BK virus DNA in CSF of immunocompetent and immunocompromised patients

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

Arch Dis Child 2003;88:174–175

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Infection with BK virus (BKV) is a common event in childhood. Thirty three per cent of children are infected by 3 years of age and more than 90% by the age of 5. JC virus (JCV) infection is not as frequent as infection with BK virus among children, but approximately 30% of the population develop antibody against JCV between 10 and 16 years of age. Recently however, BKV DNA has been detected in the brain tissue and cerebrospinal fluid (CSF) of both immunocompetent and immunocompromised individuals (mostly adults) with and without neurological symptoms. As most primary infections with BK virus occur in childhood and most primary infections with JC virus occur in adolescence, an association between primary infection and CNS disease was sought in children in these age groups. The polymerase chain reaction (PCR) microplate hybridisation method was developed for the detection of polyomavirus DNA in CSF specimens of children. CSF specimens were collected from both immunocompetent and immunocompromised patients.

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 patients with suspected encephalitis and meningitis. To investigate the possible aetiological role of BK and JC viruses in immunocompetent and immunocompromised children with suspected encephalitis and meningitis, a polymerase chain reaction and microplate hybridisation method was employed for the detection of polyomavirus DNA in 266 CSF specimens collected from immunocompetent and immunocompromised patients.

Methods: The polymerase chain reaction and microplate hybridisation method was employed for the detection of polyomavirus DNA in 266 CSF specimens collected from immunocompetent and immunocompromised patients.

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 immunocompetent 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 polyomavirus genome. The PCR reaction mixture contained digoxigenin labelled dNTPs which were incorporated into the product during amplification.

To differentiate JCV from BKV DNA, a rapid colorimetric hybridisation method was used. Digoxigenin labelled PCR products were detected using a commercially available PCR ELISA digoxigenin detection kit (Boehringer, UK). BKV DNA was detected by hybridisation to a 5′-end labelled biotin probe (BEP-1) (5′TTTTTGGGTGGTGTTGAGGTTGGA ATA TCGGTTGCT3′) specific for the 176 bp PCR product of BKV. JCV DNA was detected using a similarly labelled JEP-1 probe (5′CCTTTTACAGGTGAGGTTGGAATCCTGTGTGTT TCA3′) 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

Table 1: Detection of BKV DNA in CSF samples from children

<table>
<thead>
<tr>
<th>Patient status</th>
<th>Group I (%)</th>
<th>Group II (%)</th>
<th>Total positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukaemia</td>
<td>2/55 (3.6)</td>
<td>0/16 (0)</td>
<td>2/71 (2.8)</td>
</tr>
<tr>
<td>Encephalitis</td>
<td>1/86 (1.1)</td>
<td>2/109 (1.8)</td>
<td>3/195 (1.5)</td>
</tr>
<tr>
<td>Total</td>
<td>3/141 (2.1)</td>
<td>2/125 (1.6)</td>
<td>5/266 (1.8)</td>
</tr>
</tbody>
</table>

Group I: 2–5 years; group II: 10–16 years.

Abbreviations: BKV, BK virus; CNS, central nervous system; CSF, cerebrospinal fluid; JCV, JC virus; PCR, polymerase chain reaction; RT, reverse transcriptase.
Table 2 Clinical details of patients who were positive for BKV DNA PCR in CSF

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Group</th>
<th>BKV ELISA OD (405/492 nm) cut off: 0.24</th>
<th>JCV ELISA OD (405/492 nm) cut off: 0.21</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>F</td>
<td>I</td>
<td>0.48</td>
<td>0.07</td>
<td>CSF taken at time of diagnosis of acute lymphoblastic leukaemia, no overt CNS symptoms</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>M</td>
<td>I</td>
<td>0.45</td>
<td>0.06</td>
<td>CSF taken at time of diagnosis of acute lymphoblastic leukaemia, irritability, but no overt CNS symptoms</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>F</td>
<td>I</td>
<td>0.49</td>
<td>0.05</td>
<td>Rash, lethargic, irritability, oral ulceration, suspected encephalitis</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>M</td>
<td>II</td>
<td>0.93</td>
<td>0.04</td>
<td>Acute onset confusion, apyrexial, suspected encephalitis</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>F</td>
<td>II</td>
<td>0.36</td>
<td>0.05</td>
<td>Recurrent headache, vomiting, diplopia, intracranial pressure mildly elevated, normal CSF protein</td>
</tr>
</tbody>
</table>

Group I = 2–5 years; group II = 10–16 years.

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. 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 (2–5 years), where primary infection is more usual, and two were found among the older age group (10–16 years).

BKV and JCV DNA can be detected in bone marrow and in peripheral blood lymphocytes of the B cell lineage. It is possible that, with the entry of such infected lymphoid cells into the CNS, viral persistence in this compartment may be established. Reactivation of latent BK or JC virus could occur as a result of brain trauma, infection with another pathogen, or during immunosuppression. Thus it is possible that the BKV DNA may be present in these patients as a result of primary or reactivated infection. An alternative explanation for the detection of BKV in CSF is that the virus enters the CNS as part of the process of primary infection and is present as a “passenger” within the CNS, but does not cause neurological infection or disease. Demonstration of intrathecal antibody production was not available for these samples but is, in most instances, specific for an ongoing infection of the central nervous system; future studies should include such antibody measurements.

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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Arch Dis Child 2003 88: 174-175
doi: 10.1136/adc.88.2.174

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