Influenza-Associated Encephalopathy: No Evidence for Neuroinvasion by Influenza Virus Nor for Reactivation of Human Herpesvirus 6 or 7

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During 2 consecutive influenza seasons we investigated the presence of influenza virus, human herpesvirus (HHV) type 6, and HHV-7 in cerebrospinal fluid samples from 9 white children suffering from influenza-associated encephalopathy. We conclude that it is unlikely that neuroinvasion by influenza virus or reactivation of either HHV-6 or HHV-7 is involved.

Influenza is characterized by a broad spectrum of clinical conditions, sometimes including involvement of the brain. The presentation of the neurological manifestation, influenza-associated encephalopathy, ranges from irritability and drowsiness to seizures and coma [1]. A particular form of influenza-associated encephalopathy reported in Asian countries has recently drawn attention because of its severity and associated case-fatality rate [2].

The pathogenesis of influenza-associated encephalopathy is unclear. Influenza viruses have sometimes been detected in brain or CSF samples from affected patients, but negative results predominate. It is, therefore, uncertain whether neuroinvasion by influenza virus plays a role [1–3]. Alternatively, it has been postulated that during influenza the reactivation of human herpesvirus (HHV) type 6 or HHV-7 in the brain may result in influenza-associated encephalopathy. These viruses are known for their neurotropic properties and reside latently in the brain after primary infection. However, results from studies of the involvement of HHV-6 and HHV-7 are also inconsistent [4, 5]. Because of these inconsistencies and also because previous studies were mainly conducted with children of Asian origin, we investigated the possible role of influenza virus and of HHV-6 and HHV-7 in CSF samples from white children in The Netherlands suffering from influenza-associated encephalopathy.

Materials and methods. Patients with influenza-associated encephalopathy were recruited at both the Public Health Laboratory Friesland (serving 640,000 inhabitants) and the University Medical Centre Nijmegen (tertiary reference center for 2 million inhabitants) during 2 consecutive influenza seasons (October 2001–March 2002 and October 2002–March 2003). Patients were only included if influenza virus had been isolated from respiratory-tract specimens and a CSF sample obtained during the acute phase of illness was available for PCR assay.

Encephalopathy was defined as the occurrence of seizures and/or at least 24 h of mental or motor impairment (e.g., impaired consciousness, paresis, or aphasia). Patients who were receiving neurotropic medication or who had intrinsic brain disease were excluded. Patients were only included if neurological symptoms occurred within 48 h after the onset of respiratory illness.

Nasopharyngeal secretions and/or throat swab specimens were tested for influenza virus by routine procedures. Immunofluorescence staining was performed with monoclonal antibodies against influenza A and B viruses (Dako) on days 1 and 10 of hospitalization. All influenza virus isolates were serotyped at the Dutch reference center of the World Health Organization in Rotterdam, The Netherlands.

A commercial complement fixation assay (Clindia Diagnostics) was performed with influenza virus types A and B, parainfluenza virus types 1–3, adenosviruses, respiratory syncytial virus, enteroviruses (pools 1–3), Mycoplasma pneumoniae, Coxiella burnetii, and a Chlamydia common antigen. Presence of IgM antibodies to influenza A virus (H3N2 and H1N1), influenza B virus, and parainfluenza viruses 1–3 was assessed with a hemadsorption immunosorbent technique [6]. For detection of IgM and IgA antibodies against herpes simplex virus, varicella-zoster virus, and M. pneumoniae, in-house ELISAs were used, as described elsewhere [7]. Presence of IgG antibodies against herpes simplex- and varicella-zoster virus was also detected with in-house ELISAs, as described elsewhere [8]. For detection of HHV-6–specific antibodies, an
indirect immunofluorescence assay was used, as described elsewhere [9]. The presence of HHV-7–specific antibodies was not determined.

RNA was isolated from 200-μL samples of CSF with the High Pure RNA isolation kit (Roche Molecular Biochemicals) according to the manufacturer’s instructions, with minor modifications. Amplification was performed as described with the specific primers for either influenza A [10] or B [11].

DNA extraction from 200-μL CSF samples was performed with the MagNA Pure LC and the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Molecular Diagnostics) according to the manufacturer’s instructions. The primers and probes for HHV-6 PCR (TibMolBiol) were selected in a 111-bp region of the HHV-6 U11 gene by homologic testing for HHV-6A and HHV-6B, so both variants could be detected [12]. Amplification was performed with a LightCycler (Roche Molecular Diagnostics).

The primers for HHV-7 PCR (Isogen Life Science) amplify a 193-bp fragment from the HHV-7 region corresponding to the HHV-6 U42 gene [13]. After the first round of amplification, 10-μL of the reaction product was used for a nested PCR assay. The reaction was analyzed by gel electrophoresis.

**Results.** Nine patients fulfilled the study criteria. Their clinical and microbiological data are presented in Table 1. One patient’s case is described in more detail to illustrate the impressiveness of its clinical presentation, which, nevertheless, had a favorable outcome.

Patient 9, a previously healthy girl who was 14 months of age, was admitted to the department of child neurology with a seizure that lasted 2 h, with fixed deviation of the head and eyes, and with orobuccal automatisms. The neurological event started 1 day after onset of a flulike illness that included fever (temperature, 39°C). Influenza B virus was isolated from a throat swab specimen obtained at the time of hospitalization.

Results of additional virological studies of CSF samples and feces were negative. Results of serologic tests for respiratory and neurotropic pathogens remained negative. Detection of HHV-6 antibodies indicated prior primary infection. Results of MRI of the brain were normal, as were results of chemical and cytological tests of CSF samples, analysis of the serum C-reactive protein level, the WBC count, the differential blood count, and metabolic screening. One day after the seizure, the patient’s neurological signs abated, and recovery was complete after 1 week. At a follow-up visit 6 months later, clinical and electroencephalographic findings were normal.

All patients were of white ethnicity, were younger than 6 years of age, and presented with fever and signs of brain involvement, such as impaired consciousness and/or complex febrile seizures (Table 1). None of the patients had meningeal irritation, CSF pleocytosis, or permanent brain damage.

Influenza A virus was isolated from nasopharyngeal secretions from 7 of 9 patients; influenza B virus was isolated from nasopharyngeal secretions from the other 2 patients. All influenza A virus isolates were of the H3N2 type (A/Moscow/10/99-like), which was the predominant strain circulating in The Netherlands during the study period. In only 1 of 9 cases was HHV-6 DNA detected in CSF samples. None of the samples tested positive for HHV-7 DNA. Eight of 9 CSF samples were also tested for the presence of influenza A and B virus by PCR assay (insufficient sample volume prevented testing of 1 sample), but results were negative (Table 1).

**Discussion.** Although the existence of influenza-associated encephalopathy has been known for decades [1], it is a complication that is still considered to occur infrequently in regions outside Asia. Because our study deals with only a selected group of patients referred to clinics affiliated with our laboratories, and because it was conducted during influenza seasons when influenza activity was low, it does not provide direct infor-

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**Table 1. Summary of the clinical and microbiological data for 9 patients with influenza-associated encephalopathy.**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age in months</th>
<th>Temp., °C</th>
<th>CSF pleocytosis</th>
<th>Duration of neurological signs, days</th>
<th>Duration of hospitalization, days</th>
<th>Neurological impairment at hospital discharge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>39.7</td>
<td>No</td>
<td>Impaired consciousness</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>38.5</td>
<td>No</td>
<td>Impaired consciousness</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>40</td>
<td>No</td>
<td>Complex febrile seizure and impaired consciousness</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>62</td>
<td>40</td>
<td>No</td>
<td>Impaired consciousness</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>41</td>
<td>No</td>
<td>Impaired consciousness</td>
<td>NA</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>NA</td>
<td>No</td>
<td>Complex febrile seizure and impaired consciousness</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>39</td>
<td>No</td>
<td>Complex febrile seizure</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>23</td>
<td>40.3</td>
<td>No</td>
<td>Complex febrile seizure</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>14</td>
<td>39</td>
<td>No</td>
<td>Complex febrile seizure and impaired consciousness</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

**NOTE.** NA, data not available; NT, not tested because of insufficient volume of sample; HHV, human herpesvirus; temp., temperature.
mation on the incidence of brain involvement during influenza epidemics. Together with other reports of similar cases, however, our findings raise some doubt regarding the rarity of influenza-associated encephalopathy outside Asia.

The pathogenesis of influenza-associated encephalopathy is still unresolved. One of the mechanisms proposed is a direct invasion of the brain through hematogenous spread of the virus, although viremia does not usually occur in humans [1]. Moreover the virus has been isolated only sporadically from CSF samples, and the results of RT-PCR of CSF samples are inconsistent. In our study, influenza virus could not be detected in the 8 CSF samples that were available for testing, suggesting that a direct invasion of the brain by influenza virus is uncommon and does not provide a solid explanation for the neurological complications.

Alternatively, it has been proposed that influenza may trigger the reactivation of both HHV-6 and HHV-7, which remain latently present in the brain after primary infection. In a study from Japan, DNA of HHV-6 and/or HHV-7 was found in CSF samples of 4 of 8 children with influenza-associated encephalopathy [4]. However, these results could not be confirmed in a comparable group of Asian children [5]. We detected HHV-6 DNA in only 1 of 9 white European children with influenza-associated encephalopathy, and none of these CSF samples contained HHV-7 DNA. Taken together, it is questionable whether HHV-6 or -7 contribute to brain manifestations in influenza.

The neurological signs observed in influenza-associated encephalopathy do not seem to be related to viral infection of the brain either by direct invasion or by reactivation. Recent studies point to a possible involvement of the innate immune response by proinflammatory cytokines, which may affect the brain by indirect means. Several studies have reported that patients suffering from influenza-associated encephalopathy or febrile seizures show enhanced systemic levels of IL-6, IL-10, TNF or soluble TNF receptor-1, and type 1 interferon. As yet, no information is available on cytokine concentrations inside the brain during influenza.

In summary, our findings confirm the existence of influenza-associated encephalopathy in a non-Asian population. We found no evidence that direct infection of the brain plays a role in the pathogenesis of influenza-associated encephalopathy. However, virus-induced inflammatory (cytokine) responses within the brain may be involved. Additional studies are required to shed light on the role of cytokines in encephalopathy caused by influenza and, possibly, by other viral infections.

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References