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MAJOR ARTICLE

Rapid Dynamics of Polyomavirus Type BK in Renal Transplant Recipients

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Background. Polyomavirus type BK–associated nephropathy (PVAN) is an emerging cause of early renal transplant failure. No specific antiviral treatment has been established. Current interventions rely on improving immune functions by reducing immunosuppression. In patients with PVAN, a high BK virus (BKV) load is detectable in plasma. However, the relationship between BKV replication and disease is not well understood.

Methods. In a retrospective analysis of BKV plasma load in renal transplant recipients undergoing allograft nephrectomy ($n = 3$) or changes in immunosuppressive regimen ($n = 12$), we calculated viral clearance rates and generation times and estimated the loss of BKV-infected renal cells.

Results. After nephrectomy, BKV clearance was fast (viral half-life [$t_{1/2}$], 1–2 h) or moderately fast ($t_{1/2}$, 20–38 h), depending on the sampling density, but it was independent of continued immunosuppressive regimens. After changing immunosuppressive regimens, BKV was cleared with a $t_{1/2}$ of 6 h–17 days. Using the basic reproductive ratio, the efficacies of intervention ranged from 7% to 83% (mean, 28%; median, 22%).

Conclusion. The results emphasize that high-level BKV replication is a major pathogenetic factor that may have implications for genome rearrangements, immune evasion, and antiviral resistance.

Polyomavirus type BK–associated nephropathy (PVAN) affects 1%–10% of renal transplant recipients, with allograft failure as high as 80% [1–5]. Although PVAN likely results from multiple, partly complementary determinants [5, 6], intense immunosuppression is generally accepted as being the major risk factor [7]. Because specific antiviral treatments are not available, reducing immunosuppressive regimens is the current mainstay of intervention [7]. In the absence of intervention, PVAN relentlessly progresses to irreversible allograft failure with extensive fibrosis and tubular atrophy. Throughout these stages, a high BK virus (BKV) load has been detected in the plasma of renal transplant recipients [3, 8]. Interestingly, BKV plasma virus load has been reported to rapidly disappear after the surgical removal of renal allografts, regardless of the continuation of immunosuppressive regimens [5]. A decrease in BKV plasma load was also observed after a reduction in immunosuppressive regimens [3]. Thus, BKV virus load has been proposed as surrogate marker of PVAN, to guide diagnosis and the treatment response. However, the relationship between BKV replication and disease is not well understood [9]. In a retrospective analysis, we used mathematical modeling to analyze the BKV plasma load observed in patients after allograft nephrectomy and compared the results with those obtained after changes in immunosuppressive regimens.

METHODS

Renal transplant recipients were included in the retrospective mathematical analysis if sufficient longitudinal data were available. All BKV plasma load measurements were performed in the Transplantation Virology laboratory of the University of Basel, Switzerland, as described elsewhere [3]. For the purpose of analysis, the

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limit of detection was set at 2.69 $\log_{10}$ copies/mL. Error bars for individual data points were based on a coefficient of variation of 29.6, calculated from 168 polymerase chain reaction standard curves performed during this period.

Viral growth rates, doubling times, clearance rates, and half-lives were calculated according to the following formulas:

$$\ln(V_t) = \ln(V_0) + rt \ , \quad (1)$$

$$t_2 = \ln(2)/r \ , \quad (2)$$

$$\ln(V_t) = \ln(V_0) - ct \ , \quad (3)$$

and

$$t_{1/2} = \ln(2)/c \ , \quad (4)$$

where r denotes the exponential rate of viral increase (viral growth rate), c denotes the exponential rate of viral decrease (viral clearance rate), t_2 denotes the viral doubling time, and $t_{1/2}$ denotes the viral half-life. V_0 denotes the initial viral load, and V_t denotes the viral load after t time units. We used equations (1) and (3), solved for r and c, respectively, when only 1 or 2 consecutive sampling intervals (<4 data points) were available. Otherwise, we fitted a straight line to log-transformed virus load data to obtain the slope and the 95% confidence interval (CI) using Mathematica (version 4.1; Wolfram Research). The generation time is an estimate of the time needed to complete a full replication cycle. For a virus, this can be calculated by

$$t_g = 1/\delta + 1/c \ , \quad (5)$$

where $1/\delta$ is the average lifespan of an infected cell, and $1/c$ is the average lifespan of a virion. The basic reproductive ratio (R_0) is a measurement of the efficacy of an intervention on the reduction in viral replication. R_0 can be interpreted as the average number of secondary infected cells produced per primary infected cell [10]. For a lytically replicating virus such as BKV, which bursts from its host cell, we assumed a fixed delay of length d between the infection and viral burst. Thus, the formula is

$$R_0 = \exp(sd) \ , \quad (6)$$

where s (the slope of the virus load curve plotted on a $\log_e$ scale) represents either the net growth rate, r (if $s > 0$), or the net clearance rate, c (if $s < 0$) [11].

RESULTS

The BKV plasma load was determined in 3 renal transplant recipients undergoing allograft nephrectomy (figure 1*A* and table 1). Immunosuppressive regimens were discontinued in patient 3 but was continued in patient 1 for a simultaneous pancreas graft and in patient 2 to limit allosensitization before retransplantation. Many samples were obtained from patient 1 after surgical allograft removal, initially at 3-h intervals for the first 24 h and then daily for 4 days. After an initial 4-h increase in BKV plasma load, which was likely caused by surgical manipulations (the "washout phenomenon"), 2 consecutive intervals of viral decay could be observed. The first interval, which occurred 4–7 h after nephrectomy, yielded a clearance rate of 8.24 (±114%)/day, corresponding to an in vivo $t_{1/2}$ of 2 h, whereas the second interval, which occurred 7–10 h after nephrectomy, yielded a clearance rate of 15.4 (±61%)/day, corresponding to an in vivo $t_{1/2}$ of 1.1 h (range, 0.7–2.8 h). Averaging the data over the 6-h interval resulted in a clearance rate of 11.8 (±40%)/day ($t_{1/2}$, 1.4 h). In patient 2, the available BKV plasma loads were measured 1 week apart and yielded a clearance rate of 0.852 (±20%)/day ($t_{1/2}$, 20 h; range, 16–24 h). Fewer samples were obtained from patient 3; BKV plasma loads were determined 2 weeks apart. The clearance rate was 0.441 (±19%)/day, ($t_{1/2}$, 38 h; range, 32–47 h) In summary, fast (hours) or moderately fast (hours to 2 days) clearance rates were observed after allograft removal.

In 12 patients treated with a reduction in immunosuppressive regimens, a detailed analysis was performed of 16 intervals of viral decay (figure 1*B* and table 1). In patient 4, a clearance rate of 1.32 (±89%)/day was calculated between days 66 and 67 (line not shown; $t_{1/2}$, 13 h; range, 7–119 h). When we fitted a straight line to the data for days 84–151, an average clearance rate of 0.076 (95% CI, 0.093–0.06) was calculated, corresponding to a $t_{1/2}$ of 9 (95% CI, 7–12) days. In patient 5, 2 nonconsecutive intervals of viral decay were observed after dosages of cyclosporine A (CsA) and mycophenolate mofetil (MMF) were reduced: the first, between days 83 and 84, yielded a clearance rate of 3.01 (±39%)/day (line not shown; $t_{1/2}$, 5.5 h; range, 4–9 h); the second, between days 112 and 126, yielded a clearance rate of 0.515 (±16%)/day ($t_{1/2}$, 33 h; range, 28–39 h) with biweekly sampling. In patient 6, dosages of CsA, MMF, and prednisone (Pred) were reduced, and MMF was later replaced by azathioprine (Aza). BKV plasma loads were measured weekly and yielded a clearance rate of 0.750 (±22%)/day ($t_{1/2}$, 22 h; range, 18–29 h). In patient 7, 2 nonconsecutive intervals of viral decay were observed after the reduction of dosages of tacrolimus (Tac), MMF, and Pred. The first, between days 115 and 125, yielded a clearance rate of 0.592 (±20%)/day ($t_{1/2}$, 28 h; range, 23–35 h), and the second, between days 363 and 370, yielded a clearance rate of 0.546 (±31%)/day ($t_{1/2}$, 31 h; range,

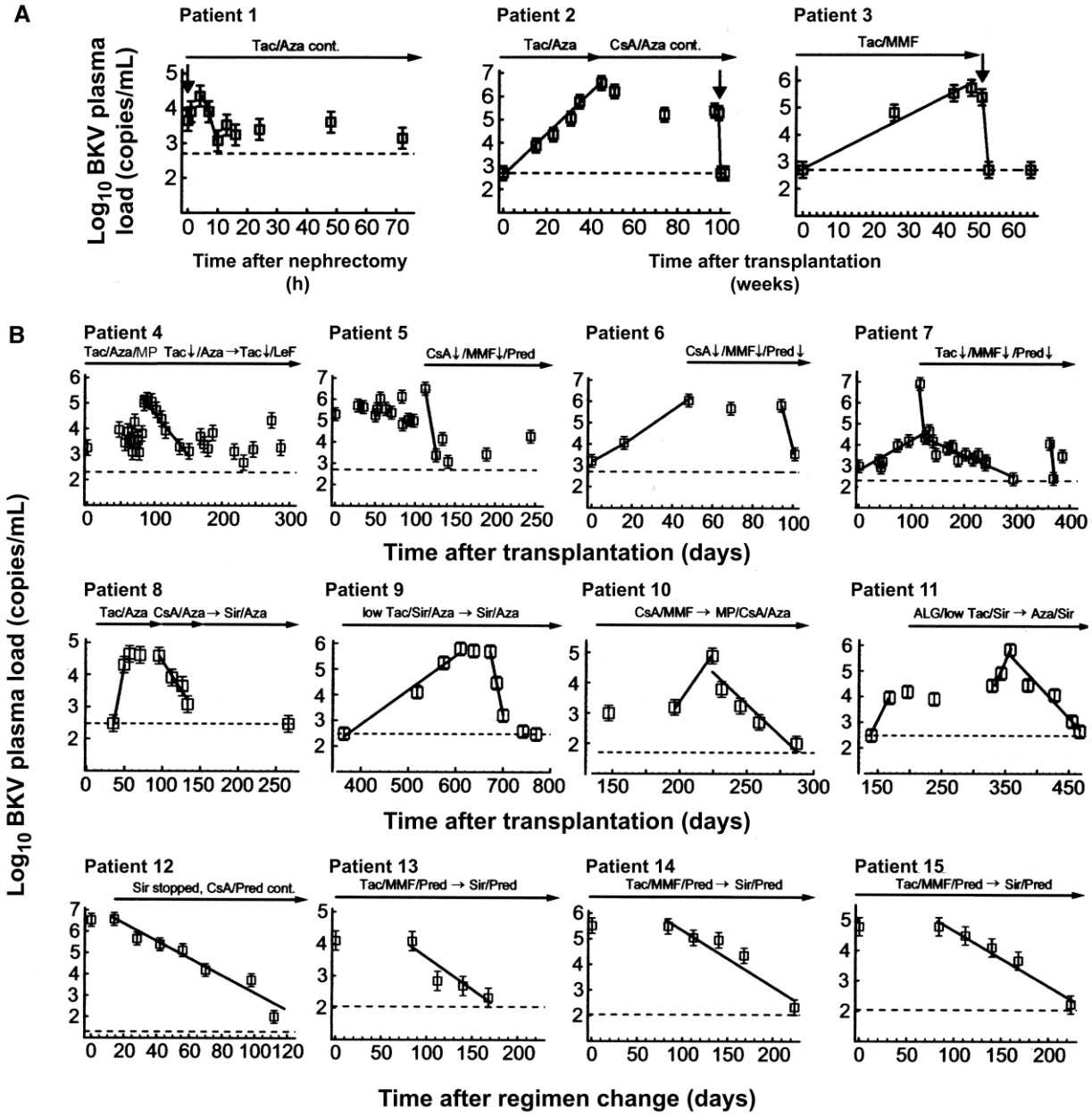

Figure 1. Longitudinal BK virus (BKV) load data and interventions. *A,* After allograft nephrectomy. *B,* After reduced immunosuppressive regimens. Vertical black arrows indicate the time of allograft removal. The horizontal arrow indicates the start of reduced immunosuppression. The dashed lines indicate the limits of detection. ↓, dose reduction; →, therapy change; ALG, antilymphocyte globulin; Aza, azathioprine; CsA, cyclosporine A; LeF, leflunomide; MMF, mycophenolate mofetil; MP, methylprednisolone pulse; Pred, prednisolone; Sir, sirolimus; Tac, tacrolimus.

23–44 h). When we fitted a straight line to the data for days 125–300, the average clearance rate was 0.024/day (95% CI, 0.032–0.017/day), with a $t_{1/2}$ of 28 days (95% CI, 22–42 days). In patient 8, Aza was continued and Tac was replaced by CsA, which yielded a clearance rate of 0.06/day (95% CI, 0.035–0.088/day), with a $t_{1/2}$ of 12 days (range, 8–20 days). In patient 9, switching from a low-dose regimen of Tac, sirolimus (Sir), and Aza to one of Sir and Aza only resulted in a clearance rate of 0.21/day (95% CI, 0.18–0.23/day), with a $t_{1/2}$ of 3.3 days (range, 3–4 days). In patient 10, switching from a regimen of CsA and MMF to CsA and Aza, combined with a methylprednisolone pulse around day 220, yielded a clearance rate of 0.094/day (95% CI, 0.035–0.16/day), with a $t_{1/2}$ of 7 days (range, 4–20 days). In patient 11, a low-dose Tac and Sir regimen was changed to one of Aza and Sir, which yielded a clearance rate of 0.083/day (95% CI, 0.025–0.14/day), with a $t_{1/2}$ of 8 days (range, 5–28 days). In patient 12, a triple regimen of CsA, Pred, and Sir was changed to a low-dose CsA and Pred regimen,

Table 1. Calculated BK virus (BKV) clearance rates and half-lives in vivo.

Patient group, no.	Sampling interval	BKV plasma load (range),[a] copies/mL	Clearance rate (range)/day	Error,[b] %	Viral half-life (range)	Intervention
Allograft removal						
1	0 h	22,220 (12,344–39,996)				Tac/Aza continued
	3 h	7959 (4422–14,326)	8.24 (pos–17.6)	114	2.0 h (1 h–pos)	
	6 h	1164 (647–2095)	15.4 (5.98–24.8)	61	1.1 h (0.7 h–2.8)	
2	0 days	195,247 (108,471–351,445)				CsA/Aza continued
	7 days	500 (278–900)	0.852 (0.658–1.02)	20	20 h (16–24 h)	
3	0 days	239,349 (132,972–430,828)				Tac/MMF stopped
	14 days	500 (278–900)	0.441 (0.357–0.525)	19	38 h (32–47 h)	
Without allograft removal but with reduced immunosuppressive regimens						
4	0 days	4549 (2527–8188)				Tac↓/Aza → LeF/Tac↓
	1 days	1220 (678–2196)	1.32 (0.14–2.49)	89	13 h (7–119 h)	
5	0 days	1,358,098 (754,499–2,444,576)				CsA↓/MMF↓/Pred
	1 days	66,998 (37,221–12,0596)	3.01 (1.83–4.18)	39	5.5 h (4–9 h)	
	0 days	3,245,286 (1,802,937–5,841,515)				CsA↓/MMF↓/Pred continued
	14 days	2402 (1334–4324)	0.515 (0.43–0.60)	16	33 h (28–39 h)	
6	0 days	669,832 (372,129–1,205,698)				CsA↓/MMF↓/Pred↓ → CsA↓/Aza
	7 days	3514 (1952–6325)	0.750 (0.58–0.918)	22	22 h (18–29 h)	
7	0 days	8,112,899 (4,507,166–14,603,218)				Tac↓/MMF↓/Pred↓
	10 days	21,715 (12,064–39,087)	0.592 (0.475–0.71)	20	28 h (23–35 h)	
	0 days	11,646 (6470–20,963)				Tac↓/MMF↓/Pred↓ continued
	7 days	255 (142–459)	0.546 (0.38–0.714)	31	31 h (23–44 h)	
8	0 days	39,251 (21,643–71,183)				Tac/AZA → CsA/Aza → Sir/Aza
	28 days	1206 (665–2187)	0.06 (0.035–0.088)	NA[c]	12 days (8–20 days)	
9	0 days	494,302 (27,256–896,431)				low Tac/Sir/AZA → Sir/Aza continued
	30 days	1608 (887–2916)	0.21 (0.18–0.23)	NA[c]	3.3 days (3–4 days)	
10	0 days	77,646 (42,815–140,813)				CsA/MMF → CsA/Aza/MP
	63 days	100 (55–181)	0.094 (0.035–0.16)	NA[c]	7 days (4–20 days)	
11	0 days	670,000 (369,445–1,215,065)				low Tac/Sir/ALG → Aza/Sir
	119 days	444 (245–805)	0.083 (0.025–0.14)	NA[c]	8 days (5–28 days)	
12	0 days	3,375,117 (1,875,065–6,075,211)				CsA/Pred/Sir → CsA/Pred
	112 days	100 (56–180)	0.093 (0.066–0.12)	NA[c]	7 days (6–11 days)	
13	0 days	12481 (6934–22,466)				Tac/MMF/Pred → Sir/Pred
	168 days	210 (117–378)	0.045 (0.014–0.10)	NA[c]	16 days (7–50 days)	
14	0 days	326,117 (181,176–587,011)				Tac/MMF/Pred → Sir/Pred
	224 days	214 (119–385)	0.051 (0.080–0.021)	NA[c]	14 days (9–33 days)	
15	0 days	60,303 (33,502–108,545)				Tac/MMF/Pred → Sir/Pred
	224 days	162 (90–292)	0.042 (0.027–0.057)	NA[c]	17 days (12–25 days)	

NOTE. ↓, dose reduction; → , therapy change; ALG, antilymphocyte globulin; Aza, azathioprine; CsA, cyclosporine A; LeF, leflunomide; MMF, mycophenolate mofetil; MP, methylprednisolone pulse; NA, not applicable; pos, positive for viral growth; Pred, prednisolone; Sir, sirolimus; Tac, tacrolimus.

[a] The BKV plasma load range was calculated on the basis of the coefficient of variation of real-time polymerase chain reaction (PCR).

[b] Related to the coefficient of variation of real-time PCR.

[c] Ranges of clearance rates and half-lives were calculated on the basis of the 95% confidence intervals.

Table 2. Calculated BK virus (BKV) growth rates and doubling times in vivo.

Patient no., period of consideration	BKV plasma load (range),[a] copies/mL	Growth rate (range)/day	Error,[b] %	Doubling time (range)
2				
0 days	500 (278–900)			
315 days	3,767,948 (2,093,304–6,782,306)	0.025 (0.019–0.031)	NA[c]	27 days (22–36 days)
3				
0 days	500 (278–900)			
336 days	529,693 (294,274–953,447)	0.019 (0.009–0.028)	NA[c]	37 days (25–77 days)
4				
0 days	1220 (678–2196)			
3 days	18,392 (10,218–33,106)	0.90 (0.51–1.30)	44	18 h (13–33 days)
0 days	1174 (652–2113)			
12 days	128,718 (71,510–231,692)	0.39 (0.29–0.49)	25	1.8 days (1.4–2.4 days)
5				
0 days	179,078 (99,488–322,340)			
6 days	10,96378 (609,099–1,973,480)	0.30 (0.07–0.53)	76	2.3 days (1–10 days)
6				
0 days	1593 (885–2867)			
47 days	1,124,186 (624,548–2,023,534)	0.14 (0.11–0.17)	18	5 days (4–6 days)
7				
0 days	1901 (1056–3422)			
49 days	811,289 (4,507,166–14,603,218)	0.17 (0.15–0.20)	14	4 days (3.6–4.7 days)
8				
0 days	300 (165–544)			
14 days	20,197 (11,137–36,628)	0.30 (0.22–0.39)	29	2.3 days (1.8–3.2 days)
9				
0 days	1000 (551–1813)			
245 days	631,424 (348,174–1,145,106)	0.026 (0.014–0.04)	NA[c]	27 days (17–50 days)
10				
0 days	1562 (861–2833)			
28 days	77,646 (42,815–140,813)	0.14 (0.097–0.18)	29	5 days (4–7 days)
11				
0 days	1901 (1056–3422)			
28 days	811,289 (4,507,166–1,460,3218)	0.10 (neg–0.38)	NA[c]	7 days (neg–2 days)

NOTE. NA, not applicable; neg, negative.

[a] The BKV plasma load range was calculated on the basis of the coefficient of variation of real-time polymerase chain reaction (PCR).

[b] Calculated from the coefficient of variation of real-time PCR.

[c] Ranges for growth rates and doubling times were calculated on the basis of the 95% confidence intervals.

which yielded an average clearance rate of 0.093/day (95% CI, 0.12–0.066/day), with a $t_{1/2}$ of 7 day (range, 6–11 days). In patients 13, 14, and 15, a regimen of Tac, MMF, and Pred was switched to a dual regimen of Sir and Pred. The average clearance rate in these patients was 0.046/day (95% CI, 0.1–0.014/day), with a $t_{1/2}$ of 15 days (95% CI, 7–50 days). Although there was no statistically significant difference between the clearance rates of patients 1–3 (allograft removed) and those of patients 4–7 (immunosuppressive regimens modified), the rates were significantly longer in patients 8–15 (2-sided $P < .01$, Mann-Whitney U test). This difference was partially due to methodological differences, because linear regressions provide average clearance rates. In summary, BKV clearance rates were moderately fast (hours to days) or slow (several days) in renal transplant recipients after immunosuppressive regimens were reduced (table 1).

Because high-level BKV replication accompanies host cell lysis in the release of infectious progeny, we estimated the loss of BKV-infected renal cells in vivo. Based on the data in table 1 and those of previous clinical studies [3, 8], we assumed a BKV plasma load of 10^4–10^6 copies/mL. This amounted to a total of 2.5×10^7–2.5×10^9 virions in a plasma compartment of 2.5 L. When we applied the fast clearance rate ($t_{1/2}$, 1 h; table 1), only 1–150 virions/day remained uncleared. Thus, in a steady-state situation, almost the entire BKV plasma load was generated every day. When we applied the moderately fast clearance

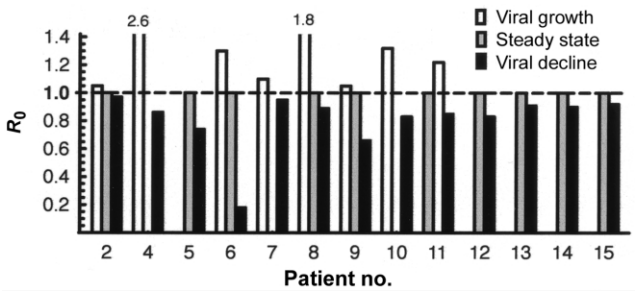

Figure 2. Efficacy of reduced immunosuppressive regimens. The basic reproductive ratio (R_0) is plotted during viral increase *(white bars),* steady state *(light gray bars),* and viral decrease *(dark gray bars).* For patient 4, the R_0 during viral growth was 2.6 ("rooftop value"). The dashed horizontal line at $R_0 = 1$ indicates the critical threshold value separating viral growth from decrease.

rate ($t_{1/2}$, 20 h), 1.09×10^7–1.09×10^9 virions were produced and cleared every day. To estimate the impact of this high in vivo turnover, we assumed a BKV burst size of 1×10^3–1×10^4 virions/lytically replicating host cell [12], which translates to 1×10^3–1×10^6 cells/day lysed through BKV replication alone. If this estimate of the number of infected cells is reasonably accurate, 4×10^4–4×10^8 BKV viruses are released from lysed tubular epithelial cells every hour.

Increases in BKV plasma load are of particular interest, because this may reflect the spread of BKV infection and the extent of tissue at risk. We found a median growth rate of 0.18/day (t_2, ~4 days), with the fastest being 0.90/day (t_2, 18 h) in patient 4 and the slowest being 0.019/day (t_2, 37 days) in patient 3 (table 2). In the densely sampled patient 1, a transient increase in BKV plasma load was noted in the first 4 h after nephrectomy, which was unlikely to have resulted from increased replication but may have been caused by surgical manipulations, as was described for Epstein-Barr virus load after resection of nasopharyngeal carcinoma [13].

On the basis of the rapid BKV clearance rate in vivo, corresponding to a $t_{1/2}$ of ~2 h, and the intracellular delay d (the viral eclipse phase between infection and the bursting of progeny virions) in vitro of 48 h [14], we calculated a BKV generation time of ~50 h. For a lytically replicating virus such as BKV, if one assumes a fixed value of d between infection and the bursting of progeny virions, the R_0 is calculated by $R_0 = \exp(sd)$ [11]. Applying a d of ~48 h, we calculated R_0 during viral growth ($R_0 > 1$) and decrease ($R_0 < 1$) after the respective interventions (figure 2). Three examples are discussed in more detail. In patient 2, the R_0 during the viral growth and decrease stages was 1.05 and 0.97, respectively. Therefore, switching from Tac and AZA to CsA and Aza around week 45 after transplantation had a 7% efficacy (R_0 = 1.04–0.97). In patient 4, we observed a sharp increase in BKV plasma load after methylprednisolone around day 80 ($R_0 = 2.6$) that was followed by a decrease in BKV plasma load over the course of 60 days ($R_0 = 0.86$), which indicated a 66% efficacy of switching from Tac, Aza, and Pred plus methylprednisolone to low-dose Tac and leflunomide (LeF) but 14% efficacy relative to that observed for the Tac, Aza, and Pred regimen before antirejection treatment with methylprednisolone. In patient 5, we observed a reduction in the R_0 of 26% (from 1 to 0.74) after the change in regimen around day 105 that did not persist; the R_0 returned quickly to ~1, although a lower level of BKV replication was maintained. In patient 6, we observed a reduction in the R_0 of 78% (from ~1 to 0.22) around day 95. In patient 11, viral growth ($R_0 = 1.34$) was observed after a steady state ($R_0 = 1$) and treatment of a steroid-refractory interstitial rejection with coexisting PVAN with anti-lymphocyte globulin and a switch from a Tac and Aza regimen to low-dose Tac and Sir. Viral growth decreased ($R_0 = 0.85$) after the switch to low-dose Sir and Aza. For patients 7–15, the respective efficacies were as follows: patient 7, 13%; patient 8, 11%; patient 9, 34%; patient 10, 37%; patient 11, 30%; patient 12, 17%; patient 13, 9%; patient 14, 10%; and patient 15, 8%. The mean (median) efficacy for all patients and regimens was 27% (17%). Under the assumption of an efficacy of 20% for reduced immunosuppressive regimens in a model patient with a quasi steady-state ($R_0 = 1$) BKV plasma load of 1×10^5 copies/mL, a viral generation time of 2 days, and a detection limit of 500 copies/mL, it would take ~7 weeks for the BKV plasma load to decrease to below the limit of detection. For a BKV plasma load of

Table 3. Contrasting BK virus (BKV) kinetics with other known viral kinetics.

Characteristic	BKV	SIV	HIV	HBV	HCV	EBV	CMV
Baseline plasma viral load, copies/mL	$\leqslant 10^7$	$\leqslant 10^8$	$\leqslant 10^7$	$\leqslant 10^{10}$	$\leqslant 10^8$	$\leqslant 10^5$	$\leqslant 10^7$
Clearance rate per day[a]	15[b]	~250[c]	~36[c]	~0.67[b]	5–10[c]	~8[b]	0.7[c]
Viral half/life	1–2 h[b]	~4 min[c]	~0.5 h[c]	19–38 h[b]	2–5 h[c]	~2 h[b]	$\leqslant$1 day[b]
Daily turnover,[d] %	>99	>99	>99	50	>99	>99	50

[a] Calculated by exponential decay slopes.
[b] Based on plasma viral DNA decay measurements.
[c] Based on plasma viral RNA decay measurements.
[d] Percentage of the total body virus population. References: simian immunodeficiency virus (SIV) [19]; HIV [17, 20]; hepatitis B virus (HBV) [21, 22]; hepatitis C virus (HCV) [20, 23, 24]; Epstein-Barr virus (EBV) [13]; cytomegalovirus (CMV) [25].

1×10^7 copies/mL, it would take 13 weeks for the virus load to decrease to below the limit of detection.

DISCUSSION

Mathematical models have contributed considerably to the understanding of viral infections in vivo, including those with hepatitis viruses, cytomegalovirus, and HIV-1 [15]. The common hallmark of these entities is progressive organ compromise through persistent viral replication in the setting of immune dysfunction. Although polyomavirus infections have not been studied so far, it is clear that the newly recognized PVAN shares these characteristics: it is a chronic progressive disorder accompanied by high BKV plasma loads in intensely immunosuppressed renal allograft recipients. In clinical practice, PVAN is still viewed as a slowly progressing disease, although the mean time from diagnosis to allograft failure is only 11 months. Our analysis indicated that rather rapid dynamics of BKV replication underlie the course of PVAN. Although effective BKV-specific antivirals are still lacking, the decrease in BKV plasma load after allograft nephrectomy provided a unique opportunity to obtain the first minimal estimates of BKV clearance as being fast ($t_{1/2}$, 1–2 h) or moderately fast ($t_{1/2}$, 20–38 h). In accordance with the data, we assumed that the replication base for BKV had been removed from the patient's body with the allograft nephrectomy. Of note, any residual replication would render these rates even faster. In patients with reduced immunosuppressive regimens, BKV clearance was moderately fast or slow, with $t_{1/2}$ ranging from 6 h to 17 days. This variability is not unexpected, given that different interventions were used and a number of complex factors may underlie this net decrease, including the individual net state of immunosuppression and the quality and quantity of BKV-specific immune effectors [16].

On the basis of the study of renal biopsies by Randhawa et al. [12], who reported that BKV-infected renal cells release, on average, 6000 virions, and on the viral clearance rates that we calculated, we estimated the daily tubular epithelial-cell loss resulting directly from BKV replication to be 1×10^3–1×10^6 cells. However, the overall impact on allograft function is likely to be underestimated, because this describes the average cytopathic aspect of PVAN without regarding the impact of focal tubular damage per nephron over the total of 1×10^6 renal nephrons and ignores other pathologic aspects of PVAN, such as immune-mediated damage.

We propose the basic R_0 as a measure to estimate the efficacy of complex interventions. The R_0 is intimately related to viral fitness in an individual transplant recipient [10, 15, 17]. The efficacies of reduced immunosuppression varied from 7% to 78% (mean, 28%; median, 22%), which are comparable to those of cidofovir and LeF in vitro [18]. Because it is independent of the plasma viral load, R_0 may prove to be an important and much-needed in vivo measurement for the evaluation of complex combined antiviral interventions in future clinical studies. If one assumes an average efficacy of 20% for an immunosuppressive regimen in a model patient with 1×10^5 or 1×10^7 BKV copies/mL at steady state, it would take ~7 or 13 weeks, respectively, for the BKV plasma load to decrease to below the limit of detection. These kinetics suggest that, for clinical management, biweekly monitoring in patients with reduced immunosuppression may be sufficient. However, we note that, after reducing immunosuppressive regimens, there may be a delay of 4–10 weeks in some patients before the BKV plasma load starts to decrease (e.g., patients 13–15 in figure 1*B*).

The limitations of our study include the varying sampling density, the relatively small sample size, and its retrospective nature. However, the results were derived from careful analysis of multiple intervals of viral decay. The estimated kinetics of BKV in PVAN lie in between the extremes of simian immunodeficiency virus [19] and HIV [17, 20], on the one side, and hepatitis B virus [21, 22], on the other (table 3), and are comparable to those of hepatitis C virus in chronic infection [20, 23, 24]. However, the smaller number of susceptible tubular epithelial host cells, in addition to the complex situation present with allograft, may explain the faster progression to end-stage kidney failure in PVAN, compared with that in chronic hepatitis C infection.

In conclusion, we report the first evidence (to our knowledge) of fast and moderately fast replication kinetics of BKV in renal transplant recipients. Our results emphasize organ cell damage as a major pathogenetic factor in PVAN. Finally, the fast BKV dynamics may explain the high frequency of BKV genome rearrangements, which are unusual for DNA viruses and may be important for immune evasion, antiviral resistance, and development of cancer.

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