LETTERS TO THE EDITOR

Detection of Epstein-Barr virus genome in peripheral leucocytes and CSF by the polymerase chain reaction in two patients with Epstein-Barr virus related to aseptic meningitis

Epstein-Barr virus, a lymphotropic herpes virus that causes infectious mononucleosis, also causes neurological disorders such as encephalitis, cerebellitis, meningitis, and the Guillain-Barré syndrome. The detection of the Epstein-Barr virus genome has been described.[1 2] We report two patients with aseptic meningitis having symptoms similar to those of infectious mononucleosis, in whom the Epstein-Barr virus genome was detected in peripheral leucocytes and CSF by the polymerase chain reaction (PCR).

Patient 1, a 34 year old Japanese man presented with fever and headache in May 1993. He had no underlying disease. Eight days after the onset of symptoms, he was admitted to our hospital. No rash, lymphadenopathy, or hepatosplenomegaly was noted. He was alert and showed mild signs of meningeal irritation. No other neurological abnormalities were found. Laboratory data showed leucocytosis (11 200), 3% atypical lymphocytes, and a slight increase in aspartate aminotransferase and γ-glutamic-pyruvic transaminase. The Paul-Bunnel test was negative. His CSF showed lymphocytic pleocytosis (65/mm^3), including 3% atypical lymphocytes and a mild increase in protein, but the CSF concentration of glucose was normal. Serum testing showed Epstein-Barr viral capsid antigen (VCA)-IgG and VCA-IgM. VCA-IgG decreased 160 times to 40 times between the acute stage and recovery (three months) and VCA-IgM also decreased (10 times to <10 times) whereas Epstein-Barr nuclear antigen increased from <10 times to 80 times. There were no significant antibody titres for herpes simplex, varicella zoster, or cytomegalovirus. Findings on chest radiographs, ECG, brain CT, and EEG were normal.

Patient 2, a 20 year old Japanese woman presented with fever and headache in June 1993. She had tonsillitis and swelling of the lymph nodes in the postauricular region. Neurological examination on admission showed only mild meningeal signs. Three per cent atypical lymphocytes were found in the peripheral blood and CSF. The Paul-Bunnel test was negative. Her CSF showed mild pleocytosis (102/mm^3), a mild increase in protein, and a normal glucose concentration. Serum and CSF EBV VCA-IgG decreased more than four times between the acute to recovery stage (serum 320 times to 40 times, CSF 4 times to <1 times). No other significant changes in viral antibody titres were found. Normal results were obtained on brain CT and EEG.

We made a presumptive diagnosis of Epstein-Barr virus meningitis in both patients on about the 10th day after onset of illness, based on symptoms resembling those of infectious mononucleosis and the presence of raised Epstein-Barr virus antibody titres. Both responded well to conservative treatment. Meningeal signs disappeared and CSF findings became normal within one month of onset of symptoms.

Peripheral leucocytes and CSF were subjected to capillary PCR.[3] The PCR analysis was performed with 1 μl of extracted DNA from about 500 μl leucocytes with buffer, and with a 50 μl sample of CSF. The primers for the EBNA1 gene have been described by Telenti *et al*[4] using the sequences: 5-GTCATCATCATC-CGGGTCTC for the plus strand and 5'-TTCGGGTTGGAACCTCCTTG for the minus strand. Amplification of DNA by rapid and high sensitivity capillary PCR was carried out for 40 cycles at 94°C for five seconds (denaturation), 42°C for five seconds (annealing), and 72°C for three seconds (extension). Products of the PCR were visualised with UV light by staining with ethidium bromide after gel electrophoresis, and Epstein-Barr virus DNA was detected as a specific 220 bp band. DNA extracted from Daudi cells was used as the Epstein-Barr virus positive control. Epstein-Barr virus DNA was detected in the CSF obtained 10 days after onset in patient 1, and eight days after onset in patient 2, and continued to be detected for three weeks after onset in both patients, whereas DNA was positive in the leucocytes four weeks after onset. Analysis by PCR was also performed for leucocytes and CSF samples from five control subjects with aseptic meningitis caused by Behçet's disease, but no Epstein-Barr virus DNA was found in these cases.

A few reports have described the detection of the Epstein-Barr virus gene by PCR in the blood and CSF of patients with neurological disorders caused by the Epstein-Barr virus. Imai *et al*[1] analysed CSF by PCR and Southern blotting, and found Epstein-Barr virus DNA in the CSF of five children with meningoencephalitis associated with infectious mononucleosis during the acute stage. Landgren *et al*[2] reported the detection of Epstein-Barr virus DNA by nested PCR in the serum and CSF of two patients with encephalitis and myelitis. In both of our cases, Epstein-Barr virus DNA was found in peripheral leucocytes and CSF during the acute stage of aseptic meningitis associated with a syndrome resembling infectious mononucleosis. Detection of Epstein-Barr virus DNA was possible for three to four weeks after onset. Due to the difficulty in isolating Epstein-Barr virus, few studies have isolated it from the CSF of patients with neurological disorders.[5] Our experience indicates that the detection of Epstein-Barr virus DNA by PCR is useful in diagnosing Epstein-Barr virus related to aseptic meningitis and in evaluating its pathophysiology.

MASAHIDE KAJI
HIROSHI SHOJI
First Department (Neurology) of Internal Medicine, Kurume University School of Medicine, 67 Asahimachi, Kurume, Japan 830

Correspondence to: Dr Masahide Kaji.

1 Imai S, Usui N, Sugiura M, *et al*. Epstein-Barr virus genomic sequences and specific antibodies in cerebrospinal fluid in children with neurologic complications of acute and reactivated EBV infection. *J Med Virol* 1993; 40:278–84.
2 Landgren M, Kyllerman M, Bergström T, Dotevall L, Ljungström L, Ricksten A. Diagnosis of Epstein-Barr virus-induced central nervous system infections by DNA amplification from cerebrospinal fluid. *Ann Neurol* 1994;35:631–5.
3 Wittwer CT, Fillmore GC, Hillyard DR. Automated polymerase chain reaction in capillary tubes with hot air. *Nucleic Acids Res* 1989;17:4353–7.
4 Telenti A, Marshall WF, Smith TF. Detection of Epstein-Barr virus by polymerase chain reaction. *J Clin Microbiol* 1990;28:2187–90.
5 Halsted CC, Chang RS. Infectious mononucleosis and encephalitis: recovery of EB virus from spinal fluid. *Pediatrics* 1979;64:257–8.

"Silent diabetes": non-ketotic hyperglycaemia presenting as aphasic status epilepticus

A 74 year old, right handed, retired messenger had been well until two weeks before admission. On that day he had telephoned his daughter, who found his speech difficult to understand. Over the subsequent days, his speech became more incoherent but he expressed his wishes by pointing, and other basic mental functions were preserved; he washed and dressed himself and found his way around the house. Immediately before admission, his walking became unsteady and relatives noticed some other errors—for example, holding his knife and fork in the wrong hands. A key feature of this history was its progressive course. There was a history of occasional drop attacks of uncertain cause before the age of 30.

On examination, appropriate verbal and motor responses were sporadic and formal assessment showed a pronounced deficit of receptive and expressive function. He could only just write his name but could find his way around the ward without guidance. General and neurological examination were otherwise normal. Routine investigations showed a blood glucose concentration of 38·7 mmol/l with serum osmolarity of 303 mmol/l but no ketonuria or systemic acidosis. Electrolytes and full blood count were normal. Glycosylated haemoglobin was 16%. Brain CT showed generalised atrophy but no focal lesion. His CSF was acellular with protein concentration raised at 1·18 g/l; there were no oligoclonal bands. Glucose concentration in CSF was 14·6 mmol/l (blood 28·7 mmol/l). Electroencephalography showed low to medium amplitude delta activity over the left hemisphere. There were scattered theta waves bilaterally and occasional sharp components in both temporal regions but no features of an encephalitis. There was a clear seizure discharge, initially at 12 Hz, slowing to 10 Hz, maximal over the left mid to posterior temporal area, lasting two minutes (figure). During this discharge, he was asked how he felt and was able to nod. There was no evidence of ictal motor activity. Intravenous diazepam resulted in drowsiness with no other change in his clinical state. Cerebral blood flow was assessed by SPECT with ^{99m}Tc-HMPAO interictally, and showed reduced uptake in the left superior temporal and inferior frontal gyri.

He was treated with insulin and phenytoin and over the next five to seven days his condition deteriorated, despite gradual normalisation of blood glucose and appropriate phenytoin treatment. He became increasingly drowsy, with motor accompaniments to his seizures appearing for the first time.