Are Sequence Variations in the BK Virus Control Region Essential for the Development of Polyomavirus Nephropathy?

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Key Words: BK virus; Noncoding control region; Sequence analysis; Nephropathy; Urine; Blood; Renal biopsy

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Abstract

BK virus replication is regulated by the noncoding control region (NCCR); major NCCR rearrangements could modify the strength of viral replication, having a role in the development of polyomavirus-associated nephropathy (PAN). Urine (n = 34), blood (n = 32), and renal biopsy samples (n = 13) from 5 transplant recipients with PAN underwent nested polymerase chain reaction to search for the NCCR region. Sequence analysis was performed on all NCCR fragments obtained. Decoy cells were evaluated semiquantitatively in urine and PAN staged in renal biopsy specimens; the results were related to the presence and type of NCCR sequence variations.

Major NCCR rearrangements were found in urine (9/75 [12%]), blood (7/30 [23%]), and renal biopsy (4/15 [27%]) samples in 3 cases; 2 cases had only unrearranged strains.

Neither the detection and number of decoy cells nor the PAN stage were related to the specific type of NCCR sequence rearrangements. NCCR rearrangements do not seem essential for the development of PAN.

Polyomavirus-associated nephropathy (PAN) has emerged as an important cause of renal dysfunction and graft loss in patients who have undergone kidney transplantation.¹,² It is caused by lytic infection of kidney tubular epithelial cells and/or the parietal cells of the Bowman capsule induced by the human polyomavirus BK virus (BKV) during primary infection or the reactivation of viral strains that latently infect renal tissue.³ The sequence of pathologic events is characterized by tubular necrosis, mixed interstitial inflammatory infiltration, and, finally, scarring and fibrosis of the renal interstitium.³ Although BKV replication is documented in 10% to 68% of renal transplant recipients,⁴ PAN is a relatively rare disease involved in 1% to 7% of cases,⁴,⁵ suggesting that conditions other than kidney transplantation are required for its development. A number of risk factors, therefore, have been suggested, including host determinants (older age, male sex, BKV-negative serostatus before transplantation),⁶,⁷ immunosuppressive therapies (tacrolimus and/or mofetil mycophenolate exposure),⁴,⁵ organ determinants (ischemia, toxic injuries, or recurring rejection episodes),⁸ and concurrent infections such as HIV, cytomegalovirus, JC virus (JCV), and simian virus 40 (SV40).⁹,¹⁰ However, the relationships between specific viral determinants and the likelihood of developing PAN or the severity of the renal damage induced by BKV have not been elucidated.¹¹

The BKV genome consists of double-stranded circular DNA with 3 functional regions: an early region coding for large (LT) and small (t) antigens; a late region coding for capsid viral proteins (VP1-3); and a noncoding control region (NCCR).¹² This last region contains the origin of replication and a number of transcription factor binding sites involved in the transcriptional regulation of the coding regions (SP-1, NF-1, and CRE), promoter-enhancing sequences that control viral
rearrangements and study the possible role of specific BKV sequence variations in the development and/or severity of PAN. Viral replication also was evaluated semiquantitatively in renal biopsy and urine specimens and related to the occurrence and type of genomic rearrangements.

**Materials and Methods**

We studied 34 urine, 32 blood, and 13 renal biopsy samples from 5 kidney transplant recipients, all of whom fulfilled the renal biopsy histologic criteria for PAN. The number of samples greatly varied for each patient owing to the different course of the disease and ranged from 1 (case 4) to 5 (case 3) renal biopsy samples, from 2 (case 1) to 10 (case 2) blood samples, and from 2 (case 1) to 13 (case 2) urine samples. In each case, the urine and blood samples were obtained simultaneously before, during, and after the renal biopsy specimens. The main clinicopathologic findings are shown in Table 1.

**Urine Samples**

The urine samples (5 mL) were cytocentrifuged at 1,800 rpm for 10 minutes, fixed in 95% ethanol, stained with Papanicolaou stain, and cytologically examined to identify the presence of decoy cells (DCs), indicating active polyomavirus replication. The number of DCs was evaluated semiquantitatively as follows: low (+), 1 to 5 cells per 5 high-power fields (HPF); intermediate (++), 6 to 10 cells per 5 HPF; or high (+++), more than 10 cells per 5 HPF.

**Renal Biopsy Specimens**

The renal tissue samples were fixed in Serra fluid (ethanol, formaldehyde, and acetic acid) for 4 hours and embedded in paraffin. We stained 4-µm-thick sections with H&E, periodic acid–Schiff, silver methenamine, Masson trichrome, and phosphotungstic acid hematoxylin. Immunoperoxidase staining was performed using a polyclonal antibody against SV40 (dilution 1:20,000; Lee Biomolecular Research Labs, San Diego, CA), which cross-reacts with human BKV and JCV. The reactions were detected by means of the streptavidin-biotin method and revealed using diaminobenzidine as the chromogen.

A histologic diagnosis of PAN was made only when epithelial nuclei showing the morphologic changes reported by Nickelet et al were identified by light microscopy and positively stained by immunohistochemical analysis. The disease was classified in early (A), intermediate (B), or late (C) stage according to the criteria proposed by Hirsch et al. Briefly, stage A is characterized by the finding of rare viral nuclear inclusions mainly in the medullary tubules without epithelial necrosis or inflammation; stage B by the presence of numerous and diffuse inclusions and a strong inflammatory response; and stage C by interstitial fibrosis and only residual viral inclusions.

**Blood Samples**

The blood samples (5 mL) were obtained and centrifuged immediately at 2,500 rpm for 10 minutes, after which the serum was separated and stored in a 1.5-mL Eppendorf tube at −20°C.

**Molecular Biology**

**DNA Extraction**

DNA was extracted from the renal tissue and blood samples as follows: We cut 4-µm-thick sections from the paraffin-embedded renal samples and placed them into 1.5-mL Eppendorf tubes. To avoid sample cross-contamination, the microtome blade was cleaned with xylene between all blocks. DNA was extracted using EDTA–sodium dodecyl sulfate–Proteinase K treatment followed by phenol-chloroform as previously reported and resuspended with 35 µL of diethylpyrocarbonate-treated and autoclaved pyrogen and RNase-free water.

DNA also was extracted from the serum samples using commercial columns (NucleoSpin virus kit, Macherey-Nagel, Düren, Germany) with a silica matrix and a high DNA

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**Table 1**

**Main Clinicopathologic Findings in Renal Transplant Recipients**

<table>
<thead>
<tr>
<th>Case No./ Sex/Age (y)</th>
<th>Age of Transplant (mo)</th>
<th>Renal Disease Leading to Chronic Renal Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/65</td>
<td>24</td>
<td>Arterionephrosclerosis</td>
</tr>
<tr>
<td>2/M/54</td>
<td>60</td>
<td>Polycystic kidney disease</td>
</tr>
<tr>
<td>3/M/58</td>
<td>24</td>
<td>Polycystic kidney disease</td>
</tr>
<tr>
<td>4/F/23</td>
<td>24</td>
<td>IgA nephropathy</td>
</tr>
<tr>
<td>5/M/65</td>
<td>12</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
binding capacity. Nested polymerase chain reaction (nPCR) of the human androgen-receptor gene was performed in all cases as a positive control in DNA extraction.²⁰

**PCR Assays**

Multiplex nPCR was performed directly on the urine samples and on the DNA extracted from all renal and serum samples to amplify the LT region of the polyomaviruses. The following primers were used: PM1+ and PM1− (outer primers), PM2− (common to all polyomaviruses), and JC+, BK+, and SV40+ (inner primers) to distinguish the different members of the Polyomavirus genus.²¹ Table 2. The amplification was performed in a total volume of 25 µL containing 2 U of BioTaq DNA polymerase (Bioline, London, England) in the presence of 1× Bioline NH₄ buffer, 2 mmol/L of magnesium chloride (1 mmol/L for the inner PCR), 10 pmol/µL of each primer (Roche Diagnostics, Milan, Italy), 0.2 mmol/L of deoxynucleotide triphosphates (Roche Diagnostics), and 10 µL of DNA extracted from blood, 5 µL of DNA extracted from renal tissue, and 2.5 µL (1 µL for the inner PCR) of urine using a Progene Techno PCR System (Techne-Duxford, Cambridge, England).

The samples were amplified by denaturation at 95°C for 5 minutes, followed by 40 cycles (35 cycles for the inner PCR) of denaturation at 95°C for 40 seconds, annealing at 61°C (55°C for the inner PCR) for 40 seconds, and extension at 72°C for 40 seconds. The cycles were terminated with a final extension at 72°C for 5 minutes. Diethylpyrocarbonate-treated and RNase-free water (Biotecx Laboratories, Houston, TX) was used as the negative control sample; the positive control samples were DNA extracted from the brain tissue with PML (for JCV), the renal tissue of a subject with histologically proven BKV nephropathy (for BKV), and SVG cell lines (for SV40).

The samples positive for the LT region of BKV underwent further amplification of the NCCR region using BKTT1 and BKTT2 as the outer primers and BRP1 and BRP2 as the inner primers Table 3. The amplification was performed in a total volume of 25 µL containing 10 pmol of each primer, 0.2 mmol/L of deoxynucleotide triphosphates, 1.5 mmol/L of magnesium chloride, 2 U of Taq DNA polymerase (Roche, Mannheim, Germany) with an appropriate reaction buffer (tris(hydroxymethyl)aminomethane hydrochloride, 100 mmol/L, pH 8.3; potassium chloride, 500 mmol/L). In the first step, 5 µL of DNA extracted from renal tissue or blood or 2.5 µL of urine was added to the PCR mixture; in the second step, 2.5 µL of template was added to the PCR mixture. The PCR was then performed using a Progene Techno PCR System. The samples were amplified by denaturation at 95°C for 5 minutes, followed by 35 cycles at 95°C for 40 seconds, annealing at 55°C in the first step and at 50°C in the second step for 40 seconds, and extension at 72°C for 40 seconds. The cycles were terminated with a final extension at 72°C for 5 minutes.

The DNA amplification products were analyzed by means of 2% agarose gel electrophoresis and visualized using ethidium bromide.

### Table 2
**Primers for Polyomavirus LT Region***

<table>
<thead>
<tr>
<th>LT Region</th>
<th>Name</th>
<th>Position</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer PM1+</td>
<td>4022-4045&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5'-TCT TCT GGR YTA AAR TCA TGC TCC-3'</td>
<td></td>
</tr>
<tr>
<td>PM1−</td>
<td>4550-4572&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5'-TTW TAG RTK CCA ACC TAT GGA AC-3'</td>
<td></td>
</tr>
<tr>
<td>PM2−</td>
<td>4388-4411&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5'-GGT AGA AGA CCC YAA RGA CTT TCC-3'</td>
<td></td>
</tr>
<tr>
<td>Inner JC+</td>
<td>4086-4108&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5'-ATA TTA TGA CCC CCA AAA CCA TG-3'</td>
<td></td>
</tr>
<tr>
<td>SV+</td>
<td>4291-4314&lt;sup&gt;5&lt;/sup&gt;</td>
<td>5'-ATA ATT TTC TTG TAT AGC AGT GCA-3'</td>
<td></td>
</tr>
<tr>
<td>BK+</td>
<td>4059-4085&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5'-GAA TGC TTT CCT TTA TAG ATG ATG-3'</td>
<td></td>
</tr>
</tbody>
</table>

LT, an early region coding for large antigens; SV, simian virus; +, positive; −, negative.
* Wobble position International Union of Biochemistry code: K, G or T; R, A or G; W, A or T; Y, C or T.
<sup>1</sup> BKV Dunlop.
<sup>2</sup> JCV complete genome.
<sup>3</sup> SV40 complete genome.

### Table 3
**Primers for the Polyomavirus NCCR**

<table>
<thead>
<tr>
<th>NCCR</th>
<th>Name</th>
<th>Position 5'-3'*</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer BKTT1</td>
<td>5106-5134&lt;sup&gt;4&lt;/sup&gt;</td>
<td>5'-AAG GTC CAT GAG CTC CAT GGA TTC TCC C-3'</td>
<td></td>
</tr>
<tr>
<td>BKTT2</td>
<td>630-657&lt;sup&gt;4&lt;/sup&gt;</td>
<td>5'-CTA GGT CCC CCA AAA GTG CTA GAG CAG C-3'</td>
<td></td>
</tr>
<tr>
<td>Inner BRP1</td>
<td>82-101&lt;sup&gt;4&lt;/sup&gt;</td>
<td>5'-TG AGA GAA AGG GTG GAG GC-3'</td>
<td></td>
</tr>
<tr>
<td>BRP2</td>
<td>339-358&lt;sup&gt;4&lt;/sup&gt;</td>
<td>5'-GCC AAG ATT CCT AGG CTC GC-3'</td>
<td></td>
</tr>
</tbody>
</table>

NCCR, noncoding control region.
* BKV Dunlop.
bromide staining. Because of the frequent NCCR rearrangements, the size of the PCR amplification fragments could be different from the expected 356 base pairs (bp) of the archetype.

Direct DNA Sequencing

DNA fragments from all NCCR-positive samples were separated by electrophoresis on 3% agarose gel. One to four fragments of each sample (ranging from 200 to 400 bp) were excised, extracted, and purified by means of PCR clean-up gel extraction (NucleoSpin).

A cycle-sequencing PCR reaction was set up using the Big Dye Terminator cycle sequencing kit (version 2.0, Applied Biosystems, Monza, Italy). The primer was added to a final concentration of 3.2 pmol/µL in a final reaction volume of 20 µL. The cycling conditions were 25 cycles of 10 seconds at 96°C, 5 seconds at 50°C, and 4 minutes at 60°C. The reaction was terminated at 4°C. The cycle-sequencing products were purified using Centri-Sep Spin Columns (Princeton Separations, Adelphia, NJ), and the DNA was sequenced using an automated, 16-capillary sequencer (ABI-Prism 3100, Applied Biosystems).

The sequences were analyzed by means of the Basic Local Alignment Search Tool (BLAST) programs using a Web site maintained by the National Center for Biotechnology Information of the United States (http://www.ncbi.nlm.nih.gov). The structure of the BKV NCCR isolates was compared with that of the “archetypal” BKV WW strain (GenBank accession No. AF123428). The linear arrangement of the BKV WW NCCR is divided conventionally into 4 blocks of sequences called P (68 bp), Q (39 bp), R (63 bp), and S (63 bp).

Results

Urine Samples

Cytologic examination revealed DCs in 22 (65%) of 34 urine samples. The number of positive samples for each patient ranged from 1 (case 1) to 8 (case 2). A high number (+++) of DCs was identified in 16 samples and a low number (+) in 6.

Amplification of the LT region was sought for all urine samples by means of nPCR, which detected polyomavirus DNA in 32 samples. The cytopathic diagnosis of polyomavirus infection was confirmed in all 22 DC-positive samples; in addition, nPCR revealed polyomavirus genomic sequences in 10 DC-negative samples. BKV was the only polyomavirus identified in 21 samples, whereas JCV/BKV coinfection was detected in the other 11.

The NCCR of BKV was amplified in all of the LT region–positive samples. Sequence analysis was performed on DNA fragments with lengths ranging from 200 to 450 bp, thus also including possible major deletions or duplications in comparison with the NCCR of the archetype isolate (255 bp). A total of 75 DNA fragments were sequenced.

Sequence analysis detected 66 fragments with unrearranged NCCR sequences and 9 fragments with major NCCR sequence rearrangements. The former were found in all cases: BKV WW archetype in cases 1, 2, and 3 (n = 46) and BKV V128-1 (GenBank accession No. AF218446) in cases 4 and 5 (n = 20). The isolates with major rearrangements identified in 9 (12%) of 75 samples were from cases 1, 2, and 5. A complete deletion of the S sequence block was found in 1 sample in case 1; a partial deletion of the P block (found in 5 samples) was the most frequent rearrangement in case 2; and a partial deletion of the Q block, partial duplication of the P block, and complete duplication of the Q block in another sample; and 2 samples from case 5 showed partially or completely deleted and duplicated blocks.

The cytologic findings were correlated with the genomic rearrangements, and the sequence variations were independent of the morphologically identified viral replication (ie, DCs) in urine samples. 5 rearranged isolates were detected in urine samples with a high number of DCs (5/16 [31%]) and 4 in DC-negative samples (4/12 [33%]).

Renal Biopsy Samples

A total of 13 renal biopsies were performed during the study, 8 of which fulfilled the histologic and immunohistochemical criteria for a diagnosis of PAN (8/13 [62%]). As shown in Table 4, PAN was identified in only 1 biopsy specimen from cases 1, 3, and 4; in 2 biopsy specimens from case 5; and in 3 biopsy specimens from case 2. On the basis of the Hirsch criteria, 2 biopsy specimens were classified as stage A, 5 as stage B, and 1 as stage C. In particular, case 2 showed...
progressive renal damage: the first 2 biopsy specimens were classified as stage B, whereas the third (obtained 7 months later) showed interstitial scarring consistent with stage C. The 5 biopsy specimens without signs of PAN showed acute cellular rejection (2 samples) and acute drug toxicity (3 samples) due to tacrolimus and cyclosporine intake. Damage due to drug toxicity was characterized by focal isometric vacuolization of the tubular epithelial cells, mainly in the pars recta of the proximal convoluted tubules.

The amplification of the LT region sought in all of the renal biopsy specimens detected polyomavirus DNA in 9 samples (Image 1A): all of the biopsy specimens with histologic features of viral infection plus 1 histologically negative sample (acute cell rejection). BKV was identified in 5 samples and JCV-BKV coinfection in the other 4.

The NCCR of BKV was amplified in 8 of 9 LT-positive cases (Image 1B). A total of 15 DNA fragments were sequenced (despite several efforts, amplification of the NCCR of 1 sample was unsuccessful).

Sequence analysis revealed 11 unrearranged BKV isolates, 5 BKV WW (cases 1-3) and 6 BKV V128-1 (cases 4 and 5). Genomic rearrangements were found in 4 DNA fragments (4/15 [27%]) from cases 1 and 5; the most frequent rearrangements were partial deletions of the Q and R blocks (Figures 2A and 2C). A 5-bp duplication was identified in the R block of 1 sample from case 5 (Figure 2C).

As shown in Table 5, sequence variations were detected in 1 biopsy sample with PAN stage A (case 1; 1/2 [50%]) and 1 with PAN stage B (case 5) (1/5 [20%]).

**Blood Samples**

The polyomavirus LT region was detected in 19 (56%) of 32 blood samples, identifying 9 BKV infections and 10 JCV-BKV coinfections (Image 1A). The NCCR of BKV was amplified from 16 of the LT-positive cases (Image 1B), and a total of 30 DNA fragments underwent sequence analysis. Attempts to amplify the NCCR were unsuccessful in 3 samples. The unrearranged BKV WW and BKV V128-1 strains were identified, respectively, in 8 samples (cases 1-3) and 15 samples (cases 4 and 5). Seven samples (7/30 [23%]) showed complex rearrangements (cases 1, 2, and 5). One sample from case 1 (Figure 2A) showed partial deletions of the Q and R blocks; 2 samples from case 2 (Figure 2B) had a partial deletion of the P block, one of which also showed a partial duplication of the S block. More complex rearrangements were identified in case 5 (Figure 2C): partial deletions of the R block in 2 samples, partial deletions of the R and Q blocks in 1 sample, and partial duplications of the P and Q blocks in 2 samples.

**Discussion**

The ability of the BKV to replicate within the organs targeted by BKV infection has been related to the genomic structure of its NCCR.13 This region is highly variable in all members of the polyomavirus family, but particularly in the human strains BKV and JCV, in which multiple rearrangements can be demonstrated depending on the source of virus isolation.22

Various rearrangements of the archetypal structure have been found in brain, kidney, blood, and tonsils, suggesting that organ- or cell-specific rearrangements might be required to allow the virus to grow in different tissues. Moreover, several areas of the NCCR (API, NF-1, CRE, and SP1) are crucial for DNA replication and the control of early and late gene transcription. Many in vitro studies have shown that deletions, duplications, or single nucleotide mutations of these sites can change the efficiency of BKV replication (reviewed by Moens and Rekvig). However, the frequency of BKV NCCR rearrangements in renal transplant recipients with PAN has been investigated in only a few studies mainly reporting single cases, and it is unclear whether specific genomic variations have a role in the pathogenesis or severity of the disease.

Randhawa et al compared the BK viral load (measured by means of quantitative PCR) in plasma, urine, and renal tissue samples among renal transplant recipients with active or resolved PAN and patients with asymptomatic viruria and found that quantitative PCR could be a useful tool in monitoring patients with BKV renal infection. In that article, no data about the presence and number of DCs in urine was reported, and, therefore, a comparison between BKV levels in urine as evaluated by molecular biologic (quantitative PCR) and morphologic (DCs) methods was not done. It is interesting that the median intrarenal viral concentration of active PAN was significantly higher than that of resolved PAN, suggesting that measure of BK viral load in renal tissue could be a way to evaluate the intensity of viral replication. However, quantitative tests are difficult to perform, and appropriate cutoff values of BK viral load should be established by single laboratories; for these reasons, such methods are not used widely.

In our study, we analyzed the sequences of the NCCR of BKV isolates detected in urine, renal tissue, and blood samples of 5 patients with histologically proven PAN and correlated them with the morphologic signs and levels of viral replication (as shown by the presence and number of DCs in urine).
Electropherogram showing the genomic structure of the noncoding control region of the BK virus (BKV) WW (A) and BKV rearranged isolate isolated from 1 urine sample in case 2 (B) (first line in Figure 2B). The X coordinate corresponds to the scan number throughout the time of data collection. The Y axis is a relative height scale for peak intensity. Traces for A residues are shown in green, C in blue, G in black, and T in red.
and the renal biopsy staging of PAN in an attempt to determine whether viral variants had greater replication ability. Major rearrangements of the BKV NCCR (consisting of deletions and duplications of partial or entire blocks of viral DNA sequences) were found in the urine (9/75 [12%]) but more frequently in the renal (4/15 [27%]) and blood samples (7/30 [23%]).

To the best of our knowledge, almost all studies evaluating the structure of the NCCR in urine samples from patients with PAN have detected the archetypal configuration (reviewed by Knowles27), but our results indicate that major rearrangements also can be found: deletions of the P and S blocks were the most frequently identified variations, followed by more complex rearrangements involving deletions and partial duplications of all 4 blocks. Because transcription factor binding sites are located within these genomic regions,14 a decrease in viral replication could be expected in the former case and an increase in the latter. However, comparison of the sequence variations with the presence and number of DCs in urine did not show any relationship between the type of rearrangements and viruria because duplications and deletions were found in the absence and the presence of large numbers of DCs. Moreover, specific BKV rearrangements did not seem to be associated with replicative advantages over unrearranged strains, which showed the same likelihood of having high replication levels: for example, cases 3 and 4 (in which no rearranged sequences were identified in the urine) had massive DC excretion. The finding of rearrangements in DC-positive and DC-negative urine samples indicates that at least some can occur primordially in infected cells from the lower urinary tract rather than from viral isolates coming from renal tubular cells during lytic BKV infection (ie, in every case associated with DC excretion2,4).

The renal biopsy specimens showed the highest frequency of NCCR rearrangements; however, an unrearranged NCCR structure was identified in 11 of 15 isolates of BKV, and only 1 had no morphologic signs of viral replication, suggesting that BKV NCCR rearrangements in the kidney are not required for the development of PAN. Recently, Randhawa et al23 studied NCCR sequence variations in 26 renal biopsy specimens with PAN and found that the most common were nucleotide substitutions in transcription factor binding sites, whereas major rearrangements similar to those seen in our series were detected in 5 cases. Therefore, they concluded that architectural variations do not seem to be essential prerequisites for the development of PAN. Furthermore, in our study, the histologic stages of PAN were not related to specific NCCR rearrangements, and, because NCCR sequence variations were detected regardless of the stage of PAN, the strength of BKV replication also does not seem to be conditioned by the presence of NCCR rearrangements.

In the blood samples, viral rearrangements were detected more frequently than in urine samples: the P, Q, and R blocks

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**Table 4**

Histopathologic Findings in Renal Biopsies

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Biopsy No.</th>
<th>PAN Stage</th>
<th>Other Histologic Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>A</td>
<td>Acute drug toxicity; arterionephrosclerosis</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>B</td>
<td>No other changes</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>C</td>
<td>No other changes</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>Not found</td>
<td>Acute drug toxicity</td>
</tr>
<tr>
<td>5</td>
<td>B</td>
<td>A</td>
<td>Acute tubular necrosis</td>
</tr>
<tr>
<td>6</td>
<td>B</td>
<td>Not found</td>
<td>Acute cellular rejection</td>
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<tr>
<td>7</td>
<td>Not found</td>
<td>B</td>
<td>No other changes</td>
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<tr>
<td>8</td>
<td>Not found</td>
<td>B</td>
<td>Acute cellular rejection</td>
</tr>
<tr>
<td>9</td>
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</tr>
<tr>
<td>10</td>
<td>Not found</td>
<td>B</td>
<td>Acute drug toxicity</td>
</tr>
</tbody>
</table>

PAN, polyomavirus-associated nephropathy. A, early; B, intermediate; C, late. For a full explanation of the stages, see the text.

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**Table 5**

Correlation Between Rearranged BKV Isolates and Morphologic Findings of Viral Replication

<table>
<thead>
<tr>
<th>Case No./Sample</th>
<th>Morphologic Diagnosis</th>
<th>NCCR Rearrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Renal biopsy</td>
<td>PAN stage A</td>
<td>2 fragments: first, del R&lt;sub&gt;31&lt;/sub&gt;; second, del Q&lt;sub&gt;34-39&lt;/sub&gt;, R&lt;sub&gt;1-19&lt;/sub&gt;</td>
</tr>
<tr>
<td>2 Urine</td>
<td>No decoy cells</td>
<td>1 fragment: del S block</td>
</tr>
<tr>
<td>2 Urine</td>
<td>Decoy cells, +++</td>
<td>1 fragment: del P&lt;sub&gt;2&lt;/sub&gt;, ins S&lt;sub&gt;1&lt;/sub&gt;, ins P&lt;sub&gt;31-49&lt;/sub&gt;</td>
</tr>
<tr>
<td>2 Urine</td>
<td>Decoy cells, +++</td>
<td>2 fragments: first and second, del P&lt;sub&gt;2&lt;/sub&gt;, ins S&lt;sub&gt;1&lt;/sub&gt;, ins P&lt;sub&gt;31-49&lt;/sub&gt;</td>
</tr>
<tr>
<td>2 Urine</td>
<td>Decoy cells, +++</td>
<td>1 fragment: del Q&lt;sub&gt;13-39&lt;/sub&gt;</td>
</tr>
<tr>
<td>2 Urine</td>
<td>No decoy cells</td>
<td>1 fragment: del P&lt;sub&gt;31-49&lt;/sub&gt;, ins Q&lt;sub&gt;13-39&lt;/sub&gt;</td>
</tr>
<tr>
<td>3 Renal biopsy</td>
<td>PAN stage B</td>
<td>2 fragments: first, del R&lt;sub&gt;31&lt;/sub&gt;, ins S&lt;sub&gt;1&lt;/sub&gt;, ins P&lt;sub&gt;31-49&lt;/sub&gt;, ins Q&lt;sub&gt;13-39&lt;/sub&gt;, ins R&lt;sub&gt;1-19&lt;/sub&gt;</td>
</tr>
<tr>
<td>3 Urine</td>
<td>Decoy cells, +++</td>
<td>1 fragment: del Q&lt;sub&gt;31-49&lt;/sub&gt;, ins S&lt;sub&gt;1&lt;/sub&gt;, ins P&lt;sub&gt;31-49&lt;/sub&gt;, ins Q&lt;sub&gt;13-39&lt;/sub&gt;, ins R&lt;sub&gt;1-19&lt;/sub&gt;</td>
</tr>
<tr>
<td>3 Urine</td>
<td>Decoy cells, +++</td>
<td>1 fragment: del Q&lt;sub&gt;31-49&lt;/sub&gt;, ins S&lt;sub&gt;1&lt;/sub&gt;, ins P&lt;sub&gt;31-49&lt;/sub&gt;, ins Q&lt;sub&gt;13-39&lt;/sub&gt;, ins R&lt;sub&gt;1-19&lt;/sub&gt;</td>
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del, deletion of base pair; ins, insertion of base pair; PAN, polyomavirus-associated nephropathy; +++ >10 cells per 5 high-power fields.
were deleted partially in 7 isolates, 2 of which also showed P and Q duplications. Dolei et al\(^8\) detected BKV with an archetypal NCCR structure in blood samples from 22% of 231 healthy blood donors, whereas Dorries et al\(^20\) and Chatterjee et al\(^30\) documented the occurrence of minor NCCR rearrangements (single base changes and small sequence deletions) in BKV isolated from the peripheral blood of an unselected population of blood donors and suggested that these might be related to the intrinsic heterogeneity of the circulating virus population. The occurrence of major NCCR rearrangements in our cases suggests 2 possible alternative explanations: highly rearranged viral isolates might pass from the kidney to the peripheral blood in patients with PAN, indicating greater viral infectiousness; or the more severe renal damage caused by viral replication could be followed by viral entry into the bloodstream. Comparison of the BKV isolates detected in the kidney and blood samples obtained simultaneously from each patient (data not shown) did not confirm the latter hypothesis, but our method (nPCR) identified only the prevailing isolates, and less represented isolates (possibly related to more severe renal damage but detectable only using cloning methods) might have been underestimated.

The evaluation of NCCR rearrangements in each patient seems to indicate that neither the presence nor a specific type of sequence variation is needed for the development of PAN. None of the BKV isolates detected in urine, blood, or renal samples from cases 3 and 4 showed NCCR major rearrangements, but both had histologically documented PAN. In contrast with our findings, in vitro studies by Ferguson and Subramani\(^31\) showed that an NF-1 mutation in the P block of BKV is associated with a 2-fold increase in viral replication, and Deyerle and Subramani\(^32\) demonstrated that the deletion of the same block reduced replication by 50%. Moreover, the strictly related JCV isolated from the brain or cerebrospinal fluid of patients with PML almost always shows a specific rearranged structure (PML-like), indicating that these sequence variations are necessary for the development of the disease.\(^15\) This suggests that the pathogenesis of PAN is different from that of JCV-induced PML.

Major rearrangements in the BKV NCCR do not seem necessary for the pathogenesis of PAN, and their presence does not seem related to significant variations in viral proliferation activity. Because the methods used in our study can identify only the prevailing isolate(s), it cannot be excluded with any certainty that less represented viral isolates with more specific rearrangements might be associated with greater viral replication efficiency.

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References


