BK and JC Viruses in Patients with Systemic Lupus Erythematosus: Prevalent and Persistent BK Viruria, Sequence Stability of the Viral Regulatory Regions, and Nondetectable Viremia

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A role for polyomaviruses in the pathogenesis of systemic lupus erythematosus (SLE) has been suggested. BK virus (BKV) and JC virus (JCV) were demonstrated in single urine specimens from 7 (16%) of 44 and 5 (11%) of 44 patients with SLE and 0/88 and 18 (21%) of 88 matched healthy controls, respectively. During a 1-year follow-up study, episodes of polyomaviruria were detected in 16 (80%) of 20 patients, BKV in 13, and JCV in 3 patients. A group of 12 (60%) of 20 patients demonstrated persistent or recurrent polyomaviruria, BKV viruria (n = 9), or JCV viruria (n = 3) in 180 (70%) of 256 specimens. Polyomaviruria was not significantly associated with immunosuppressive therapy. The BKV and JCV isolates revealed predominantly stable archetypal regulatory regions over 3 years, indicating viral persistence rather than reinfection as a cause for urinary shedding. The demonstration of nondetectable viremia and stable archetypal BKV and JCV noncoding control regions during persistent viruria argue against the urinary tract as a focus for the creation of rearranged regulatory region variants.

The human polyomaviruses BK virus (BKV) and JC virus (JCV) have independent and worldwide distributions in the human population [1]. Primary infection with BKV or JCV is most common in childhood and is followed by lifelong viral persistence. The brain and kidneys have been identified as the main target organs for polyomaviral persistence [2–4, reviewed in 5]. The presence of polyomaviral sequences in peripheral blood leukocytes (PBLs) has also been confirmed [6–8]. Clinical studies, mainly performed on immunosuppressed populations, support the general impression that reactivation of polyomavirus latency is associated with immunological impairment [reviewed in 5]. Recent studies, however, have revealed a relatively high prevalence of JCV but not BKV viruria in immunocompetent persons [9–12].

JCV has been established as the etiological agent of progressive multifocal leukoencephalopathy (PML), a rare, subacute demyelinating disease of the central nervous system in patients with impaired cell-mediated immune responses [13]. BKV has been related to a case of a fatal disseminated infection [14], some cases of urinary tract diseases, and hemorrhagic cystitis in immunosuppressed patients [reviewed in 5 and 15]. Moreover, an association between JCV [16] and, in particular, BKV [17], with human tumors has been described. These findings have not been confirmed in other studies [18, 19].

Although BKV is regarded as an opportunistic pathogen in severely immunosuppressed patients, the BKV-human cohabitation is considered to be a rather innocent relationship. So far, PML is the only clinical illness etiologically associated with a human polyomavirus [5]. However, our recent experimental and clinical studies have presented data indicating a causal relationship between the development of anti-DNA antibodies and expression of polyomavirus large T-antigen [20, 21]. A follow-up study of 20 patients with systemic lupus erythematosus (SLE) revealed a high prevalence of polyomaviruria, as well as a strong correlation between the presence of polyomaviral DNA in consecutive urine samples and anti–large T-antigen antibodies in serial serum samples, reflecting virus reactivation and large T-antigen expression [20].

The high prevalence and persistent shedding of BKV or JCV in most of these patients with SLE encouraged us to perform an extended cross-sectional study of a larger group of patients with SLE from which the earlier described 20 patients had been selected [20]. The BKV and JCV viruria frequencies were compared with a control group of randomly selected age- and sex-matched healthy subjects. We also performed polymerase chain reaction (PCR) analysis of serum or peripheral blood samples for potential polyomaviremia during persistent shedding of polyomaviruses in the urinary tract. Moreover, we examined...
whether there existed a correlation between this autoimmune disease and the type of regulatory region of the virus, since this region, at least partly, has been shown to determine several important biological properties of the virus [reviewed in 5]. Finally, we examined polyomaviruria and viral regulatory sequences in single urine specimens taken 1–3 years after the follow-up study.

Our results show a significantly higher prevalence of BKV viruria but not JCV viruria in patients with SLE compared with matched healthy controls. The prevalent BKV viruria is associated with recurrent or persistent shedding and the development of antibodies to polyomavirus T-antigen in a large number of patients with SLE, as shown in the earlier described follow-up study [20]. Persistent BKV and JCV viruria do not seem to be associated with detectable viremia in patients with SLE. Stable archetypal BKV and JCV regulatory regions that are common in the human population are also circulating in patients with SLE, as shown by DNA sequence analysis of sequential urine isolates from individual patients. These observations argue against repeated infection of the host but strongly indicate a BKV and JCV persistence in the kidney after primary infection that is characterized by intermittent or continuous viral replication and viruria.

Materials and Methods

Study population. In total, 44 patients with SLE, 41 women and 3 men, on average 45 years of age (range, 23–75 years), all fulfilling 4 or more of the American Rheumatism Association classification criteria for SLE [22], participated in a cross-sectional study in 1993–1994 analyzing the prevalence of polyomaviruria. All patients were inpatients or outpatients at the Department of Rheumatology, University Hospital of Tromsø. The BKV and JCV viruria frequencies in the patients with SLE were compared with the prevalence of polyomaviruria in a randomly chosen age- (within 2 years) and sex-matched control group of healthy subjects, 82 women and 6 men, from the Tromsø Community Health Survey [23]. A representative subgroup of 20 patients, 19 women and 1 man, on average 47 years of age (range, 23–75 years) were included in a 1-year follow-up study for determination of polyomaviruria prevalence, noncoding control region (NCCR) sequence analyses of sequential BKV and JCV isolates from individual patients, examination of potential polyomaviremia, and various serological analyses described earlier [20]. The use of immunosuppressive drugs was recorded monthly for each patient included in the follow-up study.

Urine, serum, and buffy coat samples. The 44 patients in the cross-sectional study each collected and froze 1 urine sample (20 mL) at home and brought it to the hospital, where the urine samples were stored at −70°C. The 20 follow-up patients collected urine samples in the same way every second week for 1 year, as well as monthly blood samples for serum and buffy coat preparation. Serum and buffy coat specimens were stored at −70°C. Nineteen of 20 patients in the follow-up study collected 1 urine specimen as described above during 1995, that is, 1–3 years after participating in the cross-sectional study. The urine samples from the healthy control group subjects were collected as midstream urines, stored at 4°C, and prepared for PCR analysis within 72 h. Pretreatment of urine samples for PCR analyses was as described earlier [11]. DNA from serum and buffy coat (200 µL) was purified by use of the QIAamp Blood Kit as recommended by the manufacturer (QIAGEN GmbH, Hilden, Germany).

Detection and characterization of polyomavirus DNA sequences. PCR precautions, hot start amplification conditions, detection and characterization of PCR products, and internal controls have mainly been described in detail elsewhere [11, 20, 24]. Briefly, the detection and identification of BKV and JCV DNA early gene sequences were performed by the use of PYVfor and PYVrev primers (PYV PCR) and BK- and JC-specific oligonucleotide probes or restriction enzyme analysis, respectively [24, 25]. For the detection and characterization of viral NCCRs from PYV PCR–positive specimens, we used the GPPY-1 (5′-CCTCCACATCATGTAACGTGAGC-3′) and the GPPY-2 [24] primer pair and the BKT-1/I00 primer pair [26] in a nested PCR (NCCR-PCR) for 25 and 30 cycles, respectively.

Identification and sequencing of the NCCRs of BKV and JCV. NCCR amplicons were further characterized by diagnostic restriction enzyme digestion with PflmI, Bsa36I, and SacI, which allows the distinction between BKV and JCV DNA regulatory sequence variants [11, 27]. Bidirectional sequencing of NCCR amplicons was performed by means of the Thermo Sequenase radiolabeled terminator cycle sequencing kit according to the instructions of the manufacturer (Amersham Life Science, Amersham, UK).

Statistical analysis. Fisher’s exact test was used to study differences between groups in prevalence. The tests were two-tailed, and P < .05 was significant.

Accession numbers. The sequence data that appear herein are in the GenBank database under the accession numbers AF123397–AF123431.

Results

The occurrence of BKV viruria is significantly higher in patients with SLE compared with healthy control subjects. Both we and others have previously demonstrated BKV and JCV DNA sequences in urine of patients with SLE [20, 28]. In this study we expanded the number of patients, as well as the observation period. The cross-sectional examination of single urine samples from 44 patients with SLE revealed BKV and JCV DNA in 7 (16%) and 5 (11%) specimens, respectively. In comparison, the prevalences of BK and JC viruria in the control group were 0 and 18 (21%), respectively. The occurrence of BK viruria is significantly higher in patients with SLE compared with the control group (P < .001). The prevalence difference in JC viruria between patients with SLE and control group individuals is not significant. Codetection of both BKV and JCV was not demonstrated. Both viral early gene and NCCR sequences were detected in polyomavirus-positive specimens.

High prevalence of intermittent or continuous shedding of BKV in urine in patients with SLE. A representative subgroup of 20 patients was subjected to a 1-year follow-up study. These patients could be divided into 4 groups according to the fre-
quency of polyomaviral DNA shedding in consecutive urine samples [20]. The data are presented in table 1. Briefly, group I encompassed 4 patients whose urine samples were negative throughout the entire observation period. Groups II and III included patients with a single positive urine sample (n = 2) or a few clustered positive samples (n = 2), respectively. On average, 70% (range, 30%–100%) of the urine samples of group IV patients (n = 12; mean age 47 years) were PCR positive, compared with 45% for all patients with SLE. In total, 16 (80%) of 20 patients had at least 1 polyomavirus DNA-positive urine specimen during the 1-year observation period. Three of them (15%) excreted JCV, while BKV was demonstrated in 13 patients (65%). In a single urine specimen collected 1–3 years after their participation in the cross-sectional study, 7 (37%) of 19 patients from the 1-year follow-up study tested positive for PYV PCR, 5 (26%) for BKV, and 2 (11%) for JCV DNA. All 7 patients with PYV PCR-positive urine specimens belonged to group IV in the 1-year follow-up study.

The PYV PCR method scored negative in a total of 238 (55%) urine samples in the follow-up study. To ensure the validity of negative PCR results, every other PCR-negative urine specimen from each patient was amplified with the addition of 1 fg BKV DNA, that is, 170 BKV genomes. Amplified bands of expected size were detected in 112 (98%) of 115 urine specimens spiked with BKV DNA, while single urine samples from 3 different patients showed negative results in PYV PCRs. These findings strongly indicate that negative results in PYV PCRs of urine samples are not due to inhibition of the enzymatic amplification of DNA.

**BKV and JCV viruria in relation to immunosuppressive therapy.** One reason for polyomaviruria in patients with SLE could be immunosuppressive therapy. The use of immunosuppressive drugs (corticosteroids, azathioprine, cyclophosphamide, and/or methotrexate) was therefore recorded monthly for each patient during the 1-year follow-up study and examined for any temporal linkage to episodes of polyomaviruria. Two out of 4 polyomaviruria-negative patients (group I) received corticosteroids >6 months during the 1-year follow-up. We did not detect any temporal relationship between immunosuppressive therapy and episodes of viruria in the patients with single BKV-positive urine samples (group II) or a few clustered BKV-positive samples (group III). Seven patients with persistent polyomaviruria (group IV), 6 with BKV and 1 with JCV, received corticosteroids and/or azathioprine treatment >6 months. Three group IV patients with shorter periods of immunosuppressive therapy, all shedding BKV, did not show any relation in time between therapy and viruria. The last 2 group IV patients, shedding JCV, did not receive any immunosuppressive treatment. These observations do not indicate any significant temporal relationship between immunosuppressive therapy and BK or JC viruria in patients with SLE.

**BKV or JCV shed in urine of patients with SLE have predominantly archetypal NCCRs.** The high prevalence of polyomaviruria in patients with SLE could theoretically be explained by the selection of virus strains with certain NCCRs known to predispose for reactivation [5, 29]. Therefore, the BKV or JCV NCCRs from the patients with at least 2 polyomaviral-positive urine specimens, 11 patients with BKV and 3 patients with JCV, were amplified and sequenced bidirectionally. The NCCR from the first (hereafter referred to as A isolates) and last (B isolates) polyomavirus-positive urine samples from each patient, taken on average 8.2 months apart (range, 4–10 months) during the 1-year follow-up study, were sequenced. Furthermore, the 7 positive specimens collected 1–3 years (mean, 22 months) after the follow-up study (hereafter referred as C isolates) were also analyzed.

The NCCR of the archetypal BKV (WW) strain is arbitrarily divided into P, Q, R, and S blocks consisting of 68, 39, 63, and 63 bp [29, 30], respectively, and illustrated in figure 1. This archetypal BKV (WW) NCCR was detected in 25 isolates from 10 of the 11 patients with BK viruria. Only patient 2, belonging to group III, excreted a BKV isolate with the rearranged P1±68, Q1±39-R4±9-P22±68-Q1±39-R1±68 NCCR anatomy that has not been described so far. A total of 13 different point mutations, 3 single base pair insertions, and 2 base pair deletions, as shown in figure 1, were detected, compared with the published sequence of the first WW isolate [31]. Eight different BKV (WW) NCCR DNA sequences were detected in 25 isolates from 10 different

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**Table 1.** Polyomaviral DNA in urine, buffy coat, and serum samples, as determined in 20 patients with systemic lupus erythematosus analyzed over 1 year.

<table>
<thead>
<tr>
<th>Groupa (no. of patients)</th>
<th>Urine</th>
<th>Buffy coat</th>
<th>Serum</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n (no. of patients)</td>
<td>+ (%)</td>
<td>n (no. of patients)</td>
</tr>
<tr>
<td>I (4)</td>
<td>73 (4)</td>
<td>0</td>
<td>23 (4)</td>
</tr>
<tr>
<td>II (2)</td>
<td>45 (2)</td>
<td>2 (4)</td>
<td>5 (1)</td>
</tr>
<tr>
<td>III (2)</td>
<td>55 (2)</td>
<td>9 (16)</td>
<td>9 (1)</td>
</tr>
<tr>
<td>IV (12)</td>
<td>256 (12)</td>
<td>180 (70)</td>
<td>70 (11)</td>
</tr>
<tr>
<td>Total (20)</td>
<td>429 (20)</td>
<td>191 (45)</td>
<td>107 (17)</td>
</tr>
</tbody>
</table>

a The patients were divided into 4 groups according to the frequency of polyomaviral DNA detection. I: Patients 7, 11, 19, and 21; II: patients 9 and 13; III: patients 2 and 4; IV: patients 5, 8, 10, 12, 14, 16, 17, 18, 20, 23, 24, and 25.

b BKV- or JCV-positive samples.

c We repeatedly detected BKV prototype-2 DNA sequences, both early-region and NCCR amplicons, in 2 specimens from 2 different patients (10 and 23). These findings are considered to be a contamination (see Discussion).
Figure 1. Nucleotide sequence diversity in the noncoding control region of 25 BK virus (BKV) isolates from urine specimens in 10 patients with systemic lupus erythematosus. Sequences were obtained from polymerase chain reaction–amplified DNA in urine specimens as described in Materials and Methods. The top of the figure illustrates the P-Q-R-S anatomy of the archetypal BKV (WW) strain as proposed by Markowitz and Dynan [30] and expanded by us [29]. Numbering system starts with the first nucleotide to the late side of the origin of replications assigned as 1. Nucleotide positions in the first WW strain described by Rubinstein [3] that differ from one or several BKV sequences in this study are indicated by capital letters and numbers. Unbroken lines indicate conserved sequences.

A. Comparison of A and B isolates from patients 4, 10, 17, 18, and 20. A and B isolates were the first and last BKV DNA–positive urine sample during the 1-year follow-up study in each patient.

B. Comparison of A, B, and C isolates from patients 5, 8, 16, 24, and 25. The C isolates were collected 1–3 years after the follow-up study. Nucleotide substitutions in single strains compared with the first WW isolate [31] are indicated by capital letters and numbers above and under the lines, respectively. * corresponds to the published sequence of the first WW isolate [31]. ‡, BKV strain 5A has an insertion in position nucleotide 63-C-64 and a deletion in nucleotide 176 (A). †, BKV strain 16B has an insertion in position 143-T-144. ▲ and ▼ indicate a deletion and an insertion as outlined above and under the sequence, respectively.

Patients. The NCCR DNA sequences in 13 A, B, and/or C isolates from 7 patients (patients 4, 5, 10, 17, 18, 20, and 25) were identical. Two other repetitive NCCR DNA sequences were found in 5 A and/or B isolates and 3 C isolates from 3 different patients (patients 8, 16, and 24). The A isolates of patients 5, 17, and 20 and the B isolates of patient 16 possessed a unique WW NCCR sequence.

The archetypal JCV (CY) NCCR is divided into 5 blocks according to the number of base pairs—23, 55, 66, 18, and 69 bp, respectively [32]. This archetypal CY-like NCCR was detected in 8 isolates of 3 patients (12, 14, and 23). Seven different point mutations, 1 single base pair insertion, and 1 deletion were detected, compared with the published sequence of the first CY isolate [32]. None of the patients had identical NCCR sequences. These results are presented in figure 2.

NCCR DNA sequences in sequential BKV and JCV isolates from individual patients were compared. The results are presented in table 2. The DNA sequences from the first polyomavirus-positive (A isolate) and last positive urine specimens (B isolate) collected during the 1-year follow-up study were compared in 13 patients, 10 who excreted BKV and 3 who shed JCV. Polyomavirus-positive urine specimens taken 1–3 years afterward (C isolates) were available from 7 of these 13 patients, 5 with BKV and 2 with JCV.
A total of 9 pairs of A and B isolates revealed identical NCCR sequences (BKV-positive patients 4, 8, 10, 18, 24, and 25 and JCV-positive patients 12, 14, and 23), while 3 pairs (patients 5, 16, and 20) differed in only 1 nucleotide position, and 1 pair of A and B isolates (patient 17) exhibited differences in 3 nucleotide positions (transversions C82→A and C151→G, deletion of C143). Comparison of the corresponding A, B, and C isolates showed identity (patient 25) or 1 nucleotide difference (patients 5, 8, 16, and 24) in 5 of 7 patients. The C isolate of patient 12 contained 2 mutations (deletion of A70 and transversion of C72→A), while the C isolate of patient 23 differed by 1 transversion (G203→T), 3 transitions (G233, 235, and 241 into A), and the lack of an insertion of C between nucleotides 191 and 192 as compared with the A and B isolates. The detection of conserved point mutations in sequential isolates from the same patient strongly supports the genuineness of these mutations.

**BKV and JCV DNA are not detectable by PCR in serum and PBLs of patients with SLE.** The detection of BKV or JCV in PBLs of both immunosuppressed [8, 24, 33–36] and immunocompetent individuals [6, 34, 37] prompted us to investigate serum and peripheral blood samples from patients with SLE for the presence of polyomavirus DNA. In total, 107 PBL samples and 50 serum samples from 17 and 12 patients with SLE, respectively, were analyzed with the PYV-PCR. The samples from group II–IV patients included several specimens collected during polyomaviruria. The results are summarized in Table 1. All serum specimens were negative, whereas 2 of the

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**Table 2.** Nucleotide sequence differences in BK virus (BKV) (WW) and JC virus (JCV) (CY) noncoding control regions in consecutive A, B, and C isolates from individual patients with systemic lupus erythematosus (SLE).

<table>
<thead>
<tr>
<th>Virus</th>
<th>Differences in A and B isolates</th>
<th>Differences in A, B, and C isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n0a 1 2</td>
<td>n0 1 2</td>
</tr>
<tr>
<td>BKV</td>
<td>10 6 3 1 5</td>
<td>1 4 0</td>
</tr>
<tr>
<td>JCV</td>
<td>3 3 0 0 2</td>
<td>0 0 2</td>
</tr>
<tr>
<td>Total</td>
<td>13 9 3 1 7 1 4 2</td>
<td></td>
</tr>
</tbody>
</table>

* Number of positions with different nucleotides between consecutive A, B, and C isolates in individual patients with SLE.

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**Figure 2.** Nucleotide sequence diversity in the noncoding control region (NCCR) of 8 JC virus (JCV) strains from 3 patients. The top part of the figure depicts the NCCR of the archetypal JCV (CY) strain as proposed by Yogo and coworkers [32]. Numbering system starts with the first nucleotide to the late side of the origin of replication assigned as +1 [32]. Nucleotide positions in the first published CY sequence [32] that differ from one or several JCV sequences in this study are indicated by capital letters and numbers. Unbroken lines indicate conserved sequences. A. comparison of A and B isolates from patient 14. B, comparison of A, B, and C isolates from patients 12 and 23. Nucleotide substitutions in individual strains compared with the first CY isolate [32] are indicated by letters and numbers above and under the lines, respectively. * corresponds to the published sequence of the first CY isolate [32]. ▲ and ▼ indicate a deletion and an insertion as outlined above and under the sequence, respectively.
buffy coat samples from 2 different patients were repeatedly shown to contain BKV DNA, both in PYV- and NCCR-PCR analyses. Sequencing of the NCCR revealed BKV prototype-2 DNA [31] in both specimens. Since this strain has been propagated in our laboratory and has a specific NCCR rearrangement, we prefer to consider these 2 sequences as a contamination (see Discussion).

To ensure the validity of the negative results, several controls were performed. First, the β-globin gene fragment was amplified in all buffy coat specimens, as previously described [11]. Second, to parallel negative serum or buffy coat samples, 10-fold dilutions of BKV DNA were added. PCR analysis of such spiked samples repeatedly revealed a detection limit between 30 and 40 BKV genome copies per microliter of each specimen (data not shown).

Discussion

The present study has revealed a high prevalence of BK viruria in patients with SLE. The cross-sectional examination showed a significantly higher prevalence of BK but not JC viruria in patients with SLE compared with age- and sex-matched healthy controls. These results were further strengthened by the observation of at least 1 episode of polyomavirus urinary shedding in 16 (80%) patients in the 1-year follow-up study, 13 (65%) with BKV and 3 (15%) with JCV. Twelve patients revealed recurrent or persistent polyomaviruria, 9 with BKV and 3 with JCV, in 180 (70%) of 256 urine specimens. Coshedding of BKV and JCV was not observed, although this has been reported in a small number of patients in other studies [11, 38-40]. The relatively high frequency of JC viruria and nondetectable BK viruria in the healthy control group is supported by similar findings in other countries [9, 10, 38, 40-42], as well as in other Norwegian control groups, healthy individuals [20] and hospital patients [11]. Polyomaviruria, mainly JC viruria, has been described earlier in a limited number of patients with autoimmune diseases, including SLE [28, 38]. To our knowledge, this is the first study of human polyomaviruses in a large group of patients with SLE.

Correctly performed PCR may provide the most sensitive and specific method for the detection of polyomaviruses in the absence of efficient in vitro culture techniques. However, sensitive detection of polyomaviral DNA might target latently infected cells and not a productive viral infection. The significant linkage between the development of antibodies to polyomavirus T-antigen and polyomaviral DNA detection, as well as persistent viruria [20], strongly suggests that the PCR detection of polyomaviral DNA in our study represents a productive infection in the urinary tract.

We were unable to detect polyomaviral DNA in most of the serum or buffy coat specimens from patients with SLE. The only 2 BKV DNA–positive samples possessed the specific proto-2 NCCR [31]. This strain has been propagated in our laboratory. Therefore, we regard these samples to be contaminated. Other studies have detected BKV or JCV DNA in PBLs of both immunocompetent controls [6, 34, 35, 37] and immunosuppressed individuals [6, 8, 24, 33-36]. The negative findings of BKV or JCV in PBLs in immunocompetent control subjects by use of sensitive nested PCR [8, 33] are in contrast with the frequent findings in other studies using both traditional PCR methods [6, 37] and more sensitive nested PCR approaches [34, 35]. These divergent results should encourage the use of sequence or restriction enzyme analysis of polyomaviral regions with nucleotide heterogeneity to confirm the specificity of positive findings in PBLs in future studies, as shown in this study and by others [8].

Polyomaviral NCCR rearrangements seem to be a regular feature during replication both in vivo and in vitro. Several studies have demonstrated that specific viral properties at least partly correlated with changes in the NCCR anatomy [43, 44, reviewed in 5 and 29]. Therefore, it may be suggested that the high prevalence of polyomaviruria is the result of specific NCCR variants in patients with SLE. Sequencing of urinary BKV and JCV NCCRs, however, revealed that all patients except one (patient 2) shed archetypal BKV (WW) or JCV (CY) strains with minor point mutations, suggesting that these strains are dominant in this patient group, as has been shown in urine specimens from other human populations [9, 11, 27, 28, 30, 38, 39, reviewed in 29]. Archetypal BKV (WW) strains have been reported in patients with SLE [28, 38, 45].

Some of the observed single point mutations in BKV and JCV NCCRs from patients with SLE seem to be unique. In total, 15 point mutations were detected in the NCCR from 25 BKV isolates, compared with the original BKV WW isolate [31]. Seven of these mutations (positions 52, 53, 65, 145, 146, 222, and 226) have been described earlier in naturally occurring BKV isolates [29]. Nine point mutations were detected in the NCCR from 8 JCV isolates, compared with the archetypal JCV CY strain [32]. Two of these mutations (positions 217 and 229) have been described before [24, 28, 32]. Thirteen isolates from 7 patients revealed an NCCR sequence identical to the earlier described BKV WW Tromso 1 (WWT1) [26, 27]. These results are consistent with earlier reports presenting the WWT1 type as the most prevalent BKV NCCR detected in Norway [24, 27]. Furthermore, these observations, together with the findings of 6 unique BKV WW-NCCR sequences among the other 12 isolates from 6 patients, emphasize that the high prevalence of BK viruria is a significant result and not due to a PCR contamination.

The NCCR anatomy and nucleotide sequence of consecutive A, B, and C isolates of BKV and JCV in individual patients revealed a remarkable stability over a 1- to 3-year period. Patient 2 shed a BKV isolate with a specific rearranged NCCR anatomy in 2 separate urine specimens taken 7 months apart. The 6 C isolates revealed either identity or 1 or 2 single point mutations compared with their corresponding A and B isolates.
collected 1–3 years earlier. These results are not in favor of repeated polyomavirus infection in the host but support viral persistence characterized by intermittent or continuous viral replication and viruria. NCCR sequence stability is also consistent with the observed invariability in VP-1/T-antigen intergenic region sequences in JCV isolates collected 5–7 years apart from the same patients [46].

Naturally occurring BKV and JCV isolates with rearranged NCCRs have been reported in various human specimens [4, 6, 8, 26, 47–50, reviewed in 29]. Each rearranged NCCR could be derived from the archetypal form. These observations support the hypothesis that the polyomaviral NCCR undergoes rearrangements and cell- tissue-specific selection during replication in vivo as an adaptation process to transcription and replication in alternative tissues [48, 51]. The finding of predominantly stable archetypal NCCRs during persistent viruria and the nondetectable viremia in this study argue against persistent polyomaviruses in the kidney tubuli as a focus for the creation of rearranged NCCRs disseminating throughout the infected host. A recent report describing various JCV NCCRs in multiple tissues from a pediatric patient with PML indicates that replication during primary infection contributes significantly to NCCR diversity [50].

The finding of archetypal BKV NCCRs does not support a causal relation between specific NCCR types and the high prevalence of BKV shedding in patients with SLE. What is the most likely explanation for the high prevalence of BK viruria compared with healthy individuals? First, conditions with increased steroid hormone levels [52–54] and cellular damage with subsequent cellular regeneration and differentiation [55] may enhance in vivo permissivity for polyomaviruses. Analyses of immunosuppressive drug use, including corticosteroids, in relation to the occurrence of BK viruria do not show any temporal linkage between immunosuppressive therapy and urinary shedding of BKV. Furthermore, proteinuria and increased creatinine levels were not associated with polyomaviruria (data not shown). These observations do not support any association between persistent polyomaviruria and the use of corticosteroids or kidney cellular damage. Second, human cytomegalovirus (HCMV) reactivation caused by immunosuppression may represent another factor that can reactivate latent JCV, since HCMV has been shown to induce JCV DNA replication in vitro in nonpermissive cells [56]. This seems unlikely, because only 2 out of 788 urine samples from the 20 follow-up patients contained HCMV DNA [Bendicksen S, Ghelue MV, Rekvig OP, and Moens U, unpublished data]. Third, the high prevalence of BK viruria may be the result of recurring infection rather than reactivation. Although the exact route of infection and transmission remains unknown, it is believed that after primary infection, virus spreading occurs via viremia with transport to the target organs [46, 50, 57, 58]. The viral sequences’ invariability in sequential isolates from individual patients does not support reinfection as a likely explanation. The unlikeliness in this scenario is further emphasized by the negative findings of viral DNA in serum or PBLs. Fourth, and probably the most likely explanation, is immune dysfunction. The high prevalence of BK viruria in patients with SLE is in accordance with observations in various groups of immunosuppressed patients, such as bone marrow transplantation patients [15, 33, 40, 59], renal transplant recipients [60], and human immunodeficiency virus–positive patients [24, 39], although this type of immune dysfunction may not be relevant for SLE. The importance of humoral and cell-mediated immunity in restricting human polyomavirus infection has not been examined systematically. However, the strong association between PML and disorders of cell-mediated immunity underscores the key role of appropriate T cell functions in the containment of JCV reactivations [57]. The strong association between polyomaviral DNA–positive urines and T-ag antibodies indicates appropriate T cell functions in patients with SLE in mounting a detectable antibody response to polyoma T-ag [20].

In conclusion, our results demonstrate a high prevalence of BKV but not JCV urinary shedding in patients with SLE compared with age- and sex-matched healthy controls. The BKV and JCV isolates have predominantly archetypal regulatory regions with stable specific nucleotide sequences in the same patient at intervals of 1–3 years, indicating viral persistence and not reinfection as a cause of continuous or intermittent urinary shedding. Immunosuppressive therapy was not significantly associated with periodic and persistent polyomaviruria. An unknown inherent immunological defect in patients with SLE might contribute to persistent BK viruria. Recently, on the basis of sequence variation within the VP-1/large T-antigen region, specific archetypal JCV genotypes could be identified significantly more frequently in patients with PML [61]. The biological consequence of this observation remains to be established, as well as whether such specific archetypal JCV or BKV subtypes are circulating in patients with SLE.

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