Human herpesvirus 6 in cerebrospinal fluid of patients infected with HIV: frequency and clinical significance

Simona Bossolasco, Roberta Marenzi, Helena Dahl, Luca Vago, Maria Rosa Terreni, Francesco Broccolo, Adriano Lazzarin, Annika Linde, Paola Cinque

Abstract
The objective was to evaluate the frequency of human herpesvirus 6 (HHV-6) DNA detection in the CSF of patients infected with HIV and its relation to brain disease and systemic HHV-6 infection.

Nested polymerase chain reaction (PCR) was used to analyse CSF samples from 365 consecutive HIV infected patients with neurological symptoms. When available, plasma and brain tissues from patients whose CSF was HHV-6 positive were also studied.

HHV-6 was found in the CSF of eight of the 365 patients (2.2%); two had type A and four type B; the HHV-6 variant could not be defined in the remaining two. All eight patients had neurological symptoms and signs related to concomitant opportunistic brain diseases, including cytomegalovirus (CMV) encephalitis in five patients whose CSF was also positive for CMV-DNA. Opportunistic infections but no other unexplained lesions were also found in the brain of all of the four patients who underwent neuropathological examination. Both HHV-6 and CMV were also detected in the plasma of respectively five and seven of seven patients whose CSF was HHV-6 positive.

In conclusion, HHV-6 type A or B DNA was infrequently found in the CSF of HIV infected patients, in association with both CMV brain infection and systemic HHV-6 replication. However, no certain relation between HHV-6 and brain disease was found.

Keywords: HIV infection; human herpesvirus type 6; cerebrospinal fluid; cytomegalovirus

Human herpesvirus 6 (HHV-6), variants A and B, is a ubiquitous lymphotropic virus. HHV-6 B is known to be the causative agent of exanthema subitum, but both variants have been found in immunocompromised patients. Clinical evidence supports a role for HHV-6 in causing significant CNS disease. The virus is often found in the CSF of children with febrile convulsions or other neurological symptoms complicating exanthema subitum. HHV-6 encephalitis or milder neurological symptoms have been described in both immunocompetent adults and transplanted patients, and the virus might also play an aetiopathogenetic role in multiple sclerosis. HHV-6 has also been found in the brain or CSF of adults and children with AIDS, but its role in neurological disease is still unclear.

To study the possible relation between HHV-6 and brain disease in patients infected with HIV, the presence of HHV-6-DNA in the CSF was assessed in a large group of patients with neurological disease and was correlated with clinical and neuropathology patterns.
sent for the CSF sampling in an approved manner.

A nested PCR for HHV-6-DNA detection was carried out as previously described. The positive samples were also tested by another PCR assay which gives products of different sizes for the HHV-6 A and B variants, allowing their differentiation (‘subtype’ PCR, figure). The analytical sensitivity was estimated to be 20 and 50 HHV-6 genomes for the standard and the subtype PCR, respectively. Pre-PCR treatment of CSF and plasma specimens and visualisation of amplified products was done as described. PCR examinations were done blindly with respect to the patients’ history in the 365 prospectively examined patients.

Postmortem CNS examinations were performed in three patients (time interval between the CSF sampling and necropsy was 1–90 days), and brain biopsy in one. The postmortem examinations involved eight standard tissue sections and sections from any other macroscopically visible lesion. Tissues were fixed in formalin, embedded in paraffin, and examined after haematoxylin and eosin staining. Special stainings and immunohistochemical techniques were used for the diagnosis of opportunistic diseases and HIV encephalitis, as

<table>
<thead>
<tr>
<th>DNA PCR findings</th>
<th>Patient</th>
<th>Sample (days)</th>
<th>CSF HHV-6 (variant)</th>
<th>CSF CMV</th>
<th>Plasma HHV-6 (variant)</th>
<th>Plasma CMV</th>
<th>Antiviral treatment (days)</th>
<th>CNS disease (diagnostic procedure)</th>
<th>CNS HHV-6 DNA PCR</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>a (-210)</td>
<td>NA</td>
<td>NA</td>
<td>+ (A)</td>
<td>+</td>
<td></td>
<td>Ganciclovir (from −170 to +30)</td>
<td>CMV meningoencephalomyelitis</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>b 0</td>
<td>+ (A)</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td></td>
<td>HIV encephalopathy</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c (+5)</td>
<td>+ (A)</td>
<td>+</td>
<td>+ (A)</td>
<td>+</td>
<td></td>
<td>(clinical+necropsy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>d (+14)</td>
<td>+ (A)</td>
<td>NA</td>
<td>NA</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>e (+30)</td>
<td>+ (A)</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>a 0</td>
<td>+ (ND)</td>
<td>+</td>
<td>+ (B)</td>
<td>+</td>
<td></td>
<td>Ganciclovir (from +40 to +55)</td>
<td>Toxoplasmosis, CMV encephalitis</td>
<td>+ (ND)§</td>
</tr>
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<td></td>
<td>b (+12)</td>
<td>+ (ND)</td>
<td>−</td>
<td>+ (B)</td>
<td>+</td>
<td></td>
<td>(clinical+necropsy)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c (+28)</td>
<td>+ (B)</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>d (+55)</td>
<td>+ (B)</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>a 0</td>
<td>+ (B)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td></td>
<td>None</td>
<td>Toxoplasmosis (clinical)</td>
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</tr>
<tr>
<td></td>
<td>b (+182)</td>
<td>+ (B)</td>
<td>−</td>
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<td>−</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>c (+421)</td>
<td>+ (B)</td>
<td>−</td>
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<td>+</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>4</td>
<td>a† 0</td>
<td>+ (B)</td>
<td>+</td>
<td>+ (B)</td>
<td>+</td>
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<td>Ganciclovir (from −2 to +18)</td>
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</tr>
<tr>
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<td>+ (B)</td>
<td>+</td>
<td></td>
<td>(clinical)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>c (+24)</td>
<td>+ (B)</td>
<td>−</td>
<td>NA</td>
<td>−</td>
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<tr>
<td>5</td>
<td>a 0</td>
<td>+ (A)</td>
<td>−</td>
<td>+ (A)</td>
<td>+</td>
<td></td>
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<td>Toxoplasmosis (clinical)</td>
<td>NA</td>
</tr>
<tr>
<td>6</td>
<td>a (-98)</td>
<td>−</td>
<td>−</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td>None</td>
<td>Toxoplasmosis (clinical+necropsy)</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>b 0</td>
<td>+ (B)</td>
<td>−</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>a 0</td>
<td>+ (ND)</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td></td>
<td>Ganciclovir (from −180 to +80)</td>
<td>CMV encephalitis</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>b (+100)</td>
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<td>−</td>
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<td>−</td>
<td>−</td>
<td>+</td>
<td></td>
<td>None</td>
<td>Primary lymphoma (biopsy)</td>
<td>CMV encephalitis (clinical)</td>
</tr>
</tbody>
</table>

NA=sample not available; ND=HHV-6 variant not defined; +=positive, −=negative.

*In parentheses: number of days before (+) or after (-) CSF sampling in concomitance to onset of neurological disease (0).
†Varicella zoster virus (patient 4) or Epstein-Barr virus (patient 8) was also found in the CSF.
‡CMV was demonstrated in the same periventricular areas that were found to be HHV6 positive by PCR.
§HHV-6 DNA was found in 5 of 13 sections, including cerebral cortex, basal ganglia, and periventricular areas; no additional material was available for examination by the subtype PCR.
Human herpesvirus 6 in cerebrospinal fluid of HIV infected patients

were also examined by HHV-6 PCR.\textsuperscript{11} patients whose CSF was HHV-6-DNA positive were also examined by HHV-6 PCR.\textsuperscript{11}

**Results**

HHV-6-DNA was found in the CSF of eight of the 365 patients (2.2%, table). HHV-6 A was found in two patients and HHV-6 B in four; in the remaining two patients the HHV-6 variant was not defined, because the subtype PCR yielded a negative result. Multiple CSF specimens were available from six patients: HHV-6 was found in all of the specimens from four of these patients, showing the same viral variant in each patient. CMV-DNA was found concomitantly with HHV-6-DNA in the CSF from five patients (table 1). None of the CSF samples from the 20 HIV seronegative controls was HHV-6-DNA positive.

HHV-6-DNA was found in the plasma of five of seven patients examined. In all cases, the same viral variant was found in CSF and plasma. CMV-DNA was found in plasma from all of the patients whose CSF was HHV-6-DNA positive (table). HHV-6-DNA was not found in plasma samples drawn from 22 HIV infected patients with neurological disease whose CSF was HHV-6-DNA negative.

Discordant HHV-6 PCR results between CSF and plasma were seen on six occasions: in all cases, the virus was only found in the CSF. Analysis of the CSF and plasma samples drawn after ganciclovir treatment disclosed persistence of HHV-6 in the CSF but not in the plasma (table).

Of the eight patients who had a CD4+ cell count of less than 50/µl in all of these patients clinical neurological findings that could almost always be explained by the presence of other opportunistic CNS diseases were present. Histology confirmed that none of the four patients examined had any identifiable lesions that were not attributable to opportunistic pathogens (table). However, one patient (patient 3) had mild neurological symptoms, consisting of hyposthenia and paresthesia of the limbs, both before and after the resolution of toxoplasmosis, but in the presence of a HHV-6-DNA positive CSF. In another patient (patient 4), a hemisindrome with cranial nerve deficits was still present after clearance of both CMV and VZV, but not HHV-6-DNA, from the CSF after ganciclovir therapy.

**Discussion**

The aim of this study was to assess the frequency of HHV-6-DNA detection in the CSF of patients infected with HIV, and its possible relation with neurological disease. HHV-6-DNA was found in 2.2% of 365 patients with neurological disease, a detection rate that is similar to that previously found by us for HSV-1, HSV-2, and VZV (1.6%, 0.6%, and 2.6%, respectively),\textsuperscript{14 15} but substantially lower than that found for CMV, EBV, and JCV (16%, 12%, and 9%, respectively).\textsuperscript{11} In a previous report by Liedke et al.,\textsuperscript{12} HHV-6 was found in the CSF of 30% of HIV infected patients with neurological problems. However, unlike in our study, HHV-6 was also found in a similar proportion of controls, suggesting that different detection rates may depend on the different sensitivity limits of the PCR assays, and that passenger HHV-6 positive blood lymphocytes may be detected in CSF if a very sensitive assay is used. Differently from children with primary infection and patients with multiple sclerosis, in whom the variant B of HHV-6 is predominantly identified,\textsuperscript{14 17} both variants were found in our patient group. However, and in line with our study, variant A has also been found in patients with HHV-6 encephalitis,\textsuperscript{18} as well as in children in whom HHV-6 persisted in the CSF after primary infection.\textsuperscript{18}

In most patients infected with HIV, the presence of viral genomes in the CSF is associated with clinically significant brain infection. However, we were unable to find a clear association between HHV-6-DNA detection in the CSF and virus induced neurological disease. Opportunistic diseases caused by pathogens other than HHV-6 that could totally explain the neurological symptoms were actually identified in all of our patients whose CSF was HHV-6-DNA positive. Two patients had neurological symptoms before or after the development of opportunistic infections, and in the absence of infectious agents identified other than HHV-6; however, the possibility that symptoms were initial manifestations or sequelae of underlying opportunistic infections cannot be excluded. In neuropathological studies, HHV-6 antigens have been identified in demented patients in the absence of other pathogens, thus suggesting that the virus may play a part in inducing brain disease.\textsuperscript{14} By PCR, we found HHV-6-DNA in the brain tissue of only one of our patients. Although this low detection rate might have resulted from a low sensitivity of PCR on paraffin embedded tissues, again, no relation with unidentified tissue lesions was found in the HHV-6 positive patient.

On the other hand, the fact that HHV-6 was found in almost all of the serial CSF samples taken from our patients, and that both of our PCR assays were positive in six out of eight patients suggests that, at least in these patients, the finding was not incidental. HHV-6 was often detected in plasma of our patients, suggesting that the virus may have spread to the CSF from the blood. However, the frequent discrepancies between the CSF and plasma HHV-6 findings support the hypothesis of an active brain infection by HHV-6, rather than of a passive spread through the blood-brain barrier. We therefore hypothesise that HHV-6 may be found in the CSF after reactivation in the brain or blood, without its presence necessarily inducing neuropathological changes, or clinical disease, or both.

Although no correlation between HHV-6 and HIV infections of the brain could be shown in our study, HHV-6 was found concomitant with CMV in the CSF, plasma, or both in most of our patients. HHV-6 in situ hybridisation with recent reports of tissue HHV-6 and CMV coinfection in organ transplant recipients,\textsuperscript{17 18} and of their in vitro interactions,\textsuperscript{19} all support-
ing a close interrelation between these two herpesviruses.

In conclusion, it is unlikely that HHV-6 plays a prominent part in inducing neurological disease in patients infected with HIV. However, in a small fraction of patients, the virus may reactivate in the brain, and this often occurs concomitant with active CMV brain infection.

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