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Search for herpesvirus DNA in cerebrospinal fluid of HIV patients with brain disorders: prevalence of cytomegalovirus DNA findings

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A study was carried out to search for the presence of the seven human herpesvirus DNAs in cerebrospinal fluid from 52 human immunodeficiency virus-infected patients with brain disorders. Cytomegalovirus DNA was the most prevalent with 12 positive samples; Epstein-Barr virus and varicella-zoster DNAs were detected in three and two samples, respectively, while no sample was positive for the DNA of the other herpesviruses.

Keywords: herpesviridae; CMV; EBV; HHV-6; neuro-AIDS; PCR

Introduction

Opportunistic infections of the central nervous system (CNS) are common in the course of human immunodeficiency virus (HIV) infection (Dix and Palm, 1993). Human herpesviruses, which widely infect humans, remain latent in the host after primary infection, may be reactivated in situations of immunodeficiency and show various degrees of neurotropism (Roizman, 1996). Based on these considerations, a polymerase chain reaction (PCR) study was undertaken to search for herpesvirus DNA in cerebrospinal fluid (CSF) from HIV patients admitted to the Division of Infectious Diseases of the University of Modena (Northern Italy), from January 1993 to August 1995. The investigation aimed to evaluate the presence and the eventual involvement of each herpesvirus in brain disorders affecting the patients.

Results

The results of herpesvirus DNA detection in CSF from 52 HIV patients with CNS clinical manifestations are the following. Fifteen samples were positive in PCR assays with consensus primers able to detect a conserved region of the DNA polymerase gene of human herpes simplex virus-1 (HSV-1), herpes simplex 2 (HSV-2), cytomegalovirus (CMV) and Epstein-Barr virus (EBV) (Rozenberg and Lebon, 1991). In a subsequent analysis of PCR products by restriction endonucleases *Sma*I and *Bam*HI (Rozenberg and Lebon, 1991), an undigested band corresponding to the 589 bp band of CMV was observed in 12 cases. In three cases, enzymatic digestion cleaved the PCR products into two fragments with each enzyme (100 and 424 bp with *Sma*I, 277 and 247 bp with *Bam*HI), showing that these three amplification products were from EBV DNA (Figure 1). Nested (n) PCR assays for varicella-zoster virus (VZV) DNA detection (Ozaki *et al*, 1994) gave positive results in two CSF samples. No positive samples were obtained with nPCR for human herpesvirus 6 (HHV-6) (Aubin *et al*, 1991; Dewhurst *et al*, 1993) and human herpesvirus 7 (HHV-7) (Berneman *et al*, 1992) DNA detection. In the case of HHV-6 nPCR, two samples gave a strong positive band slightly higher than the control band. Digestion of the amplification products with endonucleases *Hind*III and *Hinf*I (Di Luca *et al*, 1994) showed that the bands were aspecific.

Discussion

The search for herpesvirus DNA in CSF samples from 52 HIV patients with brain disorders disclosed a marked prevalence of CMV DNA. CMV DNA was found in twelve samples, while three samples proved positive for EBV DNA and two positive for VZV DNA (P=0.026 for CMV *versus* EBV; P=0.01 for CMV *versus* VZV with the chi-square test). This CMV DNA finding is even more relevant in the face

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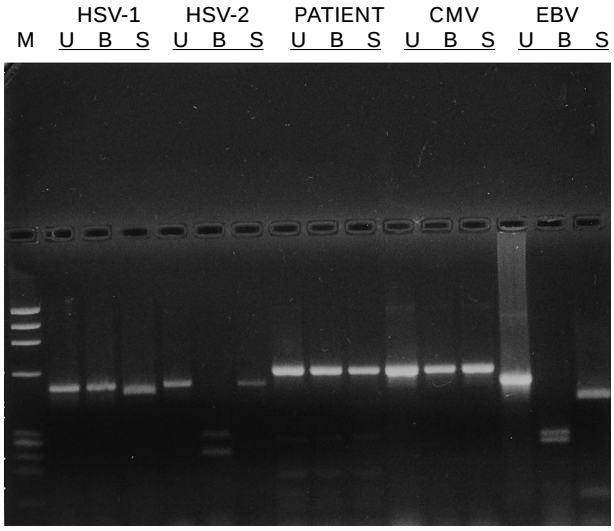

Figure 1 Consensus amplificates from a CSF sample showing the enzymatic pattern of CMV DNA. Ethidium bromide-stained agarose gel analysis: undigested (lanes U) and digested (lanes B: digestion with restriction enzyme *Bam*HI; lanes S: digestion with restriction enzyme *Sma*I) products; M: molecular weight marker (digested ∅ DNA). The consensus amplificate corresponding to CMV DNA shows undigested bands with both enzymes.

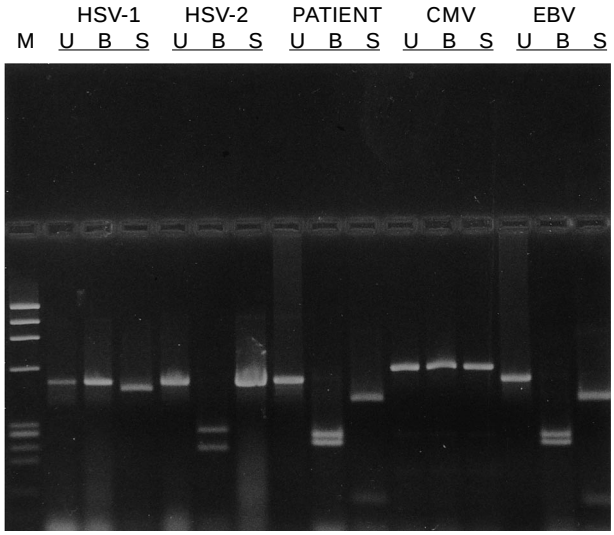

Figure 2 Consensus amplificates from a CSF sample showing the enzymatic pattern of EBV DNA. Ethidium bromide-stained agarose gel analysis: undigested (lanes U) and digested (lanes B: digestion with restriction enzyme *Bam*HI; Lanes S: digestion with restriction enzyme *Sma*I) products; M: molecular weight marker (digested ∅ DNA). The consensus amplificate corresponding to EBV DNA shows two bands originated by the cleavage of each enzyme: 277 and 247 bp with *Bam*HI, 100 and 247 bp with *Sma*I.

of the negative results obtained in the search for HSV-1, HSV-2, HHV-6 and HHV-7 DNAs.

The difference in the distribution of presumably reactivated viruses in CSF was somewhat unexpected, considering that the herpesvirus-host relationship shares features common to all members of the human herpesvirus group (Glaser and Jones, 1994; Frenkel and Roffman, 1996). With the exclusion of HSV-2, which usually has a lower diffusion, herpesviruses infect 70–80% of humans. All herpesviruses remain latent in the host after the primary infection. Excluding HHV-7, the pathogenicity of which is still unknown, the remaining herpesviruses can affect the CNS. CMV, EBV, HHV-6 and HHV-7 all establish latency in blood cells, so that any of the rescued virus can diffuse to the brain from blood. The same opportunity of reaching the CNS is shared by HSV-1, HSV-2 and VZV, all of which latently infect the same cell type.

HHV-6 and HHV-7 share the same target cells with HIV (Lusso *et al*, 1989; Frenkel *et al*, 1990). Since HIV replication is efficient in the course of symptomatic HIV infection (Ho *et al*, 1995; Wei *et al*, 1995), competition for the target cells could result in lack of expansion of the reactivated herpesvirus population and, consequently, in the absence of virus in the samples to be tested. This hypothesis could also explain the very low frequency of HHV-6 and HHV-7 isolates rescued in our laboratory from 25 peripheral blood cultures of HIV symptomatic patients. When tested for the presence of specific antigens, no cultures showed positivity for HHV-7. Only two cultures positive for HHV-6 gave rise to two virus isolates (of these, one belonged to a patient with a fever of unclear origin, and the other was from a patient who died a few days after the culture preparation). In contrast, it has been reported that HIV infection enhances HHV-6 replication in $CD4^+$ lymphocytes (Carrigan *et al*, 1990; Sieczkowski *et al*, 1995). The interaction of HIV and HHV-6 however, was studied in a condition of active replication for both viruses, and not in the situation of cells latently infected with HHV-6 which may better reflect the *in vivo* setting.

It has been reported that in immunocompetent CMV seropositive hosts, peripheral blood mononuclear cells are involved in a restricted infection that shifts, in HIV patients, to a productive infection probably as a consequence of a general activation of monocytes and T cells (Nelson *et al*, 1991). The role of tumor necrosis factor in CMV rescue *in vivo* has been documented (Döcke *et al*, 1994). High plasma levels of this cytokine are produced in the course of HIV illness (Lahdevirta *et al*, 1988; Aukrust *et al*, 1994). Thus, in the course of HIV infection, the CMV finding could be frequent as ensured by various rescue mechanisms.

In conclusion, the frequency of herpesvirus endogenous reinfections of the CNS in HIV patients differs for each member of the human herpesvirus family group, as documented by PCR procedures.

The difference probably lies in the fact that either the rescue of each endogenous herpesvirus or the resulting infectious process, or both circumstances may be differently influenced by the complex events that contribute to HIV immunodeficiency.

Information on the nervous disease cases with PCR positive for herpesvirus DNA was obtained from patient records (Table 1). CFS samples positive for CMV DNA were associated with a clinical presentation of encephalitis; of the three samples positive for EBV, two were associated with a clinical presentation of encephalitis and one of meningitis and the two samples positive for VZV with an encephalitis picture.

In AIDS patients, the etiological role of opportunistic organisms such as toxoplasma gondii and cryptococcus neoformans in brain diseases is undiscussed; on the contrary, that of reactivated latent viruses is debated.

As it concerns the nervous pictures associated with EBV and VZV (Table 1), the involvement of the viruses appears acceptable in consideration of the possibility of brain disorders in immunocompetent infected hosts (Glaser and Jones, 1994). A prerequisite for attributing disease to CMV in immunocompromised individuals rests on detection of virus from the specific organ in question (Pillay and Griffiths, 1992). In post-mortem studies of HIV patients, a CMV frequent localisation in the brain tissue has been observed (Klatt and Shibata, 1988; Vinters *et al*, 1989; Pillay *et al*, 1993). In our study, eight out of the twelve CSF samples positive for CMV DNA came from patients who had undergone autopsy (Table 1). Autopsy findings of foci of active CMV infection were detected in the brain tissue of these subjects. These results, together with the CMV DNA presence in the CSF, suggest, in agreement with the observations of others (Wolf and Spector, 1992; Gozlan *et al*, 1992; Fox *et al*, 1995), that CMV may be responsible for brain disease over the course of HIV infection. The expression of the neuropathogenetic potential of CMV in HIV patients, on the other hand, reflects the natural pattern of CMV infection, according to which virus-associated

Table 1

Patient	*Clinical features*	*Neuroimaging findings*	*Clinical diagnosis*	*Autopsy diagnosis*	*Associated herpesvirus DNA*
1	Seizures	Atrophy	Encephalitis	Micronodular encephalitis	CMV
2	Seizures, nystagmus	Negative	Encephalitis	Micronodular encephalitis	CMV
3	Coma	Atrophy	Encephalitis	Micronodular periventricular encephalitis	CMV
4	Seizures, ataxia	NP	Encephalitis	Periventricular encephalitis	CMV
5	Seizures nystagmus	Hypodense lesions in the right frontal lobe	Encephalitis	Periventricular encephalitis	CMV
6	Aphasia, headache	Hypodense lesions in the left parietal lobe	Encephalitis	Periventricular encephalitis	CMV
7	Seizures	Hypodense lesions in the left parietal lobe	Encephalitis	Micronodular encephalitis	CMV
8	Coma, seizures, nystagmus	Atrophy	Encephalitis	Micronodular encephalitis	CMV
9	Seizures	Periventricular enhancement	Encephalitis		CMV
10	Seizures, nystagmus	Periventricular enhancement	Encephalitis		CMV
11	Seizures	Periventricular enhancement	Encephalitis		CMV
12	Ataxia, cranial nerve palsies	Periventricular encephalitis	Encephalitis		CMV
13	Meningismus headache	NP	Meningitis		EBV
14	Ataxia	Periventricular encephalitis	Encephalitis		EBV
15	Headache, hemiparesis	NP	Encephalitis		EBV
16	Meningismus, headache, hemiparesis, cranial palsies	Atrophy	Encephalitis		VZV
17	Seizures, ataxia	Periventricular enhancement	Encephalitis		VZV

NP: not performed

clinical manifestations appear almost exclusively in subjects with impaired immunity, the normal immunity generally being protective and able to prevent virus-induced diseases in normal individuals.

In HIV patients under study, CMV appeared associated with brain disorders with a frequency higher than that of other human herpesviruses (P=0.026 for CMV *versus* EBV, P=0.01 for CMV *versus* VZV with the chi-square test). HSV-1, HSV-2, HHV-6 and HHV-7 DNAs were not detected in any of the CSF samples. The lack of a possible role of HHV-6 in neurological disease in the course of HIV infection that these negative results suggest agrees with the conclusions of other authors (Liedtke *et al*, 1995). On the other hand, HHV-6 has been implicated in brain as well as lung diseases affecting HIV patients on the basis of HHV-6 antigens detected in cerebral and pulmonary tissues during autopsy (Corbellino *et al*, 1993; Knox and Carrigan, 1994). The frequency of HHV-6 findings in tissues from autopsy may result from a massive reactivation of the virus in terminally ill AIDS patients. Such an event could reconcile the *post mortem* presence of HHV-6 antigens in nerve and pulmonary tissues and the absence of HHV-6 DNA in CSF from HIV patients included in this study, as well as in bronchoalveolar lavage from HIV patients with pulmonary disorders processed for HHV-6 DNA in a previous investigation (Portolani *et al*, 1996). A comparison of the incidence of HHV-6-associated organ-diseases in individuals immunocompromised by HIV infection or other causes would determine the importance of reactivated HHV-6 in inducing clinical manifestations in the course of HIV infection.

Materials and methods

CSF samples were from 52 HIV patients admitted to the Division of Infectious Diseases of the University Hospital of Modena from January 1993 through August 1995 for investigation of neurologic symptoms.

For DNA extraction, 500 μl of each CSF sample were incubated with 1 μl glycogen solution 20 mg/ml, 10 μl SDS 10%, 10 μl proteinase K 20 mg/ml at 56°C for 1 h. After inactivation of proteinase K at 95°C for 10 min, 500 μl NaCl 4 M were added and the sample centrifuged for 15 min at 5000 r.p.m. in an Eppendorf centrifuge. The DNA was precipitated from the supernatant with isopropyl alcohol (vol/vol). After overnight incubation at −20°C, the DNA was pelleted at 14 000 r.p.m. for 30 min, washed with 70% ethanol and resuspended in 20 μl Tris-EDTA buffer.

A single pair of primers selected within a highly conserved region of the DNA polymerase gene of HSV-1, HSV-2, EBV and CMV (consensus primers P1 and P2, Rozenberg and Lebon (1991) was used to detect the presence of these herpesviruses. The amplification and the subsequent analysis of PCR products with restriction endonucleases (*Sma*I and *Bam*HI) for DNA typization were carried out according to Rozenberg and Lebon (1991).

nPCR assays were used to search for the presence of DNA of VZV, HHV-6 and HHV-7 in CSF samples proved negative for consensus primers P1 and P2.

Outer and inner primers used to detect VZV DNA amplified a fragment from glycoprotein I gene according to Ozaki *et al* (1994). Primers A and C (Aubin *et al*, 1991) and HS6AE and HS6AF (Dewhurst *et al*, 1993) were employed for HHV-6 outer and inner amplification, respectively, using programmes described elsewhere (Portolani *et al*, 1996). To confirm the specificity of nPCR products obtained from 2 CSF samples, these were analyzed by endonuclease digestion with *Hind*III and *Hinf*I (Di Luca *et al*, 1994).

According to Berneman *et al* (1992), nPCR for HHV-7 was carried out with primers HV7 and HV8 for outer amplification and HV10 and HV11 for the inner amplification.

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