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Letter to the Editor

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BKV Replication and Cellular Immune Responses in Renal Transplant Recipients

To the Editor:

Hammer and colleagues report on BKV-specific T-cell responses in peripheral blood mononuclear cells (PBMC) of 15 viremic kidney transplant recipients (1). After stimulation with overlapping peptides spanning the viral capsid protein VP1, interferon-γ (IFNγ)-producing CD4+ T cells were observed in 7 patients (47%), including 2 patients (13%) with a CD8+ T-cell response. All patients with a detectable T-cell response had plasma BKV loads of >250 000 copies (c)/mL. Both patients with VP1-specific CD8+ T cells were the only ones to lose their grafts during follow-up. The authors concluded that high BKV load of >250 000 c/mL correlated with peripheral BKV-specific T-cell responses and that BKV-specific CD8+ responses indicated a risk for subsequent allograft loss (1).

Using ELISpot and flow cytometry for intracellular cytokine staining, we detected BKV-specific PBMC responses in renal transplant patients with plasma BKV loads clearly below 250 000 c/mL. In patient 1 (51 100 c/mL), both large T-antigen (LT)- and VP1-specific IFNγ-producing PBMC were detectable by ELISpot (542 and 392 SFU/10^6 PBMC). By flow cytometry, this response included LT- and VP1-specific CD4+ T cells (0.16% and 0.11%) and CD8+ T cells (0.04% and 0.06%), respectively. A similar BKV-specific cellular immune profile was found in patient 2 (300 c/mL). Patient 3 (944 c/mL) is remarkable for clear responses by ELISpot analysis which could not readily be attributed to CD4+ or CD8+ T cells. This discordance may reflect differences in sensitivity and/or an early phase of cellular immune effectors including NK-cells (2). In patient 4 (9600 c/mL), LT- and VP1-specific CD4+ T-cell, but no CD8+ T-cell responses were detectable. In patients 5 and 6 with persisting BKV viremia (38 400 and 98 000 c/mL), only very low responses were detectable by ELISpot, but none by flow cytometry. Overall, we found no correlation of BKV-specific cellular immune responses and the level of plasma BKV load. However, all patients with detectable cellular responses had a decline of BKV loads in the preceding 4–12 weeks. Interestingly, LT- and VP1-specific responses were not always concordant. Similarly, LT-specific T-cell responses were seen in patient 7 with stable allograft function after clearing BKV viremia and polyomavirus-associated nephropathy (PVAN) more than 12 months ago, and in patient 8 being on hemodialysis after allograft loss due to PVAN 15 months ago (Table 1).

In our series, VP1-specific CD8+ T-cell responses were not associated with poor allograft function. This discrepancy may be due to lower immune effector frequencies which, apart from technical differences, could reflect the stage of PVAN, immune reconstitution dynamics after reducing immunosuppression or other factors. Indeed, Hammer et al. were able to raise VP1-specific CD8+ T cells from allograft biopsies from patients without PBMC responses, all of whom maintained allograft function (1). It is unclear why the authors concluded that these cells originated in the donor rather than from efficient homing of recipient T-cells to the site of replication. It is well recognized that duration and levels of plasma BKV loads are important surrogates of viral tissue damage (3–5), which might also determine specific immune-mediated and possibly allospecific collateral damage. Thus, careful investigation of the dynamics of BKV replication, together with the qualitative and quantitative patterns of BKV-specific cellular immune responses, seems to be needed to elucidate the balance of virus and immune response and to identify the most informative tests.

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Table 1: Plasma BKV load and large T- and VP1-specific interferon-γ production in peripheral blood mononuclear cells

						PBMC		CD4+ T cells		CD8+ T cells	
		Serum creatinine		BKV load plasma	BKV replication	LT ELISpot	VP1	LT Flow cytometry	VP1	LT Flow cytometry	VP1
Patient	Age/Sex	(μmol/L)	Immunosuppression	(copies/mL)	dynamics	Δ log10	(IFNγ SFU/10^6 cells)	IFNγ (%)		IFNγ (%)	
1	36/F	113	Sir/MMF ↓	51 100	Decreasing	−3	542 392	0.16	0.11	0.04	0.06
2	61/F	141	Sir/MMF ↓	300	Decreasing	−3	97 373	0.04	0.10	0.02	0.07
3	26/M	104	CyA/Aza	944	Decreasing	−2	67 444	neg	neg	neg	neg
4	53/M	115	CyA/MMF/Pre	9600	Decreasing	−0.5	49 227	0.03	0.02	neg	neg
5	50/M	344	CyA/Tac/Lef	38 400	Stable	+0.3	10 13	neg	0.01	neg	neg
6	45/M	155	CyA/MMF/Pre	98 000	Stable	−0.2	37 156	neg	0.01	neg	neg
7	50/F	203	CyA/MMF/Pre	0	Cleared PVAN		75 276	0.02	0.07	0.09	0.07
8	65/M	Hemodialysis	No	0	Graft loss post-PVAN		20 47	0.03	0.11	0.02	0.05

Aza = azathioprine; CyA = cyclosporine; Lef = leflunomide; MMF = mycophenolate mofetil; Tac = tacrolimus; Pre = prednisone; Sir = sirolimus; PVAN = polyomavirus-associated nephropathy; LT = BKV large T-antigen; VP1 = BKV viral capsid protein-1; SFU = mean spot-forming units; IFNγ = interferon-γ; neg = negative (<0.01%).
BKV load was determined by real-time PCR as described with a cut-off of 300 c/mL plasma. ELISpots were performed in triplicate for interferon-γ detection after overnight stimulation of 2.5×10^5 PBMC with 2 μg/mL of 15-mer peptide pools of 11 amino acid overlap spanning LT or VP1 versus negative control medium. Intracellular cytokine staining for IFN-γ detection and flow cytometry was performed after 6 h stimulation with LT or VP1 peptide pools and addition of brefeldin A.

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