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Case Report

Epstein–Barr virus DNA in cerebrospinal fluid from an immunocompetent man with herpes simplex virus encephalitis

Marinella Portolani[1,2], Anna Maria Teresa Sabbatini[1], Marisa Meacci[1], Paola Pietrosemoli[1], Claudio Cermelli[2], Paolo Lunghi[3], Franca Golinelli[3] and Raffaele Stacca[3]

[1]Centre for Diagnosis of Viral Diseases, University Hospital of Modena; [2]Department of Biomedical Sciences, University of Modena; [3]Intensive Care Unit of Carpi Hospital (Modena), Italy

Herpes simplex virus 1 meningo-encephalitis was ascertained in a 63-year-old immunocompetent man. To determine the duration of the persistence of herpesvirus DNA in the central nervous system, the cerebrospinal fluid was periodically monitored by polymerase chain reaction for 53 days. In addition to HSV-1, Epstein–Barr virus DNA was detected in the cerebrospinal fluid 9 days after disease onset. The possible meaning of the Epstein–Barr virus DNA finding is discussed.

Keywords: EBV; HSV; encephalitis

Introduction

In patients with suspect herpes simplex virus (HSV) encephalitis the search for virus DNA in cerebrospinal fluid (CSF) by polymerase chain reaction (PCR) has replaced virus detection by brain biopsy since CSF–PCR is highly sensitive and specific and less invasive. PCR is used as a routine diagnostic technique at the Centre for the Diagnosis of Viral Diseases of the Modena University. We used this technique to establish a virological diagnosis of HSV-1 meningo-encephalitis in a 63-year-old immunocompetent male patient. PCR monitoring of the CSF for nucleic acid sequences of other herpesviruses (types 2–7) in addition to those of HSV-1 was also performed which showed the appearance of Epstein–Barr virus (EBV) DNA in the patient's CSF. The presence of genomic material from a second herpesvirus in the course of HSV brain infection is noteworthy.

Results

EBV DNA was detected in the CSF from a 63-year-old man 9 days after the onset of a meningo-encephalitis attributed to HSV-1 on the basis of the detection of both HSV-1 nucleic acid sequences and anti-HSV-1 IgG antibody in the patient's CSF.

As shown in Table 1, DNA sequences belonging to HSV-1 and EBV were found in CSF samples drawn 4 and 9 days, and 9 and 54 days after the onset of illness, respectively.

Table 1 also reports the results of PCR investigations carried out on peripheral blood mononuclear cells (PBMC) and the results of serological studies. At onset of symptoms, the search for serum antibodies to HSV-1 carried out with commercial ELISA kits (Bouty, Milan, Italy) showed IgG seropositivity and absence of IgM. The IgG antibody level increased significantly in subsequent serum samples: an initial OD value of 2.85 reached and remained at a value of 5.0 for at least 53 days. When ELISA IgG serum antibody levels to EBV antigens VCA and EBNA-1 (Bouty, Milan, Italy) were simultaneously measured in the earliest and latest serum specimens available, only a modest antibody increase to each antigen (0.50 of OD) was observed. This result, together with the absence of EBV IgM antibody and the detection of EBV DNA in PBMC collected 9 and 53 days after the disease onset are consistent with the mobilisation from latency of EBV in blood cells. In CSF samples, the antibody detection of HSV-1 and EBV also differed. HSV-1 IgG antibody, which first appeared on the ninth day after disease onset, was still present after 53 days. EBV antibodies were

Correspondence: M Portolani
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Table 1 HSV-1 and EBV antibody and nPCR results

	IgG antibody to:[a]						*nPCR results*		
	HSV-1		*EBV Ags in*				*HSV-1*	*EBV DNA*	
Days after	*in*		*CSF*		*Serum*		*DNA in*	*in*	
disease onset	*CSF*	*Serum*	*VCA*	*EBNA-1*	*VCA*	*EBNA-1*	*CSF*	*CSF*	*PBMC*
4 days	0.87	2.85	0.21	0.20	2.70	2.95	+	–	na
9 days	5.92	n.d.	0.13	0.31	n.d.	n.d.	+	+	+
34 days	5.89	5.15	0.10	0.24	n.d.	n.d.	–	+	na
53 days	3.82	5.0	0.14	0.18	3.25	3.51	–	–	+

Other antibody results: IgM antibody both to HSV-1 and EBV were absent in all serum and CSF samples available. IgG antibody to CMV present in serum (1.70 OD value; cut-off value: 0.20) was absent in all the available CSF samples.

Other virological results: all CSF samples proved negative for HSV-2, VZV, CMV, HHV-6 and HHV-7 DNA. No virus was isolated from CSF samples inoculated in cell cultures.

[a]=ELISA IgG antibody values at the 1 : 100 sample dilution were expressed as absorbance values (cut-off value for both HSV-1 and EBV positive results equal 1); n.a.=not available; n.d.=not done

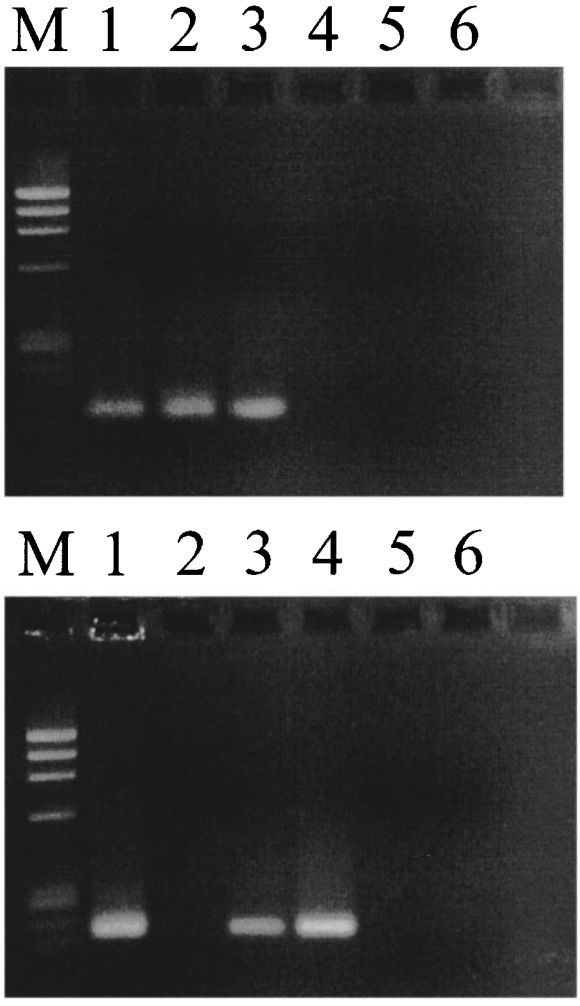

Figure 1 Amplification products from the four available samples of patient's CSF processed together by nPCR according to Oosterveer *et al* (1993), modified by Valensin. Upper picutre: PCR for HSV-1; lower picture; PCR for EBV. Lanes M=molecular weight marker (digested ϕ DNA); 1=positive control; 2–5=DNA from patients CSF samples taken on hospital admisson (2), 9 (3), 30 (4) and 50 (5) days thereafter; 6=blank.

absent throughout the period of CSF monitoring. Cytomegalovirus (CMV) IgG antibody (Biotest, AG Dreieich, Germany) present in serum was absent from CSF in accordance with the maintenance of an intact blood brain barrier.

Discussion

On the finding of HSV-1 genomic sequence and virus specific IgG antibody in the CSF, a diagnosis of meningo-encephalitis by HSV-1 was made in a 63-year-old man. The brain viral infection appeared to be the result of a HSV-1 reactivation caused by unknown events. The origin of the rescued virus, whether from peripheral or central nervous system cells, was also unknown.

The longitudinal study of CSF samples for the presence of herpesvirus DNA showed that, in addition to an HSV-1 DNA, EBV nucleic acid sequences were present. A CSF specimen collected 9 days after the onset of illness proved positive for EBV DNA. Moreover, while HSV-1 DNA disappeared from the CSF after this period, EBV DNA was still detectable at 34 days of illness.

In spite of the complexity of the virological situation, the clinical outcome of the CNS illness was favourable and 53 days after disease onset only a modest motor deficit persisted. The benign course of this case of HSV-1 encephalitis is consistent with previous literature reports. A British study suggested the existence of milder forms of HSV-1 encephalitis associated with lower mortality and increased morbidity (Klapper *et al*, 1984). The acyclovir therapy of HSV-1 encephalitis, in addition to reducing mortality, results in an increasing percentage of patients who, irrespective of age, return to normal function (Scheld *et al*, 1997).

The balance between virus replication and the host's immune response to a virus is critical to determine different rates of disease progression. In

our patient the humoral response to HSV-1 appeared vigorous (Table 1). An initially low viral load and/or a high sensitivity to acyclovir of the HSV-1 strain involved in the infectious process might, in addition, contribute to the favourable outcome of the disease. The inclusion of quantitative PCR among currently available diagnostic procedures will disclose the respective weight of each of these viral factors in order to gauge the outcome of future cases of HSV-1 encephalitis.

An intriguing aspect of the present case was the presence of EBV DNA in the patient's CSF. A report on the use of PCR for viral diagnosis of CNS diseases (Jeffery *et al*, 1997) noted that a CSF white-cell count of five or more cells/mm^3 is an independent predictor of a PCR positive result. In our patient, the later of the two EBV DNA positive CSF specimens taken 34 days after the disease onset revealed five mononuclear cells/mm^3.

Using the PCR technique, an unexpected frequency of EBV detection in brain biopsies from immunocompetent hosts with different CNS diseases has been ascertained (Pedneault *et al*, 1992). In our case, illness exacerbation which resulted in the appearance of paraplegia, coincided with the finding of EBV DNA in the patient's CSF. However, since quadriparesis may have been due to HSV alone, it is possible that the detection of EBV in CSF in occasion of the worsening of the patient's neurological symptoms is casual. Given the EBV reactivation in the patient's blood cells, possibly as a consequence of the HSV-1 encephalitis, the presence of EBV DNA in the CSF may have resulted from the migration into CSF of activated virus containing B cells in response to HSV-1 infection without pathogenetic repercussions.

In conclusion, the presence of EBV in the CSF of our patient with HSV-1 encephalitis is unlikely to be pathogenetic, but this unusual finding is noteworthy and should trigger further investigations into brain disorders of herpesvirus origin.

Materials and methods

Case report

A 63-year-old man was admitted to the Intensive Care Unit of Carpi Hospital (near Modena) with second degree respiratory failure, vascular shock and coma. Before admission, the patient had had a oral temperature of 38.5°C for 3 days followed by ataxia, tinnitus, paresthesias of the upper limbs in association with an increaes in temperature to 40°C. Neurological disease rapidly worsened with seizures, loss of consciousness and subsequent coma requiring hospital admission. Neurological examination on admission disclosed nucal rigidity, myotic pupils reactive to light, right gaze deviation and left hemiparesis. Brain CT scan showed hypodensity in the right temporal lobe involving the ipsilateral cortical-subcortical and periventricular regions. CSF analysis revealed 32 mononuclear cells/mm^3, 125 mg/dl total protein and 56 mg/dl glucose. The patient was artificially ventilated and treated with antiedema drugs (mannitol and dexametasone), antibiotics and acyclovir for possible microbial organisms and herpesvirus. As a consequence of the HSV-1 DNA detection in CSF, acyclovir therapy (750 mg every 8 h, 30 mg/kg/day) was continued for a period of 20 days. The patient's clinical condition remained stationary until the seventh day when flaccid tetraparesis appeared. An extension of the hypodensity from the right temporal to parietal lobe and the appearance of cortical hyperdensities correlated to hemorrhagic petecchia observed with CT confirmed the neurological deterioration. In spite of this, during the subsequent days, the patient slowly recovered consciousness allowing a changeover from artificial ventilation to PS ventilation. Twenty-six days after admission, when the patient was transferred to the Medical Ward of the same hospital, respiration was spontaneous and the neurological manifestations consisted of anisocoria (dx < sx), a upper motor neuron type of facial paresis on the left and hypoesthesia of the left hemisoma. CSF analysis sample 4 days after the ward change (33 days the disease onset) disclosed five mononuclear cells/mm^3, 114 mg/dl total protein and 65 mg/dl glucose.

After another 3 weeks in hospital, the patient was discharged, able to walk without assistance with only a mild left hemiparesis. An investigation able to ascertain the presence of possible cognitive deficits was not performed.

Virological studies

CSF samples taken from the patient on admission to hospital and 9, 30 and 50 days thereafter, were examined for the presence of HSV-1, HSV-2, varicella-zoster virus (VZV), CMV, EBV, human herpesvirus 6 (HHV-6) and human herpesvirus 7 (HHV-7) by nested (n) PCR. In addition, EBV DNA was searched for in PBMCs taken 9 and 15 days after disease onset. DNA was extracted from 500 μl of CSF and from PBMCs as previously described (Portolani *et al*, 1996, 1997, respectively). Outer and inner primers and nPCR procedures for the search for each herpesvirus DNA were from the following literature reports: Aurelius *et al* (1991) for HSV-1 DNA, Lynas *et al* (1993) for HSV-2 DNA, Ozaki *et al* (1994) for VZV DNA, Rawal *et al* (1994) for CMV DNA, Aubin *et al* (1991) and Dewhurst *et al* (1993) for HHV-6 DNA and Berneman *et al* (1992) for HHV-7 DNA. The presence of EBV DNA in the samples under study was ascertained by two different nPCR assays. Firstly, the samples were tested with a pair of primers (Oosterveer *et al*, 1993) with an amplification protocol modified according to Professor PE Valensin (Laboratory of Microbiology, Department of Molecular Biology, University of Siena) in order to use the EBV primers together

with a pair of primers for CMV in the same amplification programme. The external amplification programme consists of an initial denaturation at 96°C for 5 min and then 25 cycles each composed of an annealing step at 56°C for 30 sec, an extension step at 75°C for 30 sec and a denaturation step at 94°C for 35 sec; the final extension step is at 72°C for 4 min. The internal amplification programme consists of an initial denaturation at 96°C for 1 min and then 30 cycles with annealing at 58°C for 30 sec, extension at 75°C for 15 sec and denaturation at 94°C for 35 sec; final extension is carried out at 72°C for 4 min. These EBV primers amplify a region inside the BKRF1 gene. The same samples were checked for EBV DNA with two pairs of primers (PER1, PER2, PER3, PER4) which recognise a sequence of the EBV *Bam*HIW region (Laroche *et al*, 1995). A molecular control to assess the absence of amplification of HSV-1 DNA by EBV primers was included in each of the two EBV DNA amplification protocols.

The presence of infectious virus in CSF was checked by CSF inoculation into human fibroblast and VERO cell cultures.

Serum samples were examined for the presence of IgG and IgM antibodies with commercial ELISA kits: Bouty (Milan, Italy) kits were used for HSV-1, HSV-2, VZV, CMV antibodies, while Biotest kits (AG Dreieich, Germany) for EBV antibodies. In the case of HSV-1, EBV and CMV, the search for IgG and IgM antibodies was made in CSF samples as well.

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References

Aubin JT, Collandre H, Candotti D, Ingrand D, Rouzoux C, Burgard M, Richard S, Huraux JM, Agut H (1991). Several groups among human herpesvirus 6 strains can be distinguished by Southern-blotting and PCR. *Journal of Clinical Microbiology* **29:** 367–372.

Aurelius E, Johansson B, Sköldenberg B, Staland A, Forsgren M (1991). Rapid diagnosis of herpes simplex encephalitis by nested polymerase chain reaction assay of cerebral fluid. *Lancet* **337:** 188–192.

Bernemann ZN, Ablashi DV, Edger-Flechter ME, Reitz MS, Hung CL, Brus I, Komaroff AL, Gallo RC (1992). Human herpesvirus 7 is a T-lymphotropic virus and is related to, but significantly different from, human herpesvirus 6 and human cytomegalovirus. *Proceedings of the National Academy of Sciences USA* **89:** 10552–10556.

Dewhurst S, McIntyre K, Schnabel K, Hall CB (1993). Human herpesivirus 6 (HHV-6) variant B accounts for the majority of symptomatic primary HHV-6 infections in a population of US infants. *Journal of Clinical Microbiology* **31:** 416–418.

Jeffery KJM, Read SJ, Peto TEA, Mayon-White, Bangham CRM (1997). Diagnosis of viral infections of the central nervous system: clinical interpretation of PCR results. *Lancet* **349:** 313–317.

Klapper PE, Cleator GM, Longson M (1984). Mild forms of meningo-encephalitis. *Journal of Neurology, Neurosurgery and Psychiatry* **47:** 1247–1250.

Laroche C, Drouet EB, Brousset P, Pain C, Boibieux A, Biron F, Icart J, Denoyel GA, Niveleau A (1995). Measurement by the polymerase chain reaction of Epstein–Barr virus load in infectious mononucleosis and AIDS-related non-Hodgkin's lymphomas. *Journal of Medical Virology* **46:** 66–74.

Lynas C, Hill TJ, Maitland NJ, Love S (1993). Latent infection with MS strain of herpes simplex virus type 2 in the mouse following intracerebral inoculation. *Journal of Neurological Sciences* **120:** 107–114.

Oosterveer M, Markusse HM, Lennette ET, Zou JZ, Bolhuis RLH, Gratama JW (1993). Characterization of Epstein–Barr viral strains in parotid gland saliva and peripheral blood of patients with primary Sjögren's syndrome and healthy Epstein–Barr virus carriers. *Journal of Medical Virology* **41:** 261–269.

Ozaki T, Masuda S, Asano Y, Kondo K, Namazue J and Yamanishi K (1994). Investigation of Varicella-Zoster Virus DNA by the polymerase chain reaction in healthy children with varicella vaccination. *Journal of Medical Virology* **42:** 47–51.

Pedneault L, Katz BZ, Miller G (1992). Detection of Epstein–Barr virus in the brain by polymerase chain reaction. *Annals of Neurology* **32:** 184–192.

Portolani M, Fabio G, Pecorari M, Pietrosemoli P, Meacci M, Sabbatini AMT, Cermelli C, De Rienzo B (1996). Search for human herpesvirus 6 and human cytomegalovirus in bronchoalveolar lavage from patients with human immunodeficiency virus-1 and respiratory disorders. *Journal of Medical Virolgy* **48:** 179–183.

Portolani M, Meacci M, Sabbatini AMT, Pietrosemoli P, Pecorari M, Cermelli C, De Rienzo B (1997). Search for DNA in cerebrospinal fluid of HIV patients with brain disorders: prevalence of cytomegalovirus DNA findings. *Journal of Neurovirology* **3:** 192–196.

Rawal BK, Booth JC, Fernando S, Butcher PD, Powles RL (1994). Quantification of CMV DNA in blood specimens from bone marrow transplant recipients by polymerase chain rection. *Journal of Virological Methods* **47:** 189–202.

Scheld WM, Whitley RJ, Durack DT (1997). *Infections of the central nervous system.* Second Edition. Lippincott-Raven Publishers: Philadelphia, New York. pp. 83–85.