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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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Keywords: BKV; Polyomavirus-associated nephropathy; Kidney transplantation

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., 2002).

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 Real-Time Buffer (Genedia), 1 μl of each primer BK1 and BK2 (25 μM), 6 μl of 25 mM $MgCl_2$, 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

Abbreviations: BKV, human polyomavirus BK; PVAN, polyomavirus-associated nephropathy; TCR, transcription control region; PCR, polymerase chain reaction

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

Sample	Date	TCR type	Genome copies
Renal biopsy[a]	6 July 2000	Rearranged; deletions (183–192; 236–263)[b]	3275/cell
Urine a[c]	6 July 2000	Rearranged; deletions (183–192; 236–250)[b]	1.31×10^{10}/ml
Serum	6 July 2000	n.a.[d]	4.47×10^{6}/ml
Urine b[c]	5 December 2000	Rearranged; deletions (183–192; 236-250)[b]	5.63×10^{6}/ml
Urine c	24 September 2003	Archetype	3.48×10^{3}/ml
Urine d	31 March 2004	Archetype	3.83×10^{4}/ml

[a] GenBank accession number AY273186.
[b] Sequence deleted in comparison with WW-T.
[c] GenBank accession number AY273187.
[d] Not available.

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 Biosystem). For sequence comparison a BLAST programme was used.

High urinary (1.31×10^{10} genome copies/ml) and serum (4.47×10^{6} 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 and monitored for BKV DNA in serum and urine samples every 2 months. Serum was always negative and BKV urinary shedding was demonstrated in only two occasions, 19 and 25 months after the second graft (urine c and d). In both cases the BKV TCR was archetype-like (Table 1). In comparison with the strain WW-T (Sundsfjord et al., 1990), the TCR from the biopsy strain and from urine specimens a and b was characterised by a deletion from nucleotide (nt) 183 to 192, affecting the Sp1 binding site. A further deletion was observed in the biopsy strain from nt 236 to 263, and in the urinary strains from nt 236 to 250. The presence of similar substitutions in these strains suggests a common origin. A substitution at nt 290 (G → T) involved one of the NF1 binding sites.

It could be hypothesised that this patient, who was anti-BKV antibody negative before transplantation, developed a primary BKV infection, probably transmitted with the first kidney allograft, and failed to mount a protective immune response because of intense immunosuppression. The prolonged viral replication favoured the emergence of strains with rearrangements and nucleotide substitutions, that ultimately led to PVAN development and graft loss. During follow-up, 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 archetype 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.

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References

Azzi A, De Santis R, Ciappi S, Leoncini F, Sterrantino G, Marino N, et al. Human polyomaviruses DNA detection in peripheral blood leukocytes from immunocompetent and immunocompromised individuals. J Neurovirol 1996;2:411–6.

Ginevri F, Pastorino N, De Santis R, Fontana I, Sementa A, Losurdo G, et al. Retransplantation after kidney graft loss due to polyoma BK virus nephropathy: successful outcome without original allograft nephrectomy. Am J Kidney Dis 2003;42:821–5.

Hirsh HH, Brennan DC, Drachenberg CB, Ginevri F, Gordon J, Limaye AP, et al. Polyomavirus-associated nephropathy in renal transplantation: interdisciplinary analyses and recommendations. Transplantation 2005;79:1277–86.

Jorgensen GE, Hammarin AL, Bratt G, Grandien M, Flaegstad T, Johsnsen JI. Identification of a unique BK virus variant in the CNS of a patient with AIDS. J Med Virol 2003;70:14–9.

Randhawa P, Zygmunt D, Shapiro R, Vats A, Weck K, Swalsky P, et al. Viral regulatory region sequence variation in kidney tissue obtained from patients with BK virus nephropathy. Kidney Int 2003;64:743–7.

Stoner GL, Alappan R, Jobes DV, Ryschkewitsch CF, Landry ML. BK virus regulatory region rearrangements in brain and cerebrospinal fluid from a leukemia patient with tubulointerstitial nephritis and meningoencephalitis. Am J Kidney Dis 2002;39:1102–12.

Sundsfjord A, Johansen T, Flaegstad T, Moens U, Villand P, Subramani S, et al. At least two types of control regions can be found among naturally occurring BK virus strains. J Virol 1990;64:3864–71.