Persistence of Human Herpesvirus 6 According to Site and Variant: Possible Greater Neurotropism of Variant A

Caroline Breese Hall, Mary T. Caserta, Kenneth C. Schnabel, Christine Long, Leon G. Epstein, Richard A. Insel, and Stephen Dewhurst

Little is known of the persistence and pathogenicity of human herpesvirus 6 (HHV-6) after primary infection, including the role of strain variant. Over 2 to 5 years, 2,716 children and 149 adults were studied. Peripheral blood mononuclear cell (PBMC), saliva, and cerebrospinal fluid (CSF) specimens were examined for HHV-6 DNA and variant. Ninety-nine percent of isolates causing primary infection were HHV-6 variant B (HHV-6B), which predominated in 95%–98% of the variants persisting in PBMC and saliva specimens from children and adults. Of 668 CSF samples, 13% contained HHV-6 DNA; of 77 children examined after primary infection, 61% had HHV-6 DNA detected only in their CSF and 39% had HHV-6 DNA in both CSF and PBMCs. HHV-6 variant A (HHV-6A) was detected significantly (P = .0001) more frequently in CSF than in PBMCs or saliva. In children for whom HHV-6 was identified in both CSF and PBMCs, PBMCs contained only HHV-6B, while CSF contained HHV-6A or HHV-6B, not both. Thus, in patients with dual infection, only HHV-6A persisted in CSF, which suggests that HHV-6A has greater neurotropism.

Findings for adults indicate that dual infection occurs; variant persistence is similar to that for children. The frequency of HHV-6A infection increased little with age, thereby indicating that HHV-6A infection remains uncommon into adulthood. This study suggests that HHV-6 variants have different immunobiologic courses and neurotropism.

Human herpesvirus 6 (HHV-6) is a ubiquitous virus causing infection in early childhood, essentially infecting everyone by 3 years of age [1–3]. Primary infections produce variable febrile illnesses, including roseola, and are caused almost exclusively by one of the two strain groups, namely variant B (HHV-6B) [1–4]. In adults, however, the asymptomatic persistence of both HHV-6B and variant A (HHV-6A) has been detected, thus suggesting that latency of both variants occurs. Reactivation and disease due to HHV-6 infection also have been described, primarily in immunocompromised adults [5, 6]. A direct causal relationship between HHV-6 infection and disease in adults, however, remains unclear. Little information exists as to (1) how often the primary infection in infancy is followed by persistence of the virus and (2) whether the persistence continues for subsequent years and may lead to reactivation and possible clinical manifestations in adults. It is also unclear whether new infections occur in subsequent years.

The two variants may differ in their ability to maintain latency or persistence, in the sites of persistence, and, thus, in their potential pathogenicity in later life. Giving credence to the possibility that the variants have distinct biological characteristics is the observation that only one case of clinical primary infection with HHV-6A in a child has been described, despite the isolation of HHV-6A from adults [2–4]. Hidaka and colleagues [7] recently described an infant in Japan with acute roseola in whom HHV-6A infection was identified. Although the virus was not isolated from the peripheral blood, HHV-6A DNA was identified by PCR analysis of the infant’s peripheral blood mononuclear cells (PBMCs) and CSF during the acute episode of primary infection.

In our long-term prospective studies of children younger than 3 years of age who have acute febrile illnesses and present to the outpatient facilities at our hospital, we have identified >300 children with symptomatic primary HHV-6 infection via viral isolation and seroconversion [3, 8]. Although four isolates from these children had the phenotypic characteristics of HHV-6A, genetic analyses indicated that they had a mixed HHV-6A and HHV-6B genome [3, 4, 9]. Hence, we have not identified symptomatic primary infection with HHV-6A by means of viral isolation with seroconversion, thus indicating the rarity of symptomatic primary infection with HHV-6A in the United States as well as in Japan. Therefore, we prospectively examined a large cohort of children over 2 to 5 years following their primary infection with HHV-6 to determine the occurrence, frequency, and sites of persistence of HHV-6A compared with HHV-6B in their PBMCs, saliva, and CSF (CSF samples were not available for this analysis).

Received 12 May 1997; revised 8 September 1997.

Informed consent was obtained from the patients’ parents or guardians, and the guidelines for human experimentation of the U.S. Department of Health and Human Services and those of the authors’ institution were followed in the conduct of the clinical research.

Grant support: This study was supported by the National Institutes of Allergy and Infectious Diseases (RO1 A 133020-02 and KO4 A 101240).

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Clinical Infectious Diseases 1998;26:132–7
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1058-4838/98/2601–0019$03.00
obtained at the time of evaluation for their febrile illness. Family members were similarly followed up to compare the frequency and type of variant persisting in PBMCs and saliva in the parents and children.

Methods

Patients. From October 1990 to September 1996, children younger than 3 years of age who presented to our emergency and outpatient facilities because of acute febrile and nonfebrile infections were enrolled in the study if they were previously healthy and were excluded from the study if they had an underlying condition affecting their immune function. A subset of the children included in this study who were enrolled from October 1990 to July 1993 were described in previous reports [3, 10]. However, the previously reported studies did not include the subsequent follow-up and variant data for these patients that we now report in conjunction with data obtained from the children enrolled subsequently and from family members. At the initial visit, the child’s history, results of physical examination, and laboratory findings were recorded, and samples of peripheral blood and CSF (when clinically indicated) were obtained. Children and their family members were then followed up after each child’s physician was contacted. At follow-up visits, enrolled family members were interviewed, and saliva and peripheral blood samples were obtained. The study and consent forms were approved by the Research Subject Review Board of our institution.

Diagnostic criteria. Primary HHV-6 infection was diagnosed only when HHV-6 was isolated from the blood and subsequent seroconversion occurred. Children with past primary HHV-6 infection were identified by positive PCR analysis of PBMCs or saliva and/or positive serology if the child was 5 months of age or older, the age at which levels of passive maternal antibody would have diminished.

Isolation of HHV-6. PBMCs were separated from anticoagulated blood samples (0.5–3 mL) by density gradient centrifugation (Histopaque 1077; Sigma, St. Louis) and were cocultivated with stimulated cord blood mononuclear cells [8]. Positive cultures were confirmed by indirect immunofluorescence with monoclonal antibodies to HHV-6A or HHV-6B and by PCR analysis [11–13]. HHV-6 was isolated only from PBMCs.

Serological testing. IgG antibody to HHV-6 was assayed by indirect immunofluorescence with twofold dilutions of serum and HSB-2 cells infected with an HHV-6 isolate containing both HHV-6A and HHV-6B genomes [4, 8, 9].

Variant-specific DNA PCR oligohybridization. All PCR analyses were run under strict precautions to avoid contamination. All specimens before and after PCR analysis were separated physically, and materials were reserved only for work before PCR analysis. DNA from PBMCs was prepared as previously described [8, 10, 13]. Saliva samples were prepared similarly but with an additional initial centrifugation at 14,000g for 2 hours at 4°C for partial purification of the virus. Nested PCR amplification was performed with primer sequences conserved in both variants [3, 13]. Specificity was confirmed with a probe that hybridizes to both variants by Southern blot hybridization. Ten genomic copies are reliably detected [3].

Variant typing was then performed by hybridization with probes for HHV-6A (5’TGATGAAACGGTAACCGA) and HHV-6B (5’TGATGAAACGGTGTCACGCA). Assays contained HHV-6A- and HHV-6B-positive and -negative controls (including reaction mixture without DNA), and β-globin primers were coamplified as performance controls to assure that the samples contained adequate cellular material and to exclude the presence of inhibitors.

PCR detection of HHV-6 DNA and variant typing were performed on all samples obtained from family members and from all enrolled children; thus, samples from children with primary infection from whom HHV-6 was isolated by culture and who seroconverted, children who had not yet acquired HHV-6 infection, and children with prior infection were included in these analyses.

Statistical methods. χ2 analyses with use of Epi-Info (Centers for Disease Control and Prevention, Atlanta) were performed for differences in proportions.

Results

HHV-6 variants of isolates causing primary infections. All 333 HHV-6 isolates from children with primary infection were identified as HHV-6B except three (1%), which were identified as HHV-6A by monoclonal antibody reactivity (table 1). Subsequent characterization and variant typing by PCR analysis indicated that these isolates with phenotypic characteristics of HHV-6A contained a mixture of HHV-6A and HHV-6B genomes.

HHV-6 variants in children with previous infection. PCR analyses of 2,716 PBMC samples from all the enrolled children detected the HHV-6 genome in 37% of specimens (table 1), thus indicating the persistence of HHV-6 following previous primary infection. Almost all samples (97.5%) were typed as HHV-6B, but slightly more samples from children with previous infection (2.5%) were typed as HHV-6A or mixed HHV-6A/HHV-6B than isolates from children with primary infection.

Of 668 CSF samples obtained at the initial evaluation, 84 (13%) contained the HHV-6 genome (table 1). Of the 84 children for whom CSF samples were positive for HHV-6 DNA by PCR analysis, seven (8.3%) had concurrent viremia from primary infection. HHV-6 DNA was detected in both PBMC and CSF samples from 30 of the remaining 77 children (39%). HHV-6 DNA was detected only in CSF samples from the remaining 47 children (61%).
HHV-6A was identified significantly more frequently in CSF samples than in PBMC isolates from children with primary infection. Fourteen percent (or 17% when mixed HHV-6A/HHV-6B was included) of the variants in CSF were HHV-6A, compared with 1% of isolates from children with primary infection \((P = .0001)\) or 2.5% of the variants in PBMCs \((P = .0001)\) (table 1). When the type of variant in CSF samples was compared with that in PBMC samples from those children for whom HHV-6 DNA was identified in both samples, in all instances, HHV-6B was detected in PBMCs, and only HHV-6A was detected in the CSF. Thus, in those children for whom dual infection was documented by the detection of both variants, only HHV-6A persisted in the CSF.

**HHV-6 variants persisting in children.** PCR analyses of 1,767 PBMC specimens obtained from children during subsequent visits detected HHV-6 DNA in 38% of samples; 2% of the isolates were HHV-6A, similar to the proportion in PBMC samples obtained at the initial visit. The rate of persistence of HHV-6 in PBMCs was highest \((80\%)\) among children 9 to 12 months of age and diminished to 34% among those older than 2 years of age. HHV-6 DNA persisted in the saliva of 52% of children, and 3% of these samples were HHV-6A (table 1).

**HHV-6 variants persisting in families.** HHV-6 DNA was detected approximately three times more frequently in PBMC samples from children than in those from adults (table 2).

### Table 1. Proportion of 2,716 children with persistence of HHV-6 by site and variant.

<table>
<thead>
<tr>
<th>Visit, sample/finding</th>
<th>No. (%) of tested samples</th>
<th>Percent of each variant in HHV-6-positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td><strong>Initial visit</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBMCs</td>
<td>2,716</td>
<td></td>
</tr>
<tr>
<td>No. of samples cultured</td>
<td>2,716 (100)</td>
<td></td>
</tr>
<tr>
<td>Positive samples by isolation and PCR analysis</td>
<td>333 (12)</td>
<td>99</td>
</tr>
<tr>
<td>Positive samples by PCR analysis only</td>
<td>685 (25)</td>
<td>96.8</td>
</tr>
<tr>
<td>Total no. of positive samples by PCR analysis</td>
<td>1,018 (37)</td>
<td>97.5</td>
</tr>
<tr>
<td>CSF</td>
<td>668</td>
<td></td>
</tr>
<tr>
<td>Positive samples by PCR analysis</td>
<td>84* (13)</td>
<td>83</td>
</tr>
<tr>
<td><strong>Follow-up visit</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBMCs²</td>
<td>1,767</td>
<td></td>
</tr>
<tr>
<td>Positive samples by PCR analysis</td>
<td>663 (38)</td>
<td>98</td>
</tr>
<tr>
<td>Saliva²</td>
<td>258</td>
<td></td>
</tr>
<tr>
<td>Positive samples by PCR analysis</td>
<td>134 (52)</td>
<td>97</td>
</tr>
</tbody>
</table>

NOTE. HHV-6 = human herpesvirus 6; PBMC = peripheral blood mononuclear cell.

* Seven patients had concurrent viremia from primary infection.

² None of the samples were positive by culture.

Overall, 4% of the PBMC samples from families contained HHV-6A or HHV-6A/HHV-6B, and the proportion differed little between children and adults. HHV-6 DNA also was detected more frequently in the saliva of children, but the proportion of HHV-6A and HHV-6A/HHV-6B was slightly greater among adults (table 2).

In 94% of the 149 families, the variants identified in PBMC and/or saliva specimens from the adults and children were the same; in 92%, HHV-6B was detected in both samples, and in 2%, HHV-6A was detected in both samples. The variant type in samples from parents and child differed in 6% of the families. When the variant detected in saliva specimens from the adults was compared with that detected in saliva and/or PBMC specimens from the child, discordance occurred in 8% of cases; discordance occurred more frequently if the variant in the adult’s saliva was HHV-6A than if it were HHV-6B.

HHV-6 DNA persisted in both saliva and PBMC specimens from 62% of children, and in all cases, the variant was the same in both samples. However, HHV-6 was detected in both saliva and PBMC specimens from 9% of parents at any one visit, and, as for the children, the variant type was the same in both samples.

### Duration of HHV-6 persistence in saliva and PBMCs.

In sequential samples obtained from 199 children over a period of 6 months to 5 years after primary infection (average, 2.5
years; median, 2.2 years), the HHV-6 genome persisted, although sometimes intermittently, in their PBMCs. HHV-6 DNA could still be detected at 6 months to 1 year in specimens from 84% of children after primary infection, at 2 years in 78%, at 3 years in 78%, and at 4 to 6 years in 57%. HHV-6 DNA was detected in saliva 6 months to 1 year after primary infection in 70% of the children and after an interval of 2 years in 33%, 3 years in 67%, and 4 to 6 years in 50%. The variant detected in PBMC and saliva specimens obtained during the follow-up visits of all the children remained the same. Of the adults whose PBMC samples initially contained the HHV-6 genome, 33% continued to have one or more subsequent samples positive for HHV-6 DNA. Fifteen percent of the follow-up saliva specimens from adults contained HHV-6 DNA. In three-fourths of these samples, HHV-6 DNA was not consistently present but was only intermittently detected.

Discussion

The findings of this study indicate not only that persistence of HHV-6 DNA occurs in most children in the convalescent phase or the period shortly after primary infection but also that the HHV-6 genome continues to persist over the next 2 to 6 years in most normal children. Although HHV-6 DNA was less frequently and consistently detected in PBMC and saliva specimens from adults than in those from the children, persistence of HHV-6 also was common in normal adults. A similar frequency of HHV-6 persistence was reported recently by Nakata-Taya and colleagues [14] in their study of 67 Japanese children and 28 adults. Overall, HHV-6 DNA was detected in 64% of the throat swab specimens from the children. Of the children 12 to 23 months of age, who were likely to have had their primary infection recently, 20 (87%) of 23 had HHV-6 DNA in their throat swab specimens. Of the 14 children between 2 and 8 years of age, 11 (79%) had HHV-6 DNA detected. However, of the 28 adults, only nine (32%) had HHV-6 DNA detected, similar to our findings.

HHV-6B clearly predominates as the variant persisting in both children and adults. Our findings further suggest that the two variants have distinct immunologic and biological properties and possibly different pathogenic potential. Dual infection and persistence of both variants at the same and different sites within a normal individual indicate that little or no cross-immunity exists between HHV-6A and HHV-6B despite their genomic similarity [15]. Furthermore, infection with HHV-6A occurs in those individuals who have had previous infection with HHV-6B and thus occurs in the presence of good levels of antibody to HHV-6.

Infection with HHV-6A clearly appears to be much less frequent than that with HHV-6B. Although for some time HHV-6A has been found in adults, mostly immunocompromised patients (including the patient with the first reported case of HHV-6 infection) [12, 16–19], primary infection in infants has been almost exclusively with HHV-6B [2–4]. Primary infection with HHV-6A had not been identified until the recent report by Hidaka and colleagues [7]; these researchers described a 50-day-old Japanese infant with roseola in whom HHV-6A infection was identified by detection of HHV-6A DNA in the infant’s PBMCs and CSF on the second day of illness. Isolation of virus from the infant’s PBMCs was not reported. Although serological assays currently cannot differentiate between HHV-6A and HHV-6B infection, immunofluorescence assays of this infant’s sera were supportive of acute primary HHV-6 infection. The titer of antibody in the acute and convalescent-phase sera remained unchanged at 1:160 rather than decreasing, as would be characteristic of passively derived maternal antibody.

The information on the occurrence and course of infection with HHV-6A is extended by our findings that the rate of persistence of HHV-6A in both normal children and adults may be relatively low compared with the rate of persistence of HHV-6B and that the frequency of HHV-6A infection does not appear to increase with age. Hence, the lack of identification of HHV-6A infection in infants is not explained by a peak period of acquisition occurring at later ages, such as generally occurs with human herpesvirus 7 [20]. Why HHV-6A, which is so closely related to HHV-6B and human herpesvirus 7, also is not a ubiquitous infection of childhood is unclear.

HHV-6A infection conceivably could occur in young children at a greater frequency than suspected but be unrecognized because its clinical manifestations are few or varied. Somewhat against this possibility, however, are our studies of primary HHV-6 infection over the past few years at the University of Rochester Medical Center emergency department and outpatient clinics (Rochester, NY); these studies have now included the examination of >4,000 children younger than 3 years of age who have had acute febrile illnesses of varying severity and manifestations and >500 normal children without acute illness. Although a PBMC specimen from each child was cultured for HHV-6, none of the HHV-6 isolates obtained was HHV-6A. Hence, HHV-6A, in contrast to HHV-6B, has yet to be isolated from the peripheral blood of a young child.

Another potential explanation is that the differing biological characteristics of HHV-6A include poorer infectivity, resulting in a less contagious virus. The results for the families in this study may lend some credence to this explanation, since the number of other family members in whom HHV-6A was detected did not increase when there was a family member shedding HHV-6A in saliva. Another theoretical possibility is that immunity to HHV-6A does not last, and thus signs of previous infection may not remain, thereby suggesting that the HHV-6A detected in these families arose from repeated asymptomatic reinfections.

If HHV-6A does not evoke adequate immunity, the finding of its relative predominance in the CSF may be more ominous
in the face of poor immunologic control. A number of studies have suggested that HHV-6A and HHV-6B may differ in their cellular tropism and potential pathogenicity. Several studies [6, 17, 19, 21] have shown that HHV-6B tends to be more prominent in most tissues, except in healthy skin [21]. Two-thirds of lung tissue specimens from both normal adults and bone marrow transplant recipients with pneumonia were shown to contain a mixture of HHV-6A and HHV-6B [19]. In patients with Kaposi’s sarcoma, however, HHV-6A clearly was more frequent. In contrast, HHV-6B has been described more commonly in patients with Hodgkin’s lymphoma [17] and in the blood of bone marrow transplant patients [6].

Few data exist on the association of variant type with neuroinvasive potential. The relative predominance of HHV-6A in CSF specimens from children in this study, nevertheless, is suggestive of a greater neurotropic potential of HHV-6A. HHV-6 DNA was detected relatively frequently in the CSF specimens from these normal children, and CSF was the only specimen in which the HHV-6 genome was detected for 61% of the 77 children examined after infection (for the other 39%, HHV-6 DNA was detected in both CSF and PBMCs). Of note is the observation that in those children in whom infection with both variants was concurrently present, only HHV-6A persisted in the CSF, while HHV-6B persisted in PBMCs, thus suggesting the neurotropic preference of HHV-6A.

The association of roseola with CNS manifestations has long been suggested by the clinical descriptions of infants with roseola complicated by such CNS manifestations as a bulging anterior fontanelle, encephalopathy, meningoencephalitis, and meningitis [22, 23]. Infection with HHV-6 has been associated with severe or fatal CNS disease, including encephalitis and meningoencephalitis, in both children and immunocompromised adults [24–27]. HHV-6 has also been identified in brain tissue specimens from immunocompromised patients as well as in autopsy specimens from adults who died of unrelated causes [28, 29].

The most frequent complication associated with primary HHV-6 infection in normal children has been febrile seizures [2, 10, 30, 31]. HHV-6 DNA has been detected in CSF specimens from children with viremic primary infection and in specimens from those with past HHV-6 infection, and some studies have suggested that the presence of HHV-6 DNA in the CSF is associated with a greater risk for manifestations of CNS disease, including the risk of recurrent seizures [3, 10, 23, 30, 31].

The significance of HHV-6 infection in the CNS and any role that variant type may have are far from delineated, especially in view of the observation that HHV-6 DNA has been detected in autopsy specimens of brains in adults who died of causes unrelated to CNS pathology [29]. Proving an association of HHV-6 infection with subsequent neuropathogenicity will be a difficult task considering that the documentation of the presence of HHV-6 in the CNS is not generally feasible, particularly prospectively, and its association with potential pathogenicity may require a prospective evaluation over decades.

References


