Case report

BK virus regulatory region sequence deletions in a case of human polyomavirus associated nephropathy (PVAN) after kidney transplantation

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Abstract

The pathogenesis of polyomavirus-associated interstitial nephropathy (PVAN) in kidney transplant recipients is likely to depend upon multiple risk factors, which may include viral characteristics. We report a case of interstitial nephropathy in a young kidney allograft recipient, associated with a BK virus (BKV) strain with a rearranged transcription control region (TCR). BKV strains with deletions and nucleotide substitutions in the TCR were present in a kidney biopsy and urine samples. After retransplantation, following loss of renal function, a BKV strain with an archetypal TCR was detected in association with asymptomatic reactivation.

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1. Case report

The pathogenesis of polyomavirus BK-associated nephropathy (PVAN), as well as of other BKV-related diseases, is likely to depend upon multiple risk factors, including host and virus properties (Hirsh et al., 2005). The anatomy of the TCR region may strongly influence the biological characteristics of a BKV strain and changes at this level could have important consequences for viral pathogenicity, by analogy with the polyomavirus JC. Sequence heterogeneity in kidney biopsy tissues with viral nephropathy has been documented (Randhawa et al., 2003). Archetype and rearranged BKV strains were detected in the kidney of a leukaemia patient with tubulointerstitial nephritis and meningoencephalitis (Stoner et al., 2002), whereas unique rearrangements characterised the BKV TCR found in brain and CSF (Jorgensen et al., 2003; Stoner et al., 2003). We describe a case of interstitial nephropathy associated with a BKV strain with rearranged TCR in a young kidney allograft recipient. As previously reported (Ginevri et al., 2003), PVAN developed at 32 months post-transplant in an 18-year-old caucasian male. BKV DNA was detected in paraffin-fixed kidney tissue sections and urine and serum samples taken at the same time, by a nested PCR, which amplifies sequences in the TCR (Azzi et al., 1996). A SYBR Green real time PCR was used in order to assess the viral load. The target of the quantitative reaction was the same sequence, 386 base pairs long, amplified by the inner primers of the nested PCR. Ten microliters of extracted DNA were used for real-time amplification in a final reaction volume of 50 μl. The PCR mixture consisted of: 15 μl of each primer BK1 and BK2 (25 μM), 6 μl of 25 mM MgCl₂, 2.5 U of Taq Gold, 1U of UDG heat-labile and water to obtain a 40 μl volume. After 10 min at 20 °C and 6 min at 94 °C, 2 cycles of 95 °C for 1 min, 55 °C

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for 1 min, 72 °C for 1 min were performed, followed by 40 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s. Fluorescence was measured automatically during each PCR cycle through the entire amplification period. The reading was performed at 82 °C for 10 s (iCycler iQ real-time PCR system; Bio-Rad).

Melting curve analysis was performed to assess the specificity of the PCR products. BKV DNA (DUN strain) cloned in pBR322 (ATCC 45025) was used as a standard for the real-time PCR.

The PCR-amplified BKV TCRs were analysed by sequencing in both directions using the second round PCR primers and the BigDye terminator sequence kit (Applied Biosystems). For sequence comparison a BLAST programme (Applied Biosystem) was used.

High urinary (1.31 × 10^4 genome copies/ml) and serum (4.47 × 10^3 genome copies/ml) viral loads were detected and BKV strains with rearranged TCR were demonstrated in both the kidney biopsy and the urine sample (a) taken on the same day, as well as in a urine sample (b), taken 5 months later (Table 1). Following loss of renal function, after 18 months haemodialysis, the patient was successfully retransplanted (Table 1). Following loss of renal function, after retransplantation, a BKV strain with an archetypal TCR, probably transmitted by the second graft, was associated with asymptomatic reactivation and low urinary viral load. The BKV-specific immune response, developed by the patient at the time of withdrawal of immunosuppression upon first graft failure (Ginevri et al., 2003), could have contributed to the control of the archetypic BKV strain. In the case described here, BKV damage was limited to the kidney tissue where a rearranged strain, with some nucleotide substitutions, was dominant. Different variations in the BKV TCR may occur during intense viral replication in the kidney and other tissues in immunocompromised patients. BKV with TCR organisation better adapted to the specific host cell machinery may be selected in the different tissues, to become the dominant strain.

Acknowledgments

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References


Table 1: Pattern of BKV-TCR and viral load in the different samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Date</th>
<th>TCR type</th>
<th>Genome copies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal biopsy*</td>
<td>6 July 2000</td>
<td>Rearranged; deletions (183–192, 236–263)</td>
<td>3.27 × 10^6</td>
</tr>
<tr>
<td>Urine a</td>
<td>6 July 2000</td>
<td>Rearranged; deletions (183–192, 236–250)</td>
<td>1.31 × 10^7</td>
</tr>
<tr>
<td>Serum</td>
<td>6 July 2000</td>
<td>n.a.</td>
<td>4.47 × 10^4</td>
</tr>
<tr>
<td>Urine b</td>
<td>5 December 2000</td>
<td>Rearranged; deletions (183–192, 236–250)</td>
<td>5.63 × 10^3</td>
</tr>
<tr>
<td>Urine c</td>
<td>24 September 2003</td>
<td>Archetypal</td>
<td>3.48 × 10^3</td>
</tr>
<tr>
<td>Urine d</td>
<td>31 March 2004</td>
<td>Archetypal</td>
<td>3.35 × 10^3</td>
</tr>
</tbody>
</table>

* GenBank accession number AY273186.

b Sequence deleted in comparison with WW-T.

GenBank accession number AY273187.

d Not available.
