

# Prevalence of Polyomavirus BK and JC Infection and Replication in 400 Healthy Blood Donors

Adrian Egli,<sup>1</sup> Laura Infanti,<sup>3</sup> Alexis Dumoulin,<sup>1,2</sup> Andreas Buser,<sup>3</sup> Jacqueline Samaridis,<sup>1</sup> Christine Stebler,<sup>3</sup> Rainer Gosert,<sup>1</sup> and Hans H. Hirsch<sup>1,2,4</sup>

<sup>1</sup>Transplantation Virology, Institute for Medical Microbiology, Department of Biomedicine, and <sup>2</sup>Division of Diagnostics, Institute for Medical Microbiology, University of Basel, <sup>3</sup>Blutspendezentrum Schweizerisches Rotes Kreuz beider Basel, <sup>4</sup>Infectious Diseases and Hospital Epidemiology, University Hospital of Basel, Switzerland

**Background.** The replication of BK virus (BKV) and JC virus (JCV) is linked to polyomavirus-associated nephropathy, hemorrhagic cystitis, and multifocal leukoencephalopathy in immunodeficient patients, but the behavior of these viruses in immunocompetent individuals has hardly been characterized.

**Methods.** We used EIA to study samples obtained from 400 healthy blood donors aged 20–59 years for BKV- and JCV-specific antibodies against virus-like particles. We also studied BKV and JCV loads in plasma and urine among these individuals by use of real-time polymerase chain reaction.

**Results.** IgG seroprevalence was 82% (328 of 400 donors) for BKV and 58% (231 of 400) for JCV. As age increased (age groups were divided by decade), the seroprevalence of BKV decreased from 87% (87 of 100) in the youngest group (aged 20–29 years) to 71% (71 of 100) in the oldest group (aged 50–59 years) ( $P = .006$ ), whereas the seroprevalence of JCV increased from 50% (50 of 100) in the youngest group to 68% (68 of 100) in the oldest group ( $P = .06$ ). Asymptomatic urinary shedding of BKV and JCV was observed in 28 (7%) and 75 (19%) of 400 subjects, respectively, with median viral loads of 3.51 and 4.64 log copies/mL, respectively ( $P < .001$ ). Unlike urinary BKV loads, urinary JCV loads were positively correlated with IgG levels. The shedding of JCV was more commonly observed among individuals who were seropositive only for JCV, compared with individuals who were seropositive for both BKV and JCV, suggesting limited cross-protection from BKV immunity. Noncoding control regions were of archetype architecture in all cases, except for 1 rearranged JCV variant. Neither BKV nor JCV DNA was detected in plasma.

**Conclusions.** Our study provides important data about polyomavirus infection and replication in healthy, immunocompetent individuals. These data indicate significant differences between BKV and JCV with respect to virus-host interaction and epidemiology.

The human polyomaviruses BK (BKV) and JC (JCV) infect immunocompetent individuals without specific signs or symptoms and remain latent thereafter in the renourinary tract [1]. In seropositive individuals with altered immunity, viral replication can reactivate and progress to serious organ diseases such as polyomavirus-

associated nephropathy, hemorrhagic cystitis, and progressive multifocal leukoencephalopathy [2–4]. Because no specific antiviral drugs are available to treat polyomavirus disease, treatment depends on improving immune functions to regain control over viral replication [5].

Polyomavirus-associated nephropathy (PVAN) in individuals who have undergone kidney transplantation is primarily caused by BKV replication, with rates ranging from 1%–10% [6, 7]. The stringent sequence of high-level viremia of  $>7$  log genome equivalents (GEq)/mL followed by viremia and histologically manifest disease after transplantation has become a paradigm of polyomavirus pathogenesis and diagnosis [8, 9]. The dynamics of BKV replication are rapid, with a half-life of 2 h–12 h in plasma and urine [10, 11]. The extensive cytopathic wear of  $4 \times 10^7$  cells per day is further accelerated by more pathogenic BKV variants with rearranged non-

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Reprints or correspondence: Hans H. Hirsch, MD, MSc, Transplantation Virology, Institute for Medical Microbiology, Department of Biomedicine, University of Basel, Petersplatz 10, CH-4003 Basel, Switzerland (Hans.Hirsch@unibas.ch).

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coding control regions (NCCRs) that increase viral replication capacity [12].

High-level urothelial BKV replication is also observed in >50% of patients after hematopoietic stem-cell transplantation, but only 10% develop severe polyomavirus-associated hemorrhagic cystitis [13, 14]. The key JCV-mediated disease is progressive multifocal leukoencephalopathy, which was diagnosed in  $\leq 7\%$  of patients with HIV or AIDS prior to the advent of combined antiretroviral therapy [15–17]. JCV viremia seems not to correlate with histologically confirmed JCV disease, and JCV viremia is exceptional and low level [18–21].

The behavior of BKV and JCV in immunocompetent individuals has hardly been investigated, and some results seem contradictory. Ling et al. detected JCV but not BKV in urine, and peripheral blood mononuclear cells (PBMCs) were uniformly negative for both viruses [22]. By contrast, Dolei et al. reported detecting BKV and JCV DNA in 7% and 0.9%, respectively, of 231 DNA preparations of PBMCs from healthy blood donors [23]. Zhong et al. detected BKV and JCV DNA in centrifugation-enriched urine pellets from 351 presumably immunocompetent outpatients and 99 healthy volunteers, and the percentage of subjects in whom the viruses were detected increased with age, from 14% to 44% and 10% to 72%, respectively [24]. None of these studies conducted antibody testing to distinguish between reactivation and primary replication. Research that used hemagglutination inhibition assays (HIA) indicated that the overall seroprevalence rates were 81% for BKV and 35% for JCV, but urinary viral shedding was not investigated [25]. Thus, there is considerable uncertainty about the BKV and JCV detection rates and the viruses' respective seroprevalence. For the analysis of samples obtained from 400 healthy blood donors at the time of blood donation, we used current real-time polymerase chain reaction (PCR) and EIA for antibodies to virus-like particles (VLP) to identify BKV and JCV DNA in urine and plasma and to determine antibody responses in plasma.

## SUBJECTS AND METHODS

**Blood donor population.** We enrolled 400 consecutive healthy blood donors from the blood donation center in Basel, Switzerland (Schweizerisches Rotes Kreuz [Swiss Red Cross]) from February 2007 to January 2008. Participants gave written informed consent to the protocol (267/06) approved by the local institutional review board. Participants were enrolled into 1 of 4 age groups of 100 persons each (divided by decade into age 20–29, 30–39, 40–49, and 50–59 years); each group consisted of 50 women and 50 men (total in all 4 age groups, 200 men and 200 women). From each participant, citrate-anticoagulated blood (CPT Vacutainer; Becton Dickinson) and urine were collected at the time of regular blood donation. The donors fulfilled all criteria required for blood donation, including serologic and PCR negativity for hepatitis B virus (HBV), hepatitis C virus (HCV), HIV, and *Treponema pallidum*.

**Polyomavirus molecular diagnostic assays.** BKV and JCV loads were quantified after DNA extraction from citrate-anticoagulated plasma or urine using the Magnapure reagents and robotics (Roche). The real-time PCR protocols for detection of BKV and JCV DNA in urine and plasma samples have been described elsewhere [8, 12, 21, 26]; the primers and probes used are listed in table 1. For the purpose of the study, the limit of detection was arbitrarily set to 3 log GEq/mL, which is the limit used routinely in our diagnostic laboratory.

**EIA for antibodies to BK and JC VLP.** The VP1 coding sequence of BKV and JCV was inserted into a pFastBac vector (Invitrogen) by using the restriction enzymes *Bam*HI-*Xho*I and *Bam*HI-*Hind*III (New England Biolabs). Recombinant baculovirus genomes containing the BKV or JCV VP1 gene sequence were generated by using the Bac-to-Bac expression system (Invitrogen) and transfected in Sf9 insect cells, in accordance with the manufacturer's instructions. The 3-dimensional structure and quality of BK VLP and JC VLP was confirmed by electron microscopy. BK VLP and JC VLP were used as antigens in a standard EIA assay, as described elsewhere [27]. The optical density at 492 nm ( $OD_{492}$ ) obtained with PBS alone was taken as background and subtracted. The cutoff defining a positive serologic response was  $OD_{492}$  0.110 for BKV- and JCV-specific IgG and IgM.

**Sequencing of viral NCCR and BK VP1 gene.** To determine the architecture of the BKV and JCV NCCRs, we performed nested PCR using BKV- and JCV-specific primer sets (table 1). The amplicons were analyzed with 2% Tris, sodium acetate, and EDTA–agarose gel electrophoresis to identify archetypal structure, as well as deletions and/or insertions. Bands of altered mobility were extracted and sequenced as described elsewhere [12]. The BKV serotype-defining VP1 sequences [28] were identified by cycle sequencing on an AB3130 Genetic Analyzer, using the primers shown in table 1.

**Statistical analysis.** For statistical analysis, we used SPSS (version 13; SPSS). Data were summarized as medians and ranges or as mean ( $\pm$ SD), as appropriate. If the Kolmogorov-Smirnov Z Test indicated a nonnormal distribution, we used nonparametric tests such as Mann-Whitney *U* tests, Kruskal-Wallis tests, Spearman's  $\rho$  correlation analyses, linear regression analysis, and paired Wilcoxon tests. Categorical data were analyzed by using Fisher's exact test or Pearson's  $\chi^2$  test, depending on sample size. All tests were 2 sided and  $P < .05$  was considered statistically significant. Bonferroni correction was used where appropriate.

## RESULTS

**General characteristics of the study population.** More than 95% of blood donors were white. Their blood and rhesus groups corresponded to the distribution found in central Europe; group A was the most common, observed in 194 (49%) of 400 subjects, followed by group O in 146 subjects (37%), group B in 23 subjects

**Table 1. Primers and probes used for detection of BK and JC polyomavirus (BKV and JCV) in plasma and urine from 400 healthy blood donors.**

Analysis	Sequence
PCR quantification	
BKV	
LT-forward	5'-AGCAGGCAAGGGTCTATTACTAAAT-3'
LT-reverse	5'-GAAGCAACAGCAGATTCTCAACA-3'
Probe	FAM-AAGACCCTAAAGACTTTCCCTCTGATCTACACCAGTTT-TAMRA
JCV	
LT-forward	5'-CTAAACACAGCTTGACTGAGGAATG-3'
LT-reverse	5'-CATTTAATGAGAAGTGGGATGAAGAC-3'
Probe	FAM-TAGAGTGTGGGATCCTGTGTTTCATCATCACT-TAMRA
NCCR rearrangement	
BKV	
Outer 1, BKTT5	5'-GAGCTCCATGGATTCTTC-3'
Outer 2, BKTT6	5'-CCAGTCCAGGTTTTACCA-3'
Inner 1, BKTT7	5'-CCCTGTTAAGAACTTTATCCATTT-3'
Inner 2, BKTT8	5'-AACTTTCACTGAAGCTTGTCGT-3'
JCV	
Outer 1	5'-AGGCCTAATAAATCCATAAGCTCCA-3'
Outer 2	5'-GTTCCACTCCAGGTTTTACTAATT-3'
Inner 1	5'-TTTTAGCTTTTTGCAGCAAAAAATTA-3'
Inner 2	5'-CCTGGCGAAGAACCATGGCCAG-3'
BKV VP1 sequencing	
Outer 1	5'-GTGCAAGTGCCAAACTACTAATAA-3'
Outer 2	5'-TGCATGAAGGTTAAGCATGCTAGT-3'
Inner 1	5'-CAACCAAAAGAAAAGGAGAGTGTC-3'
Inner 1	5'-TCCTCCACCATGCTCATGCACT-3'

**NOTE.** LT, large T antigen; NCCR, noncoding control region; PCR, polymerase chain reaction.

(6%), and group AB in 37 subjects (9%). Of 400 subjects, 331 (83%) were positive for rhesus factor.

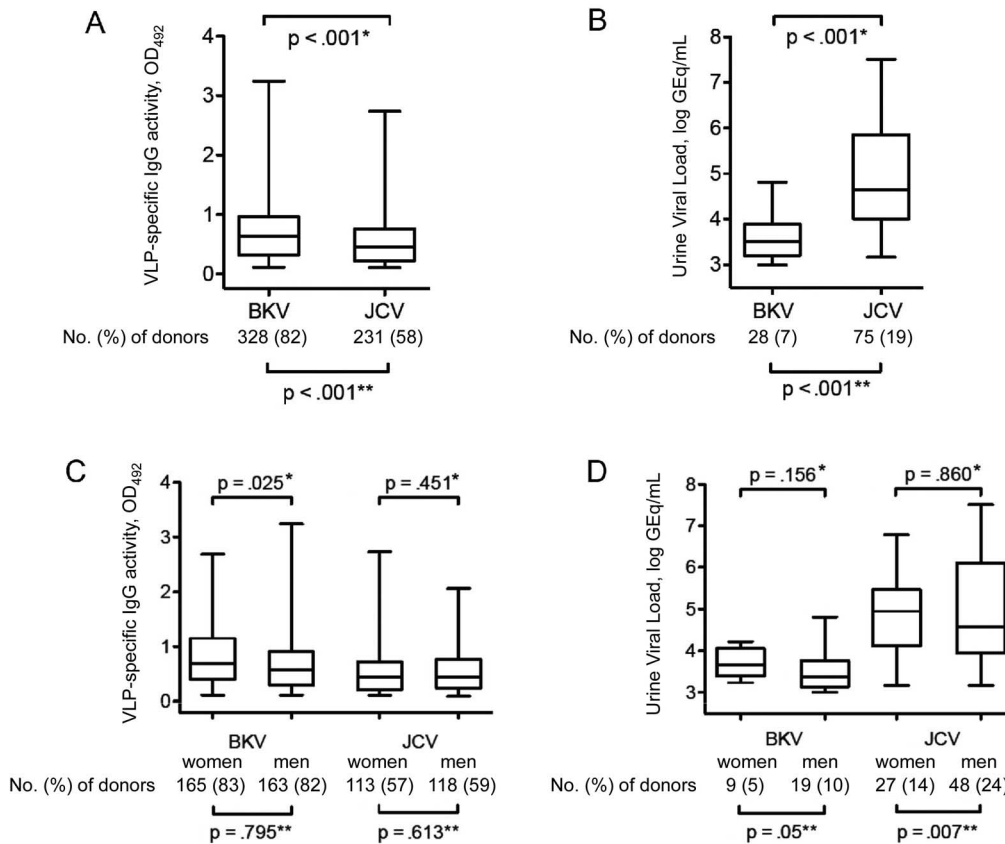
**Prevalence of BKV and JCV antibodies, DNAemia, and DNAuria.** BKV IgG seroprevalence among healthy blood donors was 82% (328 of 400), significantly higher than the corresponding JCV IgG seroprevalence of 58% (231 of 400) ( $P < .001$ , by  $\chi^2$  test; figure 1A). The mean IgG level as defined by OD<sub>492</sub> was higher for BKV than for JCV ( $P < .001$ , by Mann-Whitney  $U$  test). No significant difference was detected in IgM seroprevalence, which was 1% (4 of 400) for BKV and 0% (0 of 400) for JCV. All of the IgM-positive individuals were also IgG positive.

BKV was detected in urine samples from 28 (7%) of 400 healthy blood donors, a detection rate lower than that for JCV, which was detected in samples from 75 (19%) of the donors ( $P < .001$ , by  $\chi^2$  test; figure 1B). Median urinary viral loads were also lower for BKV than for JCV (3.5 log vs. 4.8 log GEq/mL;  $P < .001$ , by Mann-Whitney  $U$  test). Four (1%) of 400 healthy blood donors had both BKV and JCV DNA detected in the urine. No difference in urinary BKV or JCV load was detectable when we compared individuals who shed just one of the viruses with individuals who shed both viruses (table 2). There was no association between polyomavirus shedding and ABO blood group. However, individuals who shed BKV in the urine were more

likely to be positive for rhesus factor than were those who did not (27 of 331 vs. 1 of 69;  $P = .047$ , by  $\chi^2$  test), whereas no association between rhesus factor positivity and JCV shedding was found (60 of 331 vs. 15 of 69;  $P = .484$ , by  $\chi^2$  test). Of note, neither BKV nor JCV DNA was detected in any of the 400 plasma samples.

**Sex and age dependent prevalence of BKV and JCV antibodies and viruria.** BKV IgG seroprevalence was not different between female and male blood donors (165 [83%] vs. 163 [82%] of 200;  $P = .795$ , by  $\chi^2$  test) (figure 1C). However, the level of BKV IgG activity was higher for women than for men ( $P = .025$ , by Mann-Whitney  $U$  test). No difference in JCV IgG seroprevalence or the level of JCV IgG activity was apparent between women and men (113 [57%] of 200 vs. 118 [59%] of 200;  $P = .613$ , by  $\chi^2$  test;  $P = .451$ , by Mann-Whitney  $U$  test) (figure 1C). Both BKV and JCV were more frequently detected in urine samples obtained from men, compared with samples obtained from women ( $P = .05$  for BKV and  $.007$  for JCV, by  $\chi^2$  test) (figure 1D). However, there were no significant differences between the sexes with respect to urinary BKV or JCV loads (figure 1D).

When we compared the antibody responses in the different age groups, we found that IgG seroprevalence and the level of



**Figure 1.** Box-whisker plots showing overall prevalence of BK and JC polyomavirus (BKV and JCV) antibodies and viruria. *A*, BK and JC virus-like particle (VLP)-specific IgG seroprevalence ( $N = 400$ ). *B*, Urinary BKV and JCV load ( $N = 400$ ). *C*, BK-VLP-specific and JC-VLP-specific IgG seroprevalence, according to sex ( $n = 200$ , for each sex). *D*, Urinary BKV and JCV load, according to sex ( $n = 200$ , for each sex). Boxes span the interquartile range; the line within each box denotes the median, and whiskers indicate the minimum and maximum values. \* $P$  calculated by Mann-Whitney  $U$  test; \*\* $P$  calculated by  $\chi^2$  test. GEq, genome equivalents; OD<sub>492</sub>, optical density at 492 nm.

BKV IgG activity decreased significantly with increasing age, from 87% (87 of 100) in the youngest group (aged 20–29 years) to 71% (71 of 100) in the oldest group (aged 50–59 years) ( $P = .006$ ; figure 2*A*), whereas a strongly opposite trend was found for JCV IgG ( $P = .06$ ) (figure 2*A*). BKV shedding was not associated with age group ( $P = .429$ , by  $\chi^2$  test) (figure 2*B*). By contrast, JCV shedding was more frequent in older age groups, increasing from 9% (9 of 100) in the youngest group to 28% (28 of 100) in the oldest group ( $P = .02$ , by  $\chi^2$  test), but no correlation between age and urinary viral load was found (figure 2*B*). When we compared individuals who shed just one virus with those who shed both viruses, we found that individuals who shed only BKV tended to be younger than those who shed only JCV. All individuals who shed both BKV and JCV belonged to the oldest age group (50–59 years old).

**Serologic responses and viral loads.** To investigate the relationship between antibody response and the level of urinary viral shedding, we divided subjects into the following 3 strata, according to their urinary viral load: undetectable ( $<3$  log GEq/mL), low (3–6 log GEq/mL), and high ( $>6$  log GEq/mL). BKV loads were undetectable in 372 (93%) of the donors and low in

the remaining 28 (7%). JCV loads were undetectable in 325 (81%), low in 61 (15%), and high in 14 (4%) of the donors (figure 3). There was no correlation between BKV antibody level and urinary BKV load. For JCV, the IgG level was significantly higher in individuals in the high-shedding group, compared with those in the low-shedding group ( $P < .001$ ) (figure 3). Similar results were obtained for JCV by using a Spearman  $\rho$  test and a linear regression model (Spearman  $\rho$ , 0.284 [ $P = .017$ ]; and linear regression coefficient [ $R^2$ ], 0.139 [ $P = .001$ ]), whereas only a trend was observed for BKV (Spearman  $\rho$ , 0.423 [ $P = .027$ ]; and  $R^2$ , 0.122 [ $P = .081$ ]). No cross-correlation was found between BKV loads and JCV IgG activity level (data not shown).

**Cross-reactivity of BKV and JCV IgG responses.** Seropositivity for both BKV IgG and JCV IgG was found in 182 (46%) of 400 individuals. When we compared the IgG antibody level in individuals who were seropositive for only one virus with that in individuals who were seropositive for both viruses, we found that the BKV IgG level was significantly higher in individuals who were seronegative for JCV than in individuals who were seropositive for both BKV and JCV ( $P = .012$ ; table 3). Conversely, individuals who were seropositive only for

**Table 2. Age and viral status of blood donors evaluated for BK and JC polyomavirus (BKV and JCV), according to virus detected.**

	BKV and JCV (n = 4)	BKV only (n = 24)	JCV only (n = 71)	None (n = 301)	P
<b>Demographic characteristic</b>					
Age, median (range), years	55 (53–56)	39 (22–58)	46 (20–59)	38 (20–59)	<.001 <sup>a</sup>
<b>Viral data</b>					
<b>BKV</b>					
VLP-specific IgG, median (range)	0.4415 (0.0974–0.9066)	0.8079 (0.0858–1.7773)	0.3012 (0.0001–1.876)	0.5353 (0.0035–3.2405)	.001 <sup>a</sup>
Urinary viral load, median (range), GEq/mL	2812 (1020–5660)	3270 (1000–64,900)	0	0	.59 <sup>b</sup>
<b>JCV</b>					
VLP-specific IgG, median (range), OD <sub>492</sub>	1.2752 (0.1753–2.062)	0.0774 (0.0205–0.9538)	0.6596 (0.0306–2.0533)	0.1101 (0.0035–2.7329)	<.001 <sup>a</sup>
Urinary viral load, median (range), GEq/mL	5,469,450 (31,997–2.1 × 10 <sup>7</sup> )	0	42,251 (1471–32.7 × 10 <sup>6</sup> )	0	.132 <sup>b</sup>

**NOTE.** GEq, genome equivalents; OD<sub>492</sub>, optical density at 492 nm; VLP, virus-like particles.

<sup>a</sup> By Kruskal-Wallis test.

<sup>b</sup> By Mann-Whitney *U* test.

JCV showed a higher JCV IgG level than did those who were seropositive for both BKV and JCV ( $P = .028$ ; table 3). When we compared the rate at which urinary BKV shedding occurred, we found no difference between individuals who were seropositive for BKV and those who were seropositive for BKV and JCV ( $P = .169$ ). Interestingly, urinary shedding of JCV was significantly less common among individuals who were seropositive for both viruses, compared with those who were seropositive only for JCV ( $P < .001$ ; table 3). When we compared the BKV IgG level and the JCV IgG level, we found no positive correlation (figure 4A). By contrast, some limited correlation suggestive of cross-reactivity was apparent between BKV and JCV IgM activity levels (figure 4B). Taken together, these data indicated that BK-VLP and JC-VLP IgG responses were specific to the respective polyomavirus capsid and that cross-reactivity was not a major concern.

**Types of BKV detected in urine.** To investigate the serotype-defining genotype of BKV in the urine of healthy blood donors, we sequenced the relevant VP1 region. Although urinary BKV loads were low, 25 of 28 samples positive for BKV by PCR could be sequenced. BKV type 1 was most common, occurring in 18 (80%) of 25 samples, followed by type 4 (in 5 samples [20%]), and type 2 (in 2 samples [8%]). BKV type 3 was not found. No differences in median urinary BKV loads were found between the subtypes (the median [range] BKV load for type 1 was 3.57 log GEq/mL [3.0–4.8 GEq/mL], for type 2 it was 3.27 GEq/mL [3.0–4.2 GEq/mL] and for type 4, it was 3.27 GEq/mL [3.2–3.8 GEq/mL]). When we compared the BKV IgG level in individuals who shed BKV, we found a significantly higher BKV IgG level in individuals who shed BKV type 1, compared to those who shed non-type 1 BKV (figure 4C). When the BKV IgG level among the 18 individuals who shed BKV type 1 was compared according to viral subtype (i.e., subtypes 1a [ $n = 5$ ]), 1b-1 [ $n = 6$ ], and 1b-2 [ $n = 7$ ]), it showed a trend

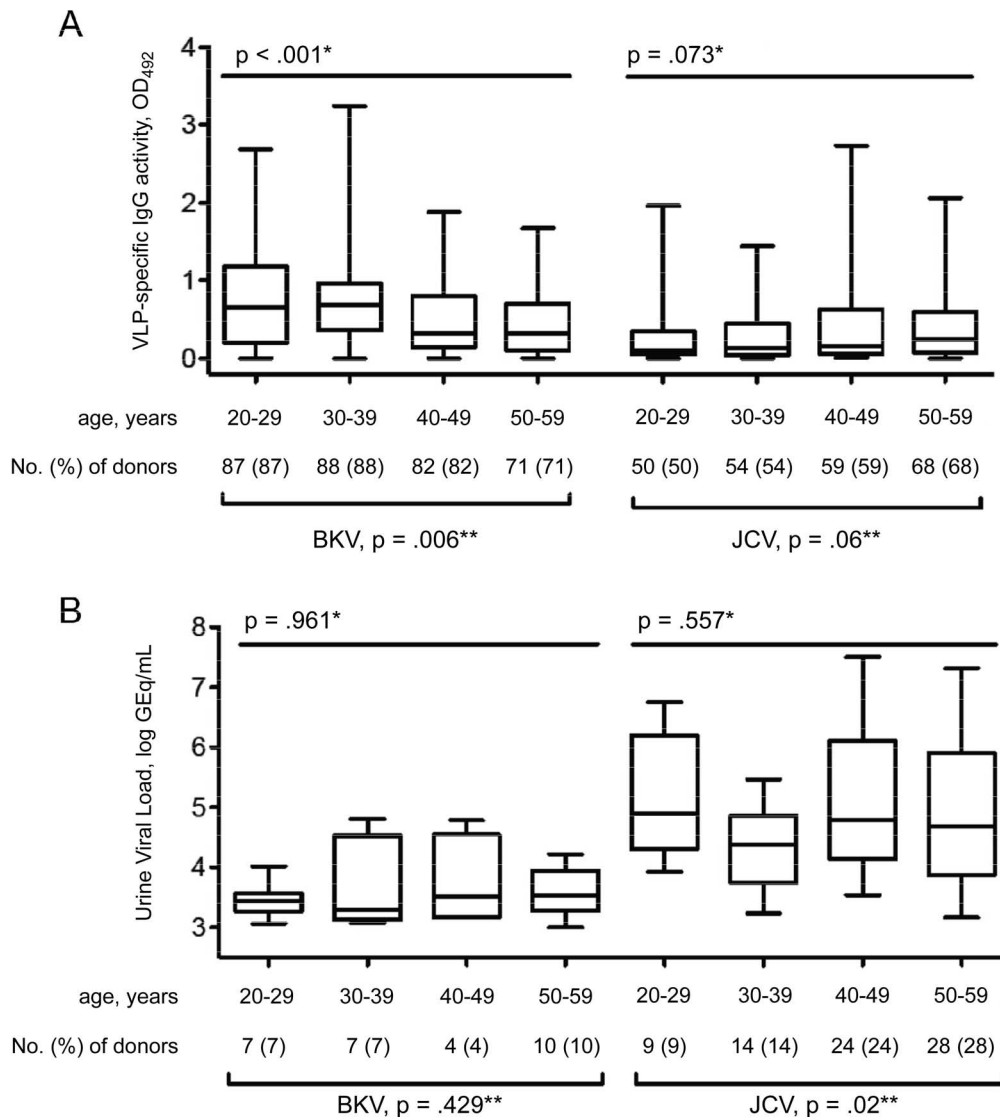
toward increase among patients who shed BKV subtype 1-b2 (figure 4D).

**Architecture of BKV and JCV NCCR in subjects who shed virus.** Given the role the polyomavirus NCCR plays in viral replication capacity in transplant recipients, we examined the NCCR architecture of BKV and JCV found in the urine of healthy blood donors. In 20 of 28 BKV-positive urine samples, the NCCR could be amplified and sequenced but no NCCR rearrangement was identified. In 70 of 75 JCV-positive urine samples, the NCCR could be characterized, and in only 1 case was a rearranged JCV NCCR identified. The JCV NCCR rearrangement was confirmed in 2 subsequent urine samples obtained 1 and 2 months after the original sample, which revealed identical insertion in the F-block (GeneBank accession numbers FJ233068–70). In the first sample, the urinary JCV load was 4.78 log GEq/mL; in the second, it was 3.78 log GEq/mL; and in the third, it was 3.25 log GEq/mL.

## DISCUSSION

This comprehensive study of samples from 400 consecutive blood donors provides important baseline data on human polyomavirus infection and replication. First, the overall IgG seroprevalence for BKV was 82% (328 of 400), which was significantly higher than the 58% (231 of 400) seroprevalence observed for JCV. Second, urinary shedding of BKV was observed in 28 subjects (7%), which was significantly fewer than the 75 subjects (19%) observed to shed JCV. Third, the median urinary BKV load was 3.51 log GEq/mL, which was significantly lower than the median urinary JCV load of 4.64 log GEq/mL. Fourth, analysis of the NCCR of all urinary BKV and JCV genomes revealed archetype architecture in all but 1 case, which involved a rearranged JCV NCCR. Finally, neither BKV nor JCV was detected



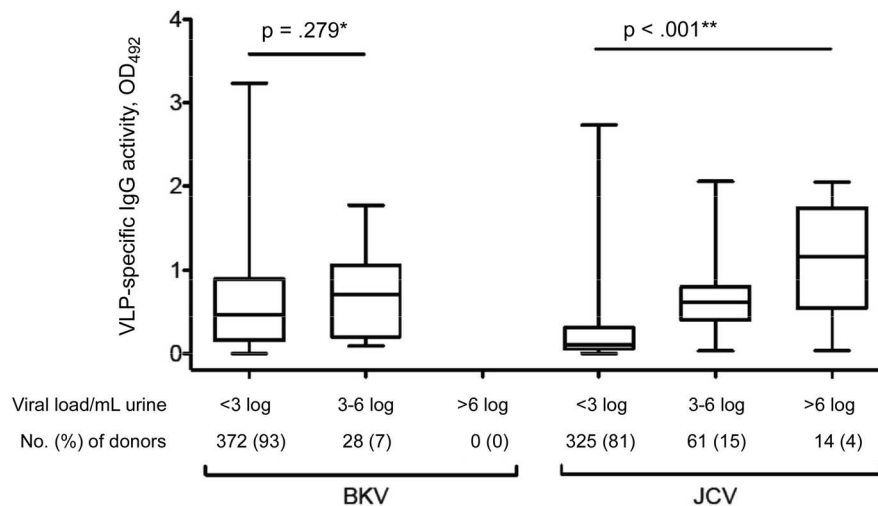


**Figure 2.** Box-whisker plots showing age-dependant prevalence of BK and JC polyomavirus (BKV and JCV) antibodies and viruria. *A*, BK and JC virus-like particle (VLP)-specific IgG levels, according to age group. *B*, Urinary BKV and JCV load, according to age group. Boxes span the interquartile range; the line within each box denotes the median, and whiskers indicate the minimum and maximum values. \**P* calculated by Kruskal-Wallis test; \*\**P* calculated by  $\chi^2$  test. GEq, genome equivalents; OD<sub>492</sub>, optical density at 492 nm.

in plasma under conditions routinely used to monitor viremia in patients undergoing kidney and/or hematopoietic stem-cell transplants. Unlike earlier studies, we studied healthy donors at the time of regular blood donation that was conducted in accordance with international standards and was not confounded by pregnancy, clinical and laboratory abnormalities, or infections with *T. pallidum*, HIV, HBV, or HCV. Thus, we may conclude that BKV and JCV infection and replication are common in bona fide healthy, immunocompetent adults, but the rates of seropositivity and urinary viral shedding point to significant differences in the epidemiology of BKV and JCV infection.

We noted that the BKV IgG seroprevalence was highest in the youngest age group (aged 20–29 years) at 87% (87 of 100) and decreased with older age to 71% (71 of 100) in the oldest group

(aged 50–59 years), whereas the opposite trend was observed for JCV IgG seroprevalence, which increased from 50% (50 of 100) in the youngest group to 68% (68 of 100) in the oldest group. Knowles et al. [25] used HIA in a large study of 2435 serum samples obtained in England in 1991 and observed a similar age-related decrease in BKV seroprevalence from 91% to 74% ( $P < .01$ ; overall mean seroprevalence, 81%), whereas JCV seroprevalence increased with age from 34% to 45% ( $P < .01$ ; overall mean seroprevalence, 35%). We may conclude that no gross alterations in the epidemiology of BKV infection seem to have occurred in the last 15 years. Similar data have been reported for immunocompetent Japanese outpatients [24]. The data indicate that the puzzling increase in the incidence of PVAN is not related to changes in the epidemiology of BKV infection in



**Figure 3.** Box-whisker plots showing virus-like particle (VLP)-specific IgG activity for BK and JC polyomavirus (BKV and JCV), according to urinary viral load. Subjects were stratified as follows: <3 log genome equivalents (GEq)/mL (undetectable), 3–6 log GEq/mL (low), and >6 log GEq/mL (high). Boxes span the interquartile range; the line within each box denotes the median, and whiskers indicate the minimum and maximum values. \**P* calculated by Mann-Whitney *U* test; \*\**P* calculated by Kruskal-Wallis test. OD<sub>492</sub>, optical density at 492 nm.

the general population, but rather to other modulating factors, of which increased immunosuppression following kidney transplantation is the key suspect.

The decline in BKV IgG antibody level with age is also of interest, as it suggests that significant (re)exposure to BKV does not seem to occur in most healthy, immunocompetent individuals during adult life. This is in line with our data showing low-level urinary shedding of BKV in only 28 individuals (7%). By contrast, significant increases in BKV-specific antibody titers have been observed in patients undergoing kidney transplantation who had high-level viremia and viremia [27, 29–33]. Unlike BKV, JCV starts with a lower IgG seroprevalence that increases with age. Initial serological studies indicated that BKV infection occurs earlier during childhood, whereas JCV infection follows

later, which is supported by our data as well. This suggests that significant exposure to JCV leads to primary and secondary infections during adult life. This observation is of interest for the as yet unresolved pathogenesis of progressive multifocal leukoencephalopathy in patients with AIDS and suggests the hypothesis that exogenous infection and replication may be the cause in some profoundly immunosuppressed individuals.

Our data show that urinary shedding of JCV was not only more common but occurred in significantly greater quantities than BKV shedding, with 14 (4%) of 400 participants having JCV loads >6 log GEq/mL. By contrast, the urinary BKV load was observed to be <5 log GEq/mL for all subjects. Thus, compared to immune control of BKV, JCV-directed humoral and/or cellular immunity seems less efficient at controlling viral repli-

**Table 3.** IgG activity levels and viral shedding in the urine of 400 healthy blood donors evaluated for BK and JC polyomavirus (BKV and JCV), according to serologic results.

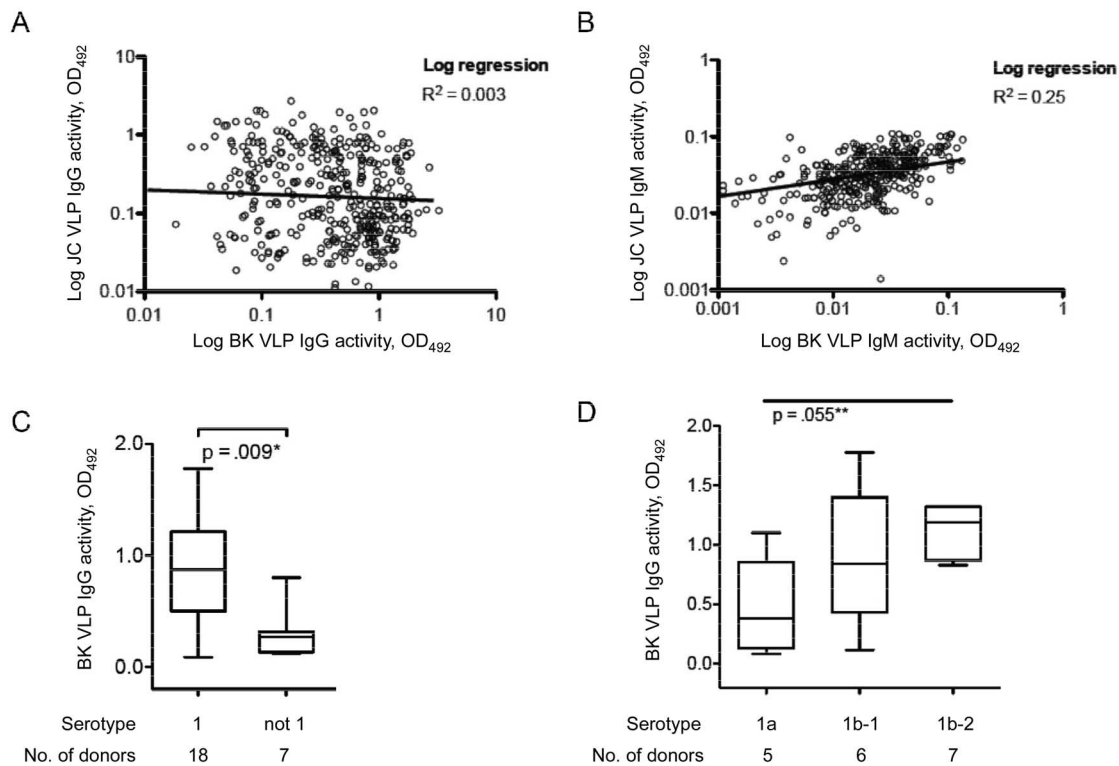
Viral data	BKV <sup>-</sup> /JCV <sup>-</sup> (n = 23)	BKV <sup>+</sup> /JCV <sup>-</sup> (n = 146)	BKV <sup>-</sup> /JCV <sup>+</sup> (n = 49)	BKV <sup>+</sup> /JCV <sup>+</sup> (n = 182)	<i>P</i>
VLP-specific IgG, OD <sub>492</sub>					
BKV	0.0586 (0.0035–0.1002)	0.7709 (0.1141–3.2405)	0.0776 (0.0001–0.1065)	0.5241 (0.1142–2.6862)	.012 <sup>a</sup>
JCV	0.0398 (0.0035–0.094)	0.0523 (0.0059–0.1085)	0.6096 (0.1144–2.0533)	0.4343 (0.1101–2.7329)	.028 <sup>b</sup>
Virus detected in urine, proportion of subjects					
BKV	0/23	15/146	2/49	11/182	.169 <sup>c</sup>
JCV	0/23	4/146	19/49	52/182	<.001 <sup>c</sup>

**NOTE.** OD<sub>492</sub>, optical density at 492 nm; VLP, virus-like particle.

<sup>a</sup> Comparison of BKV<sup>+</sup>/JCV<sup>-</sup> and BKV<sup>+</sup>/JCV<sup>+</sup>, by Mann-Whitney *U* test.

<sup>b</sup> Comparison of BKV<sup>-</sup>/JCV<sup>+</sup> and BKV<sup>+</sup>/JCV<sup>+</sup>, by Mann-Whitney *U* test.

<sup>c</sup> Comparison of individuals who shed virus, by  $\chi^2$  test.



**Figure 4.** Virus-like particle (VLP)-specific Ig levels in serum samples from 400 healthy blood donors evaluated for BK and JC polyomavirus (BKV and JCV). BK-VLP-specific and JC-VLP-specific IgG levels (*A*) and IgM levels (*B*) in samples were plotted against each other, and regression analysis was performed to check for statistical evidence of cross-reactivity. Note that log scales differ in *A* and *B*. *C*, Comparison of BK-VLP-specific IgG levels between donors who had BKV serotype 1 in urine and donors who had non-serotype 1 (i.e., serotypes 2–4) BKV in urine. *D*, BK-VLP-specific IgG levels in donors with BKV serotype 1 in urine were further compared among BKV subtypes 1a, 1b-1, and 1b-2. Boxes span the interquartile range; the line within each box denotes the median, and whiskers indicate the minimum and maximum values. \**P* calculated by Mann-Whitney *U* test. \*\**P* calculated by Kruskal-Wallis test. OD<sub>492</sub>, optical density at 492 nm; R<sup>2</sup>, regression coefficient.

caution. The absence of IgM in practically all individuals who shed virus indicates that these events represent persistent or reactivated replication. Given the role of cellular immune control in other viral infections that have a propensity for latency and reactivation, such as cytomegalovirus infection, we suspect that these differences most likely reside in the efficiency of cellular immune surveillance [7, 34]. However, BKV and JCV are closely related to each other with 72%–81% nucleotide homology in the regions of the 5.1-kb DNA genome, yielding amino acid homologies of 59%–83% [35]. We observed that JCV shedding was significantly more common among individuals who were positive only for JCV, compared with those who were seropositive for both BKV and JCV, suggesting limited cross-protection from a BKV-induced immune response. A precedent for limited cross-protection has been established for herpes simplex type 1 and type 2, which are ~70% homologous to one another [36]. Moreover, cross-reacting CD8<sup>+</sup> T cells have been described recently for some closely related peptide epitopes in the large T antigen and in the VP1 capsid protein [37–39]. Because T cell immunity requires major histocompatibility complex presentation by infected cells or processing by antigen-presenting cells, we suspect that JCV shedding is not necessarily a sign of T cell

dysfunction but may instead reflect differences between BKV- and JCV-infected cells with respect to presentation, anatomic location, and/or accessibility to T cells (e.g., in mucosal sites) [6]. Our data suggest that male sex and older age may be contributory factors. In this respect, Drachenberg et al. noted that reducing immunosuppression in patients undergoing kidney transplantation who had JCV-mediated PVAN resulted in clearance of allograft involvement but not clearance of urinary JCV loads [21]. Clearly, further studies are needed to address these differences in the respective polyomavirus-host balance.

Our serological analysis indicated that there is little cross-reactivity between BKV and JCV IgG when baculovirus-expressed VLP are used as EIA antigens. This result is in line with VLP studies by Hamilton et al., Viscidi et al., and Stolt et al., who also included preabsorption studies [40–42]. We noted a higher BK-VLP IgG level in individuals who shed BKV serotype 1 compared with those who shed virus that was not serotype 1. Among those who shed serotype 1 virus, the individuals who shed subtype 1-b1—which was used to generate the BK VLP—showed higher levels of IgG activity. Of note, (sub)serotypes 1-b1 and 1-b2 only differ by 1 amino acid in the VP1 gene (position 1908). These differences suggest that the IgG response is highly specific for the re-



spective BKV subtype, leading to underquantification of IgG levels in response to other BKV (sub)types. Another explanation could be that secondary exposure to BKV subtype 1-b1 is more prevalent, and therefore the cognate antibody responses are boosted. Although none of the individuals who shed BKV subtypes other than 1-b1 were seronegative, our data indicate that further studies are needed to investigate the diagnostic and pathogenic relevance of BKV subtype-specific IgG. For JCV, only a single capsid serotype has been reported.

Urinary polyomavirus shedding among immunocompetent, healthy blood donors provided a unique opportunity to investigate the architecture of the regulatory NCCR of both BKV and JCV. In all but 1 case of JCV infection, the BKV and JCV NCCR were of archetype architecture. Earlier *in vitro* studies showed that rearrangements of the NCCR (rr-NCCR) arise rapidly during polyomavirus replication [43]. Typically, partial duplications (insertions) and deletions are found, which confer increased early gene expression and higher replication capacity. JCV and BKV with rr-NCCR have been detected *in vivo* in pathology samples of affected brain and transplant tissue, as well as in the cerebrospinal fluid and blood of patients with PML and PVAN, respectively [44]. In patients undergoing kidney transplantation, the emergence of rr-NCCR BKV in plasma is associated with higher plasma BKV loads and more advanced pathology both *in vivo* and *in vitro* [12, 45, 46]. In healthy individuals, little data is available on the prevalence of rearranged viruses. In urine samples from pregnant women, Markowitz et al. found predominantly archetypal BKV in 18 of 127 samples and predominantly archetypal JCV in 5 of 76 samples [47]. Thus, our data support the hypothesis that high-level replication *in vitro* or *in vivo* in severely immunocompromised patients seems to be linked to the frequent emergence of polyomavirus variants with rr-NCCR [44].

We could not detect BKV or JCV in plasma. Dolei et al. examined PBMC DNA from 205 blood donors and 26 workers in a hematology unit and reported that 21.6% of PBMCs tested positive for BKV, whereas JCV DNA was found in 0.9% by use of nested PCR [23]. By contrast, Ling et al., who did not use nested PCR, failed to detect BKV or JCV DNA in any of 30 blood samples; no BKV was found in urine samples but JCV was detected in 47% of samples and was more common in samples obtained from individuals >40 years old ( $P = .03$ ) [22]. Thus, we cannot exclude the possibility that more sensitive PCR protocols might allow the detection of low levels of polyomavirus DNA, but viral genome integrity, the cell types involved, and contamination needs to be addressed to elucidate the potential significance of such results [48].

In summary, our study provides important baseline data on polyomavirus infection and viral replication in healthy, immunocompetent individuals. These data indicate significant differences between BKV and JCV with respect to virus-host interaction and the epidemiology of infection, which may have an

impact on the pathogenesis of infection in immunocompromised patients.

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