subacute encephalitis
					202	+ (NT, NT)	
HL16 (M)[d]	4 yr	+ (B)	NT	2 (15)	0[i]	+ (B, 4.6)	Fever, convulsions
HL17 (M)	4 yr	+ (B)	NT	1 (0)	0[i]	+ (B, 2.6)	Convulsions, encephalopathy
HL18 (F)[d]	12 yr	+ (A)	NT	5 (553)	0	+ (A, NT)	Encephalitis
HL19 (M)[d,f]	23 yr	+[g] (B)	+[g] (NT0)	6 (1,701)	0	+ (B, NT)	Epstein-Barr virus encephalitis
					25	+ (B, 3.6)	
HL20 (F)	52 yr	+ (B)	NT	1 (0)	0[i]	−	HSV-1 encephalitis
					18	+ (B, 3.7)	
HL21 (F)[f]	58 yr	+[g] (B)	+[g] (B[h])	2 (3)	1	+[g] (B, 5.5)	Meningitis, Parkinson's disease

[a] Abbreviations: NT, not tested; M, male; F, female; NK, not known.
[b] High level, >3.5 $\log_{10}$ copies/ml in serum; >6.0 $\log_{10}$ copies/ml in whole blood.
[c] High level in every sample tested.
[d] See reference 47 for more detail.
[e] Mother has high-level serum HHV-6 DNA.
[f] HHV-6 chromosomal integration identified by fluorescent in situ hybridization and ≥1 HHV-6 DNA copy/hair follicle cell (46).
[g] ≥1 HHV-6 DNA copy/cell or lysed cell (46).
[h] Detected in lymphoblastoid cell line derived from this patient's blood.
[i] Onset date unknown, so day of first sample defined as 0.

HHV-6 DNA in CSF. Five of the 9 children (56%) had HHV-6 DNA detected in the CSF. In the 4 cases where there was sufficient CSF for further tests, the mean concentration was 2.4 $\log_{10}$ copies/ml (95% confidence limits, 1.0 to 3.7) and the variant was B. The cell count was known for all but one of the CSF samples tested (median, $<1 \times 10^3$ leukocytes/ml; range, <1 to 2).

HHV-6 DNA in serum. Seven of the 9 children had low-level HHV-6 DNA, i.e., <3.0 $\log_{10}$ copies/ml, detected in the acute-phase serum; in each case, this was variant B. Viral DNA was not detected in any convalescent-phase serum.

HHV-6 DNA in the CSF, serum, and whole blood of immunocompetent children and adults with viral chromosomal integration. Table 2 shows the results of testing for viral DNA in CSF, whole blood, and serum from 21 children and adults with high level HHV-6 DNA in serum and/or whole blood, i.e., viral chromosomal integration (see "Definitions" in Materials and Methods); these comprised 9 patients found to have such high-level viral DNA in group 1 (routine diagnosis patients HL8 to HL11, HL14, HL16 to HL18, and HL 20) and all of the 12 referred HHV-6 patients in group 2 (HL1 to HL7, HL12, HL13, HL15, HL19, and HL21). In all, 4 of these 21 patients had HHV-6A and 17 had HHV-6B. The patients' neurological illnesses were diverse, ranging from fever and convulsions to suspected encephalitis (confirmed in 2 patients as herpes simplex encephalitis).

HHV-6 DNA in CSF. HHV-6 DNA was detected in 20 of the 21 patients' CSF (24/26 CSF samples) (Table 2). Of the 26 CSF samples tested, the leukocyte count was known for 17 (median, 5×10^3 leukocytes/ml; range, <1 to 65). There were four samples with a cell count of $<1 \times 10^3$/ml, and these included the 2 samples in which HHV-6 DNA was not found. In patient HL20, who had herpes simplex encephalitis, HHV-6 DNA was not detected in the first CSF sample, which contained $<1 \times 10^3$ leukocytes/ml but was found in the later CSF sample with many more leukocytes, namely, 50×10^3 leukocytes/ml. In

TABLE 3. Prevalence of HHV-6 DNA in CSF of immunocompetent patients

No. of patients tested	Age (yr)	No. of patients with HHV-6 DNA in CSF (% prevalence[a])	No. of patients with HHV-6 DNA in CSF and HHV-6 primary infection (% prevalence[a])	No. of patients with HHV-6 DNA in CSF and high-level serum HHV-6 DNA[b] (% prevalence[a])	No. of patients with HHV-6 DNA in CSF and indeterminate status (% prevalence[a])
200	<2[c]	14 (7.0)	5 (2.5)	4 (2.0)	5[d] (2.5)
310	≥2[e]	6 (1.9)	0 (0)	4 (1.3)	2[f] (0.6)

[a] No. with HHV-6 DNA/no. tested.
[b] >3.5 $\log_{10}$ HHV-6 DNA copies/ml.
[c] Median age, 0.9 years (range, 0 to 1.9 years).
[d] In 2 cases, serum was not available. In 1 case, high-level serum HHV-6 DNA was excluded. In 1 case, primary infection was excluded. In 1 case, high-level serum HHV-6 DNA was excluded and primary infection was excluded.
[e] Median, 37 years (range, 2 to 88 years).
[f] In 1 case, serum not available. In 1 case, high-level serum HHV-6 DNA was excluded and primary infection was excluded by antibody tests.

patients HL6, HL9, and HL15, samples had been taken long after the onset of illness, i.e., between 107 and 371 days later, yet all contained HHV-6 DNA.

In 16 samples, there was sufficient CSF to quantify the HHV-6 DNA, and the mean concentration was 4.0 $\log_{10}$ copies/ml (95% confidence limits, 3.5 to 4.5); this is the equivalent of an average of at least one copy/CSF leukocyte and at least a log higher than the mean HHV-6 DNA CSF concentration in primary infection ($P = 0.008$, paired t test; $P = 0.02$, Mann-Whitney two-sample rank sum test). In 2 cases, there was sufficient material to carry out a β-globin PCR and, hence, estimate the HHV-6 DNA copies/CSF leukocyte for each individual sample; in both cases, this was ≥1.

HHV-6 DNA in serum and/or whole blood. All 21 patients had high-level HHV-6 DNA in every sample tested. For 12 of these, persistent high-level HHV-6 DNA was demonstrated, since more than one sample was available (Table 2); in the case of HL19, high-level persistence was documented in serum for over 4 years.

Prevalence of HHV-6 DNA in the CSF of immunocompetent patients with neurological illness. Table 3 shows the results for the 510 patients in group 1 (routine diagnosis patients); these comprised 200 children <2 years old and 310 older children and/or adults. Of the young children whose CSF contained HHV-6 DNA, 5 (P3, P5, P6, P8, and P9) (Table 1) had primary HHV-6 infection, and 4 (HL8 to HL10 and HL14) (Table 2) had high-level serum HHV-6 DNA and thus viral chromosomal integration (see "Definitions" in Materials and Methods). Of the older children and/or adults whose CSF contained HHV-6 DNA, there were no cases of primary infection, but 4 patients (HL16 to HL18 and HL20) (Table 2) had high-level serum HHV-6 DNA, i.e., viral chromosomal integration. The prevalence of HHV-6 DNA in CSF associated with HHV-6 chromosomal integration was 2.0% (95% confidence limits, 0.6 to 5.0) in the young children and 1.3% (95% confidence limits, 0.4 to 3.3) in the older children and/or adults; the overall prevalence for all ages was 1.6% (95% confidence limits, 0.7 to 3.1).

DISCUSSION

In this study of HHV-6 DNA in CSF, we compare the results for primary infection with those for viral chromosomal integration. Primary infection, always with HHV-6B, occurs early in life in almost all persons and may sometimes be accompanied by symptoms, i.e., fever and convulsions with or without a rash. In contrast, no disease has yet been causally linked to HHV-6A, nor has the time of first infection with this variant ever been identified. In primary infection with variant B, viral DNA appears transiently in serum at a low level, followed by antibody seroconversion (41, 47). Thereafter, HHV-6 becomes latent, virus is not detected in serum, and viral DNA is present at the low level of around 1 copy per 10^4 to 10^5 leukocytes (7) but not in other cell types, such as hair follicles (46).

On the other hand, in HHV-6 chromosomal integration, both peripheral blood leukocytes and hair follicle cells have a high viral load (37), and as we have recently shown, this results from ≥1 copy of HHV-6 DNA in each of these cell types (46), suggesting that every cell in the body contains virus. In fact, it seems that cases of chromosomal HHV-6 integration are usually inherited in the germ line and passed from parent to child (10, 37). Other notable features of viral chromosomal integration are the presence not only of variant B but sometimes variant A instead (8, 11, 37, 46) and persistent high-level HHV-6 DNA in whole blood and serum (46), which in the latter case, is at least 100-fold higher than that observed briefly in primary infection (47).

Thus, 21 subjects with HHV-6 chromosomal integration were identified in this study because of high viral DNA load in whole blood and/or serum (Table 2). Indeed, for 12 of the patients, persistence of this high-level HHV-6 DNA was also documented. In 4 patients, chromosomal integration was confirmed by fluorescent in situ hybridization on chromosome preparations and ≥1 copy of HHV-6 DNA/cell or lysed cell in hair follicles and whole blood/serum, respectively (8). A fifth patient had ≥1 copy of viral DNA/cell or lysed cell in whole blood and serum, but hair follicles and chromosome preparations were not tested. Moreover, another 2 patients must have inherited viral integration from their mothers, since they also had high-level serum HHV-6 DNA.

Turning now to our findings on CSF in primary infection, HHV-6 DNA was only detected in about half (56%) of CSF samples and at a low mean concentration of 2.4 $\log_{10}$ copies/ml. This presumably reflects the concurrent and similarly brief low-level HHV-6 DNA in serum or plasma seen in the first few days of primary infection (6, 36, 47). Indeed, it has been previously reported that HHV-6 DNA appears transiently in CSF at this time (35).

Notably, almost all of the CSF samples (92%) from the 21

patients with viral chromosomal integration contained HHV-6 DNA, and unlike primary infection, where more than one CSF was available for testing, HHV-6 DNA was shown to persist in every case, even up to a year after the original onset of illness. The mean CSF viral DNA concentration for all samples was 4.0 $\log_{10}$ copies/ml and more than 10-fold higher than that in primary infection. On the basis of the median CSF cell count, it could be calculated that, as expected for viral integration, there was, on average, at least one HHV-6 copy/CSF leukocyte, and this was also confirmed for two individual samples by comparing HHV-6 and human β-globin concentrations. HHV-6 DNA could even be detected in normal CSF with no evidence of inflammation, i.e., the CSF cell count was $\leq 5 \times 10^3$/ml. However, when the cell count was at its lowest, i.e., $<1 \times 10^3$ cells/ml, only half of the samples tested contained detectable HHV-6 DNA, presumably because our test was at the limit of sensitivity.

HHV-6 DNA in CSF is usually taken to indicate active infection, but in chromosomal viral integration, there is no evidence to date of virus replication (27, 38). Thus, many of our patients with integrated virus were initially given a misdiagnosis of HHV-6 encephalitis because of viral DNA in CSF, although they had disparate symptoms, final diagnoses, and outcomes, including 2 patients with confirmed cases of herpes simplex encephalitis, one of whom died (Table 2). It is also worth noting that ganciclovir therapy with its frequent adverse effects was initiated unnecessarily in one case because of such a misdiagnosis (submitted for publication).

Similar confusion has probably arisen in the literature. Several cases from a large study of patients of all ages with focal encephalitis were attributed to HHV-6 because viral DNA was detected in CSF (28), but in the absence of quantitative measurement of HHV-6 DNA, it is difficult to differentiate primary infection from viral integration and the role of the virus is unclear. Turning to older individuals in whom primary infection is extremely unlikely, there are a few case reports describing, in all, 11 immunocompetent adults and 1 teenager. All had clinically defined encephalitis and HHV-6 DNA in their CSF (4, 5, 13, 21, 26, 29, 30, 33, 39), and in each case, the diagnosis was given as HHV-6 encephalitis but viral integration had not been considered. Viral DNA load in CSF was measured in 7 patients; low level HHV-6 DNA was found in 2 of these but in both primary infection was excluded (29, 30) suggesting viral reactivation. The remaining 5 had high viral DNA levels ($\geq$4.0 $\log_{10}$ copies/ml) (5, 21), which although not noted in the reports, is equivalent to at least 1 viral copy/CSF leukocyte, strongly suggesting chromosomal HHV-6 integration. Moreover, in 1 of these 5 patients HHV-6 DNA persisted in CSF for at least 55 days despite therapy with ganciclovir and was also in serum at a high level, in this case $\geq$5.0 $\log_{10}$ copies/ml (5). Thus, unrecognized chromosomal viral integration may often account for HHV-6 DNA in CSF.

Additional support for the idea that chromosomal HHV-6 integration commonly results in viral DNA in CSF in immunocompetent individuals comes from instances in which the HHV-6 variant was typed. There are a remarkable number of cases of encephalitis in immunocompetent adults attributed to variant A, 6 in all (4, 13, 26, 29, 30, 39), as opposed to one attributed to variant B (5, 28). As previously discussed, HHV-6B, not HHV-6A, is responsible for primary infection in childhood, whereas either variant A or B can be integrated into human chromosomes. These findings are once again confirmed in the present paper, where only HHV-6B was detected in the CSF in primary infection and variant A was found in the CSF of some of our patients with evidence of integrated virus and variant B in others. It may therefore be concluded that, when HHV-6A rather than B DNA is detected in CSF, this most likely indicates viral integration. Such integration may well account for the mistaken idea (18) that, since HHV-6A tends to persist in CSF, it is more neurotropic than variant B.

Regarding the 1.6% prevalence of HHV-6 DNA in the CSF due to chromosomal integration in our immunocompetent patients, there was no appreciable difference between young children <2 years old and older children and/or adults, i.e., 2.0 and 1.3%, respectively, findings suggesting that viral integration is always acquired very early in life and consistent with vertical transmission, i.e., inheritance. As expected for a common cause of neurological disease in young children (41), primary infection was also responsible for viral DNA in the CSF of this age group. In contrast, viral chromosomal integration was the main cause of HHV-6 DNA in the CSF of older children and adults who were past the age for primary infection.

Of the prior surveys of the prevalence of HHV-6 DNA in the CSF of predominantly immunocompetent populations, all, as in our case, were based on samples referred for diagnosis of suspected viral CNS infection. The results were 0.05% (1/2,161) (31), 0.3% (1/320) (15), 1.2% (9/753) (12), 1.5% (6/407) (34), and 2.8% (3/106) (20). All but one of these surveys included at least some young children but without analysis for differences due to age. However, two further studies did analyze their results in this way. In one case (2), a prevalence of 2.0% (3/148) was found in children <2 years, but all 3 subjects were less than 2 months old, suggesting acquisition at birth, i.e., chromosomally inherited HHV-6. Isaacson et al. (21), divided their results into those for young children, $\leq$3 years old (0%, 0/98), and older children and adults (0.4%, 4/902), thus excluding HHV-6 DNA in CSF due to primary infection in the latter group. In summary, although not considered a possibility by the authors, the prevalences in all the above studies are of the same order as that attributed by us to HHV-6 chromosomal integration, suggesting that, in most of these instances, HHV-6 DNA in CSF resulted from this phenomenon.

Although neither our survey of HHV-6 DNA in CSF nor those discussed above included normal controls, the prevalence in such controls due to viral integration should be between 0.7% (95% confidence limits, 0.2 to 2.1) and 1.5% (95% confidence limits, 0.7 to 2.8) as judged by our two separate reports of high level serum or plasma HHV-6 DNA, i.e., presumed chromosomal integration, in control populations (24, 47). Further support for these estimates comes from the reported prevalences of between 0.9 to 1.6% for congenital HHV-6 infection (1, 9, 17), i.e., presumed chromosomal integration. All of these frequencies are remarkably similar to the prevalence of 1.6% (95% confidence limits, 0.7 to 3.1) HHV-6 DNA in our CSF samples from immunocompetent patients of all ages with various neurological symptoms. Davies et al. (12) attempted to solve the problem of controls in their study of suspected CNS infections by using clinical and laboratory criteria to define a group of patients unlikely to have CNS viral infection and found a prevalence of 0.3% (95% confidence

limits, 0.01 to 2.8) for HHV-6 DNA in CSF, a finding no different from ours. The only other information comes from controls without CNS disease (40) in whom 1 individual of 107 (95% confidence limits, 0.02 to 5.1) had HHV-6 DNA persisting in two CSF samples collected 4 months apart, presumably because of viral chromosomal integration. From the limited evidence available, the prevalence of HHV-6 DNA in the CSF due to viral chromosomal integration in those with suspected viral CNS infection is the same as that in controls, suggesting that such integration does not predispose to neurological disease.

It may be concluded, therefore, that where HHV-6 DNA is detected in the CSF of immunocompetent patients, it is commonly due to chromosomal viral integration resulting in a mistaken association with encephalitis. This is especially so for older children and adults in whom primary infection, the other common cause of HHV-6 DNA in CSF, is exceptionally rare. A recent editorial commentary (49) asked "Human herpesvirus 6 infection of the central nervous system: is it just a case of mistaken association?" The present findings go a long way toward answering this question; our results show that any diagnosis of HHV-6 encephalitis should not be made without first excluding chromosomal HHV-6 integration by measuring the DNA load in CSF, serum, and/or whole blood. Further support for such integration would be provided by the detection of HHV-6 DNA in hair follicle cells.

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