Evidence of Human Herpesvirus 6 Infection in 4 Immunocompetent Patients with Encephalitis

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(See the article by Birnbaum et al. on pages 887–9 and the editorial commentary by Whitley and Lakeman on pages 894–5)

We describe 4 patients with encephalitis due to possible reactivation of human herpesvirus 6 (HHV-6) infection who were enrolled in the California Encephalitis Project. All were immunocompetent and had HHV-6 loads determined in cerebrospinal fluid specimens. Tests for detection of HHV-6 should be considered for individuals with encephalitis.

Human herpesvirus 6 (HHV-6) was first isolated in 1986 from PBMCs obtained from HIV-infected individuals and was later found to cause roseola infantum [1, 2]. HHV-6 has been categorized into variants A and B [3]. Evidence suggests that HHV-6B primarily appears in children with roseola or other febrile illnesses. HHV-6A is primarily found in patients with lymphoproliferative disorders and in immunocompromised hosts with CNS disease [4]. We describe 4 patients with encephalitis due to possible reactivation of HHV-6 infection who were enrolled in the California Encephalitis Project (CEP).

Case reports. A 38-year-old previously healthy woman (patient 1) was initially admitted to a hospital outside of California with meningitis that followed a seizure. She had speech difficulties and personality changes. Within 2 weeks after admission, patient 1 was transferred to a hospital in California.

A brain MRI revealed abnormal findings in the left temporal lobe, and electroencephalography revealed changes consistent with herpes simplex virus (HSV) encephalitis. Although PCR of CSF obtained on the day of transfer was negative for HSV type 1 (HSV-1), the patient was given intravenous acyclovir for 21 days and was discharged home receiving oral valacyclovir.

Six weeks later, patient 1 was readmitted to the hospital with worsening neurological symptoms, including emotional lability and language difficulties. A second MRI revealed new right and persistent left temporal involvement. A lumbar puncture showed 98 WBCs/mm³, a glucose level of 65 mg/dL, and a total protein level of 111 mg/day (table 1). Treatment with intravenous acyclovir was restarted. PCR of CSF specimens sent to 2 reference laboratories tested negative for HSV-1.

Specimens were sent to the CEP for analysis. Herpesvirus-consensus PCR (Argene Biosoft) of CSF specimens was positive for HHV-6 and negative for HSV-1, HSV type 2 (HSV-2), varicella-zoster virus, Epstein-Barr virus, and cytomegalovirus. Paired serum specimens collected on the first day of the second hospital admission and 8 days later were negative for HHV-6 IgG and IgM antibodies (table 1). IgG titers for HSV in serum specimens indicated past infection. Intrathecal antibodies to HHV-6 and varicella-zoster virus were not detected. However, HSV IgG antibodies were detected in a CSF specimen. This sample was forwarded to the Diagnostic Virology Laboratory at the University of Alabama, Birmingham, for further testing. PCR of the CSF specimen was positive for HHV-6, and viral quantification was performed using standard curves generated by TaqMan probes and primers in an ABI 7700 machine. Results are summarized in table 1.

After PCR confirmed the presence of HHV-6, patient 1 received a 6-week course of oral valganciclovir. Neurological symptoms improved, but neurocognitive tests performed 1 year later continued to demonstrate neurological deficits.

A 66-year-old previously healthy woman (patient 2) presented to her primary care physician with a 6-week history of respiratory symptoms and a 2-week history of headache. Amoxicillin-clavulanate was prescribed. The next day, patient 2 presented to an emergency department with a worsening headache; a CT scan of the head showed no abnormalities. She received outpatient treatment with a macrolide antibiotic.

Thirteen days later, she was admitted to the hospital with malaise and a persistent headache. Physical examination revealed photophobia, a mild tremor, and hyperreflexivity. A
Table 1. Demographic, clinical, and laboratory data for 4 patients with evidence of encephalitis associated with human herpesvirus 6 (HHV-6).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, sex</th>
<th>Clinical presentation</th>
<th>CSF findings</th>
<th>Other findings by test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WBC count, cells/mm³</td>
<td>Protein level, mg/dL</td>
<td>Glucose level, mg/dL</td>
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<tr>
<td>1</td>
<td>38, F</td>
<td>Ataxia and seizures</td>
<td>16</td>
<td>111</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>66, F</td>
<td>Tremors</td>
<td>58</td>
<td>66</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20, F</td>
<td>Headache and blurred vision</td>
<td>LP1, 130; LP2, 10; LP3, 117</td>
<td>LP1, 48; LP2, ND; LP3, 533</td>
</tr>
<tr>
<td>4</td>
<td>14, M</td>
<td>Seizures</td>
<td>535</td>
<td>282</td>
</tr>
</tbody>
</table>

**NOTE.** CDHS, Viral and Rickettsial Disease Laboratory at the California Department of Health Services, Richmond; EIA, electroencephalography; LP, lumbar puncture; ND, not done; UAB, Virology Diagnostic Laboratory at the University of Alabama, Birmingham.

³ Several segmental areas of narrowing in the right colosso marginal artery and branches, suggesting possible cerebral vasculitis.

⁴ Testing of paired serum specimens showed no significant change in IgG antibody titer.
brain MRI showed no abnormalities, apart from a mild gliosis. Results of blood chemistry analysis and 2 sequential complete blood cell counts were within normal limits. A lumbar puncture revealed 58 WBCs/mm³ (with 99% lymphocytes), a glucose level of 99 mg/dL, and a total protein level of 66 mg/dL (table 1). Acyclovir therapy was initiated for possible HSV-1 encephalitis. Four days after admission, PCR of CSF was negative for HSV-1, and acyclovir therapy was discontinued.

Five days after hospital admission, the patient was discharged with remarkable improvement. Although memory deficits improved, the patient still had concentration difficulties, fatigue, and a slight tremor.

Specimens were forwarded to the CEP for diagnostic analysis. Herpesvirus-consensus PCR of CSF was positive for HHV-6 and negative for HSV-1, HSV-2, varicella-zoster virus, Epstein-Barr virus, and cytomegalovirus. Results of serological analysis of serum specimens were negative for IgM and nonspecific for IgG. Intrathecal antibodies to HHV-6 and HSV-1 were not detected. The CSF sample was forwarded to the Diagnostic Virology Laboratory at the University of Alabama for further testing (table 1).

Data for 2 other patients with evidence of HHV-6 in CSF are summarized in table 1. A 20-year-old woman (patient 3) presented with headache and visual disturbances and was hospitalized for 22 days. A 14-year-old boy (patient 4) presented with seizures and was hospitalized for 6 days.

To test the sensitivity of the herpesvirus-consensus PCR used by CEP for detection of HHV-6, a total of 328 randomly selected extracts (stored at −70°C) were sent to Quest Diagnostics Nichols Institute (San Juan Capistrano, CA) for HHV-6–specific testing with real-time PCR involving an AB 7900 instrument. High viral loads (>10⁷ copies/mL) were identified in samples obtained from patients 2 and 4. One sample tested positive, with a viral load of <500 copies/mL that was not identified by our assay. This finding suggests that the assay used by the CEP can detect virus loads of at least 10⁶ copies/mL but not less than 500 copies/mL.

Discussion. The CEP was initiated in 1998 to identify the etiological causes and characterize the epidemiological features of encephalitis. Physicians can refer specimens to the CEP for diagnostic testing if a patient is >6 months of age, is immunocompetent, has been hospitalized with encephalopathy, and has ≥1 of the following signs and/or symptoms: fever, seizure, focal neurological findings, CSF pleocytosis, and electroencephalography or neuroimaging findings consistent with encephalitis.

CSF specimens collected from such patients and forwarded to the CEP are tested for herpesviruses by means of a herpesvirus-consensus PCR kit, which is specific for HSV-1, HSV-2, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, and HHV-6 [5]. Serological testing of paired serum specimens for detection of HSV and varicella-zoster virus was performed with an EIA. Serological testing was also conducted on CSF by EIA to detect IgG antibodies to HSV, varicella-zoster virus, HHV-6, and measles. Additional EIA is performed on serum specimens for detection of arboviruses, influenza virus A and B (from October through March), Epstein-Barr virus, adenoviruses, *Mycoplasma pneumoniae*, and *Chlamydia* species; PCR for detection of enteroviruses and *M. pneumoniae* is performed on CSF and respiratory specimens [6].

Of 1000 patients enrolled in the CEP, only 4 tested positive for HHV-6 by means of the herpesvirus-consensus PCR. The ages of all 4 patients were well above the mean age (≤3 years) of persons in whom acute infection is more likely to occur. It is not certain whether these cases represent primary infections or reactivation, but given the ages of these patients, reactivated infection is more likely. Two patients (age, 9 months and 2 years) who were enrolled in the CEP yet who were not discussed in this article were PCR-negative for HHV-6 but had serological evidence of acute HHV-6 infection. Of the 98 patients ≤3 years of age who were enrolled in the CEP, none had CSF specimens that were PCR-positive for HHV-6.

HHV-6 has been shown to cause a variety of severe neurological clinical manifestations, including seizures, meningitis, and encephalitis. Evidence of reactivated HHV-6 infection leading to encephalitis has been well documented in immunocompromised hosts, including HIV-positive patients; recipients of bone marrow transplants, liver transplants, and renal transplants; and persons with lymphoproliferative disorders [1, 4].

The 4 patients described in this report were immunocompetent and had no previous neurological disease. However, the significance of these detections of HHV-6 in CSF specimens is unclear.

The high CSF viral loads support the possibility that HHV-6 was a cause of illness in our 4 patients (table 1). Generally, HHV-6 loads of this magnitude, which were presumably associated with reactivated infection, have been detected in samples of saliva or the affected tissues of patients with clinically significant disease, such as lymphoma [7]. In addition, with the exception of patient 1, the patients we described had no evidence of other agents that have been associated with encephalitis. Evidence of HSV-specific IgG intrathecal antibody might suggest reactivated HSV infection in patient 1.

Previous studies have also reported detection of HHV-6 in patients with encephalitis. In one study, 9 of 138 immunocompetent patients with focal encephalitis had CSF specimens that were PCR-positive for HHV-6 [8]. Three published case reports of encephalitis, all of which involved immunocompetent adults >50 years of age, have implicated reactivation of HHV-6 infection, based on PCR detection of HHV-6 in CSF but not PBMCs [9], in CSF and plasma [10], and in white matter lesions [11].

Alternatively, it can be argued that detection of HHV-6 in
CSF is not a significant finding; one study demonstrated that HHV-6 was detected in brain tissue specimens obtained from 10 of 31 previously healthy persons [12]. These subjects had no evidence of clinical signs associated with CNS disease.

Furthermore, there were inconsistent clinical presentations for the cases presented in this report. The 4 patients presented with disease of wide-ranging clinical severity and differing hospital courses and illness durations. However, this variation has also been supported by McCullers et al. [8], who described 9 of 138 patients with focal encephalitis, a diagnosis of HHV-6 infection, and outcomes ranging from full recovery to moderately severe impairment and death.

Additional studies are needed to understand the role of HHV-6 infection in patients with encephalitis. The evidence associating HHV-6 with cases of encephalitis of unknown cause has grown increasingly persuasive, with multiple case reports involving adult patients. Given the established association between other human herpesviruses and this condition, it is reasonable to consider HHV-6 testing for individuals with encephalitis.

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