Polyomavirus BK-Specific Immunity after Kidney Transplantation

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Failure to mount or maintain a protective immune response may influence the development of polyomavirus BK (BKV)-associated nephropathy (PVAN). However, limited data are so far available on BKV-specific immunity after kidney transplantation. BKV-specific cellular immune response was retrospectively analyzed in kidney recipients with or without BKV infection/reactivation by measuring the frequency of interferon (IFN)–γ-secreting cells in peripheral blood. Patients with BKV-active infection and good renal function (n=6) had a mean BKV-specific lymphocyte frequency 2 log lower than healthy controls and in the same range as BKV-seropositive recipients without active infection (n=7). Patients with PVAN (n=5) revealed undetectable levels of BKV-specific cells. However, two patients from the latter cohort treated with immunosuppression reduction showed the emergence of specific immunity, with IFN-γ production in the same range as healthy controls. Our preliminary data suggest that lack of protective immunity toward BKV may favor the occurrence of BKV active infection and influence the progression to PVAN.

Keywords: Kidney transplantation, Polyomavirus BK infection, Cellular immunity, IFN–γ-secreting lymphocytes, Serology.

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Polyoma BK virus-associated nephropathy (PVAN), a relevant cause of reduced allograft survival in kidney-transplant (KTx) recipients (*1–6*), occurs as the result of BK virus (BKV) infection/reactivation in the setting of immunosuppression (*6*). As demonstrated for other viral infections relevant to transplantation (*7*) and as suggested by the increased incidence of reactivation and clinical disease linked to the degree of immunocompromise (*1*), failure to mount a protective immune response may be a central event in the development of BKV-active infection and progression to the related pathology. However, as hypothesized for cytomegalovirus-related interstitial pneumonia (*8*), T cells may also play a negative role in the pathogenesis of graft damage caused by PVAN because interstitial inflammation has been described in patients with overt BKV nephropathy (*9*).

Our understanding of the immune response to BKV is limited. Although immunoglobulin G antibodies (Abs) specific for human polyomaviruses can be detected in up to 90% of adult population (*6*), the humoral immune response does not appear to play a major role in the containment of polyomavirus-related disease because anti-polyomavirus JC Abs are incapable of preventing progressive multifocal leukoencephalopathy (PML) (*10*), and KTx recipients with measurable anti-BKV Abs progress to BKV nephropathy. Conversely, the crucial role of T-cell immunity in the control of human polyomavirus infections is suggested by recent work underscoring a role for polyomavirus JC-specific cytotoxic T lymphocytes (CTL) in the containment of PML in affected humans (*10*, *11*). Studies of the cellular immune response against BKV have been scarce (*12*). In particular, no data are available on cell-mediated immunity after solid-organ transplantation. To explore the role of specific cellular immunity in the development of BKV-related disease, we retrospectively analyzed the frequency of BKV-specific, interferon (IFN)–γ-secreting lymphocytes in peripheral blood mononuclear cells (PBMC) of kidney recipients, stimulated with BKV-pulsed autologous dendritic cells (DC) in a enzyme-linked immunospot (ELISPOT) assay (*13*).

Eighteen kidney-allograft recipients with or without BKV infection/reactivation and PVAN were evaluated for BKV-specific immunity. The clinical characteristics of the patients are summarized in Table 1. Nine healthy individuals were also evaluated as controls. This study was performed in accordance with institutional guidelines, and patients or guardians gave informed consent. BKV nephropathy was diagnosed by the presence of intranuclear viral inclusion bodies in tubular epithelial cells and was confirmed by immunohistochemistry and quantitative polymerase chain reaction (PCR) for BKV performed on paraffin-fixed tissue section from renal biopsy. BKV DNA detection in serum, urine, and biopsy samples was performed by a previously described qualitative PCR assay (*14*). Samples found BKV DNA positive by the nested PCR were quantitated by a BKV-specific real-

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TABLE 1. Clinical characteristics of patients enrolled in the study

Pt. no.	Age at Tx	Baseline IS	IS at BKV dx	BKV serology R/D	Time Tx-BKV dx (months)	Time Tx-IE (months)	BKV-DNA at IE		Renal biopsy (grade[a]/BKV load)	Clinical outcome
							U	P		
1	2	CsA-PDN	CsA-PDN	+/+	na	+36	−	−		−
2	12	CsA-PDN	CsA-PDN-MMF	+/+	na	+44	−	−		−
3	9	tac-PDN	CsA-PDN	+/+	na	+24	−	−		−
4	13	CsA-PDN-MMF	CsA-PDN-MMF	+/+	na	+20	−	−		−
5	17	tac-PDN (+αCD25)	tac-PDN	+/+	na	+6	−	−		−
6	23	CsA-PDN-MMF	CsA-PDN-MMF	+/+	na	+5	−	−		−
7	6	CsA-PDN (+αCD25)	CsA-PDN	−/−	na	+4	−	−		−
8	12	CsA-PDN	CsA-PDN-AZA	+/+	+62	+86	+	−		Good GF
9	17	CsA-PDN-MMF	CsA-PDN-MMF	−/+	+18	+24	+	−		Stable GF
10	17	tac-PDN	tac-PDN	+/+	+7	+31	+	−		Good GF
11	13	tac-PDN-MMF (+αCD25)	tac-PDN-MMF	+/+	+3	+27	+	−		Good GF
12	5	tac-PDN (+αCD25)	tac-PDN	−/+	+1	+1	+	−		Good GF
13	15	tac-PDN (+αCD25)	tac-PDN-MMF	+/+	+20	+21	+	−		Good GF
14	9	CsA-PDN	CsA-PDN	−/+	+32	+55	+	+	Grade A 1550	Stable GF. sCr: 1.2
15	24	CsA-PDN-AZA (+αCD25)	CsA-PDN-AZA	+/−	+29	+31	+	+	ND	Stable GF. sCr: 1.9
16	15	CsA-PDN-MMF	CsA-PDN-MMF	+/+	+3	+4	+	+	Grade B 1320	Stable GF. sCr: 1.4
17	18	tac-PDN-MMF	tac-PDN-MMF	+/+	+4	+6	+	+	NE 2050	Stable GF. sCr: 1.4
18	18	tac-PDN (+αCD25)	tac-PDN	−/+	+1	+1.5	+	+	Grade B 3120	Stable GF. sCr: 1.5

[a] Grading: according to Drachenberg et al. (*18*).

Tx, kidney transplantation; IS, immunosuppressive regimen; BKV dx, diagnosis of BKV infection/reactivation or disease (first positive viral DNA); R, recipient; D, donor; IE, specific immunity evaluation; U, urine; P, plasma; CsA, cyclosporine A; PDN, prednisone; tac, tacrolimus; MMF, mycophenolate mofetil; αCD25, anti-CD25 monoclonal antibody; AZA, azathioprine; sCr, serum creatinine (mg/dL); na, not applicable; NE, not evaluable (unsuitable tissue sampling); ND, not performed because of patient refusal.

time PCR. The target of the quantitative reaction was the same 386 bp sequence amplified by the inner primers of the qualitative reaction. Patients' and donors' sera were titrated against BKV by hemagglutination inhibition (HI) test. HI titers 1:20 or greater were considered indicative of past infection.

ELISPOT assays to determine the frequency of IFN–γ-secreting PBMC were performed following a method previously described (*13*). Ninety–six-well multiscreen filter plates (MAIPS 4510, Millipore, Bedford, MA) were coated with 100 μL of primary Ab (IFN-γ, Mabtech, Nacka, Sweden) at 2.5 μg/mL and incubated overnight at 4°C. PBMC, isolated by Ficoll-Hypaque density gradient centrifugation and cryopreserved before use, were then seeded at 3×10^5/well or 1×10^5/well (depending on cell recovery) in the presence of irradiated DCs pulsed with BKV antigen (DC-BKV) or mock-antigen (E:S ratio 10:1) (*13*). Samples were assayed in triplicate. Controls included wells plated with DC-BKV alone. After incubation for 24 hours at 37°C, 100 μL of biotinylated secondary Ab (Mabtech, 0.5 μg/mL) was added, and plates were then processed according to standard procedure. The number of spots per well was calculated after subtraction of assay background, quantitated as an average of 24 wells containing only sterile complete medium, and specific background, quantitated as the sum of cytokine spots associated with responders alone, DC-BKV alone, and responders plated with DC pulsed with mock antigen. Median assay background was zero spots per well.

We sought to use a methodological approach that would allow a quantitative measurement of BKV-specific lymphocytes present in the peripheral blood at the time of analysis. Because the technique we previously developed (*13*), based on the use of repeated stimulations with BKV-pulsed DC before plating, might induce primary responses in otherwise naïve lymphocytes, we decided to perform an ELISPOT assay where responder cells consisted of previously unmanipulated PBMC. As shown in Figure 1, the mean frequency of BKV-specific, IFN–γ-producing cells in the seven BKV-seropositive healthy controls was $151/10^6$ cells (range 30–430), a magnitude approximately 10-fold less than that reported for other latent viruses relevant to transplantation such as Epstein-Barr virus (*15*). In the two BKV-negative controls and one BKV-negative KTx recipient, ELISPOT was always negative, demonstrating the specificity of this assay.

Patients with BKV-active infection and good renal function (group 2: viruria+/viremia−; n=6) had a mean BKV-specific lymphocyte frequency 2 log lower than healthy controls ($5.5/10^6$ cells; range 0–9) and in the same range as BKV-seropositive KTx recipients without evidence of active infection (group 1 viruria−/viremia−; n=6; frequency $4/10^6$ cells; range 0–9). The two BKV-seropositive patients without viral reactivation that were analyzed within the first 6 months after the transplant had undetectable levels of IFN–γ-secreting lymphocytes. One of these two patients, tested at 12 months, when immunosuppression load had been significantly decreased, showed a measurable frequency of BKV-specific cells (from $0/10^6$ cells to $9/10^6$ cells).

The five patients with PVAN (group 3: viruria+/viremia+) revealed undetectable levels of BKV-specific cells at the time of diagnosis (Fig. 1). However, two patients from the latter cohort, treated with immunosuppression reduction and evaluated prospectively, showed the emergence of specific immunity, with IFN-γ production in the same range as healthy seropositive controls (Figs. 1 and 2). The emergence of BKV-specific cellular immunity coincided with viral clearance in renal tissue or plasma, reduction of viral load in urine, and stabilization of renal function (Fig. 2). In accordance

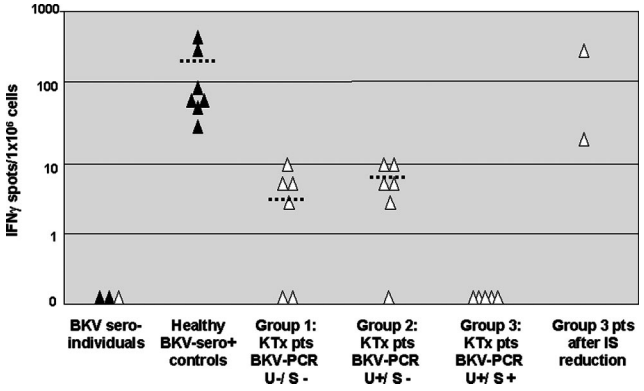

FIGURE 1. BK virus (BKV)-specific interferon (IFN)-γ production in healthy individuals and kidney transplant recipients. Results are reported as number of spots/10^6 peripheral blood mononuclear cells (PBMC) on a logarithmic scale. Evaluated groups: healthy individuals (*filled triangles*) seronegative or seropositive for BKV; kidney-transplant (KTx) recipients (*empty triangles*) without evidence of BKV infection/reactivation at the time of immunity assessment (group 1), with evidence of BKV infection/reactivation in urine exclusively at the time of immunity assessment (2 seronegative and 4 seropositive Tx recipients included in group 2) and patients with viral load in urine and blood and evidence of polyomavirus BK (BKV)-associated nephropathy (PVAN) at the time of immunity assessment (3 seronegative and 2 seropositive Tx recipients included in group 3). The column on the extreme right shows results obtained in two patients who belong to group 3, after immunosuppression (IS) reduction. PCR, polymerase chain reaction.

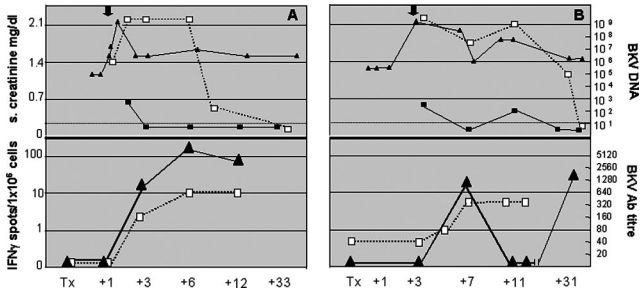

FIGURE 2. Viral load, serum creatinine, and BKV-specific immunologic parameters in cases 18 (A) and 16 (B). Horizontal axis in each frame: time course in months after renal transplantation. Upper frame in each panel: serum creatinine levels (mg/dL) (vertical axes on the left side; *filled triangles*) and viral load (copies/μL) (vertical axes on the right side) in urine (*empty squares*) and blood (*filled squares*). Time of diagnosis (*arrows* above the top panel). Lower frame in each panel: BKV-specific IFN-γ production (spots/10^6 cells) (vertical axes on the left side; *filled triangles*) and antibody (Ab) response (expressed as reciprocal titers) (vertical axes on the right side; *empty squares*).

with cellular immunity data, both patients seroconverted or increased BKV-specific Ab titer upon reduction of immunosuppression secondary to BKV infection. However, the latter patient, who had fluctuations of the frequency of BKV-specific IFN–γ-producing cells coincident with viremia relapses and variations of graft function, maintained sustained Ab levels throughout the follow-up, suggesting that, in a context of immunosuppression, variations of specific Ab titres may not be sufficiently sensitive to guide clinical decisions.

Two BKV-seronegative patients who experienced a primary infection after transplant were able to mount a protective cellular immune response that likely contributed to the containment of viral spread. However, the patient who developed an early primary BKV infection immediately after an acute rejection episode requiring steroid pulses (pt. 18) was at first unable to activate BKV-specific immunity and progressed to PVAN (Fig. 2A). Notably, notwithstanding the early presence of cellular immunity directed toward BKV, the other patient (pt. 12) did not show a concomitant seroconversion, and at 6 months from the onset of infection, BKV-specific Abs were still undetectable (data not shown).

Antigen exposure was not always able to induce significant expansion of the BKV-specific lymphocyte pool in the presence of high immunosuppressive load. Significantly, patients with BKV-associated nephropathy, whose antigen exposure was maximal, showed undetectable levels of BKV-specific IFN–γ-producing lymphocytes at diagnosis. This finding, in line with recent observations demonstrating a significant correlation between gene polymorphism linked to low IFN-γ expression and development of BKV nephropathy (*16*), seems to suggest that the degree of immunocompromise may be a crucial factor in the onset of PVAN.

It could be argued that the low/absent frequency of BKV-specific lymphocytes found in the peripheral blood of patients with PVAN at the time of diagnosis is the result of specific T cells homing at the site of BKV localization in the graft, suggesting a causative role for specific cellular immunity in the pathogenetic process leading to PVAN. However, the observation that, in patients from the PVAN cohort, the emergence of a measurable cellular immune response upon immunosuppression reduction leads to decrease in viral load and, more importantly, to stabilization of graft function, represents strong evidence against the hypothesis of a role for cellular immunity in the pathogenesis of the disease. On the basis of our preliminary data, an alternative scenario could be that the absence of specific immunity favored BKV spread in renal tissue, where viral antigens, presented in the context of an allogeneic environment, would elicit the vigorous cytotoxic response detected by gene-expression studies of PVAN biopsies (*17*), mainly mediated by human leucocyte antigen-unrestricted T cells. Indeed, in a recent study, where we demonstrated the feasibility to reactivate BKV-specific CTL for an adoptive immunotherapy approach to PVAN, we observed that BKV-specific CTL lines from KTx recipients with BKV-nephropathy displayed strong major histocompatibility (MHC)-unrestricted, BKV-directed killing, partly abrogated by depletion of CD3+/TCRγδ+ T cells (*13*). In this view, early infusion of autologous, MHC-restricted, BKV-specific CTL, following a strategy of preemptive cell therapy guided by BKV DNA levels (*4*) in combination with quantitative analysis of BKV-specific T cells, may counterbalance the increased risk conferred by defective development of BKV-specific immunity, with positive effects on the outcome of BKV-related disease. However, given the small number of patients included in our retrospective study, the value of specific cellular immunity for prediction of PVAN and to monitor treatment efficacy has to be further evaluated in prospective follow-up studies (*18*).

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