BK virus DNA in CSF of immunocompetent and immunocompromised patients

A Behzad-Behbahani, P E Klapper, P J Vallely, G M Cleator

Materials and methods

Patients and specimens
A total of 266 CSF specimens were collected from two groups of children: group I (2–5 years), and group II (10–16 years). CSF samples were originally submitted to the Clinical Virology Laboratory, Manchester Royal Infirmary, for investigation of possible virus meningitis or encephalitis (group I: n = 86; group II: n = 109), or CSF was taken for monitoring of the efficacy of chemotherapy in leukaemia patients who were immunocompromised (group I: 2–5 years; group II: n = 109), or CSF was taken for monitoring of the efficacy of chemotherapy in leukaemia patients who were immunocompromised with suspected encephalitis and meningoencephalitis.

Results:
BK virus DNA was detected in three (2.1%) CSF samples taken from patients aged 2–5 years; two were patients with acute lymphocytic leukaemia without overt neurological symptoms, the other was a patient with suspected encephalitis. BK virus DNA was also detected in two (1.6%) CSF samples taken from older children in the age range 10–16 years; both children had suspected encephalitis. JC virus DNA was not found in any CSF sample from either age group.

Conclusions:
Detection of BK virus in the CSF of immunocompromised and immunocompetent patients with suspected neurological disease suggests that this virus may have had a pathogenic role in the aetiology of this condition.

DNA extraction for PCR
All CSF specimens were stored at −20°C. DNA was extracted using the guanidine thiocyanate method. PCR amplification was performed according to the method of Arthur and colleagues using 20-base oligomer primers (PEP-1 and PEP-2) specific for sequences within the “large T” and “small T” regions of the polyoma virus genome. The PCR reaction mixture contained digoxygenin labelled dNTPs which were incorporated into the product during amplification.

To differentiate JCV from BKV DNA, a rapid colorimetric hybridisation method was used. Digoxygenin labelled PCR products were detected using a commercially available PCR ELISA digoxygenin detection kit (Boehringer, UK). BKV DNA was detected by hybridisation to a 5’-end labelled biotin probe (BEP-1) (5’TTTTTTGTTGTTAGTGTAGTTGAGTTGGTGTTTCTGCTGTTGCT3′) specific for the 176 bp PCR product of BKV. JCV DNA was detected using a similarly labelled JEP-1 probe (5’CCTTTTATGTTGTTGATTTGTGTTGGATGTTGTGGAGTTGGCGCT3′) specific for the 173 bp PCR product of JCV. The results were expressed as net absorbance (405/492 nm) after the optical density of the substrate blank was automatically subtracted for each microwell. The “cut off” net absorbance values between positive and negative samples were calculated as follows: the mean of the net absorbance of 20 CSF samples negative for BKV and JCV DNA by PCR was determined and found to be negative for herpes simplex virus 1 and 2, varicella zoster virus, and cytomegalovirus DNA by PCR and for enterovirus RNA using RT-PCR.

Abbreviations:
BKV, BK virus; CNS, central nervous system; CSF, cerebrospinal fluid; JCV, JC virus; PCR, polymerase chain reaction; RT, reverse transcriptase
together with standard deviation of the mean. The “cut off” point was defined as: mean + 3 standard deviations. The cut-off point between positive and negative for detection of BKV and JCV DNA by PCR-ELISA in CSF samples was found to be 0.24 and 0.21 respectively.

RESULTS
Sensitivity of the PCR and PCR-ELISA
The limit of sensitivity of the BKV and JCV PCR-ELISA was found to be 6 copies of the BKV or 9.5 copies of the JCV genomes respectively.

Detection of BKV and JCV DNA in CSF
BKV DNA was detected in the CSF of three children aged 2–5 years; two were bone marrow transplant patients who developed neurological symptoms, the other was a previously healthy patient with suspected encephalitis. BKV DNA was also detected in two children in the age range 10–16 years; both were non-leukaemic patients with suspected encephalitis. JCV DNA was not detected in any CSF samples from either age group (table 1). Table 2 shows clinical details of patients who were positive for BKV DNA PCR in CSF.

DISCUSSION
In many cases of encephalitis, particularly among children, a viral aetiology is suspected but not identified.1 In this study, BKV DNA was detected in CSF samples of three immunocompetent patients presenting with mild encephalitis and in two immunocompromised patients without neurological symptoms. One of the BKV positive samples in the encephalitic patients was found in the younger age group (10–16 years). One of the BKV positive samples in the younger age group (10–16 years), the other was a previously healthy patient with suspected encephalitis. BKV DNA was also detected in two children in the age range 10–16 years; both were non-leukaemic patients with suspected encephalitis. JCV DNA was not detected in any CSF samples from either age group (table 1). Table 2 shows clinical details of patients who were positive for BKV DNA PCR in CSF.

JCV DNA was not detected in any CSF samples. Thus, although this virus is known to be able to infect the CNS, there is no evidence from this study to suggest that it is involved in meningoencephalitis in childhood.

This study is the first to provide evidence for a possible role of BK virus in childhood meningoencephalitis and adds support to the hypothesis that encephalitis might be one of the outcomes of primary infection with BK virus during childhood. To confirm this finding it will be essential to investigate further cases. Definition of the role of BK virus in infection of the CNS is important. If infection is benign and the virus does not damage brain cells, such knowledge would be of benefit to the clinician. On the other hand, if the infection is not benign then the late consequence of infection might be a chronic degenerative progressive multifocal leucoencephalopathy-like disease or other more subtle neurological damage, and aggressive antiviral chemotherapy might be contemplated. Further studies concerning the role of BKV as an agent of neurological disease in children are clearly warranted.

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