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BKV and JCV large T antigen-specific CD8+ T cell response in HLA A*0201+ kidney transplant recipients with polyomavirus nephropathy and patients with progressive multifocal leukoencephalopathy

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Abstract

Background—BK virus (BKV), which causes polyomavirus-associated nephropathy (PVN) in kidney transplant recipients (KTx), has 75% homology with JC virus (JCV), the etiologic agent of progressive multifocal leukoencephalopathy (PML). The large T-antigen (T-ag) is the main regulatory protein of polyomaviruses that is expressed early in the viral cycle.

Objectives—To characterize epitopes of BKV and JCV T-ag recognized by $CD8^+$ T-cells and explore the role of these cells in containing polyomavirus infection.

Study design—We tested peripheral blood mononuclear cells of HLA $A*0201^+$ BKV- and JCV-seropositive individuals, including patients with active BKV or JCV infection and healthy control subjects in a cross-sectional study.

Results—$CD8^+$ T-cells that recognized the nonamer BKV T_{p579}, which is identical to JCV T_{p578}, were detected by tetramer staining in 10/13 (77%) healthy individuals, 3/10 (30%) KTx/PVN, and 4/9 (44%) patients with PML and/or HIV-infection. Conversely, BKV T_{p398} and T_{p410}-specific $CD8^+$ T cells were detected in 3/13(23%) and 1/13(8%) healthy individuals only.

Conclusion—These data suggests that, as it is the case for the VP1 protein, the same population of $CD8^+$ T-cells may recognize epitopes located on the BKV and JCV T protein. The overall cellular

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immune response against polyomavirus T-ag, however, is lower than against the VP1 protein and is more frequently detected in healthy individuals than in patients with active BKV or JCV infection.

Keywords

BK virus; JC virus; large T antigen; cytotoxic T lymphocytes; tetramer; polyomavirus nephropathy; progressive multifocal leukoencephalopathy

Introduction

The cellular immune response to BK virus (BKV) VP1 is detectable in 80% of BKV-seropositive healthy individuals and plays an important role in the containment of BKV in kidney transplant recipients with polyomavirus nephropathy (KTx/PVN) (Binggeli et al., 2007, Chen et al., 2006, Krymskaya et al., 2005, Sharma et al., 2006). T-ag, which is the main viral regulatory protein, is the first viral protein to be expressed once BKV enters the host cell. Investigators have explored the immune response to BKV T-ag in healthy individuals (HI) or KTx recipients without PVN (Binggeli et al., 2007, Li et al., 2006, Provenzano et al., 2006, Randhawa et al., 2006). In this study, we investigated the specific cellular immune response against three BKV T-ag epitopes presented to $CD8^+$ T cells cytotoxic T lymphocytes (CTL) by the A*0201 molecule. One of these BKV epitopes had a sequence identical with a corresponding epitope of JCV. We report for the first time testing patients with progressive multifocal leukoencephalopathy (PML) for the presence of CTL against T-ag, and comparing their responses to those of KTx/PVN patients.

Methods

We studied 32 HLA-$A*0201^+$ individuals, including 13 healthy individuals (HI), 10 biopsy-proven KTx/PVN, and a group of 9 subjects (group PML/HIV), including 4 with PML (biopsy-proven or with CSF PCR positive for JCV: 3 HIV^+ and 1 HIV^-), and 5 HIV^+-patients with other neurological diseases. All study subjects were shown to be BKV and JCV-seropositive except one HI whose serology could not be assessed for technical reasons. All KTx/PVN and 10/13 HI were the same as those reported in our study of the CD8+ T-cell response against BKV VP1 protein (Chen et al., 2006), and were tested in parallel using the same fresh blood samples. A total of four nonamer peptides of BKV T-ag, p410, p398, p579 and p570, predicted by the published algorithms (http://bimas.cit.nih.gov/molbio/hla_bind/, and http://www.syfpeithi.de) to bind the HLA-A*0201 molecule were synthesized, and the respective tetramers were constructed, as previously described(Du Pasquier et al., 2003, Koralnik et al., 2002). BKV T_{p579} LLLIWFRPV and T_{p570} ILQSGMTLL had complete homology with the corresponding epitopes of JCV T_{p578} and T_{p569}, while BKV T_{p398} CLLPKMDSV and T_{p410} FLHCIVFNV had 2 and 3 amino acid (aa) difference with JCV T_{p397} CLLP*Q*MD*T*V and T_{p409} FL*K*CIV*L*N*I*, respectively.

HLA typing, hemagglutination inhibition assay, ^{51}Cr functional lysis and tetramer staining assays, and quantitation of BK viral load DNA by quantitative PCR were performed as previously described(Chen et al., 2006).

Results

No tetramer staining of $CD8\alpha\beta^+$ T-cells from any of the fresh blood samples was observed (data not shown). However, after 10–14 days of *in vitro* stimulation in the presence of peptide, $CD8^+$ T-cells recognizing BKV T_{p579}, T_{p398} and T_{p410} were detected in 10, 3 and 1 of 13 healthy HI, respectively, while only $CD8^+$ T-cells recognizing T_{p579} were detected in 3/10 KTx/PVN and 4/9 of the PML/HIV group. No study subjects had a T_{p570} response (Table 1).

The percentage of $CD8^+$ T-cells staining with the tetramers was low in all groups, between 0.2 and 3.5 % (Figure 1).

In KTx/PVN, the percentage of cells staining with T_{p579} tetramer was comparable to the results obtained in the same subjects with BKV $VP1_{p44,}$ but lower than for $VP1_{p108}$ (patients 2,3 and 10, Fig 4 (Chen et al., 2006)). In HI the T-ag-specific response was globally lower than the VP1-specific response (Chen et al., 2006) in 7/13, comparable in 5/13, and in one subject only a T-ag, but no VP1-specific response, was detected. Finally, in the PML/HIV group the T-ag response was always at least one log below that of the VP1 response (data not shown).

As shown in table 1, 12/13 HI (92%) had $CD8^+$ T cells recognizing either one or more of the T epitopes tested, which was significantly higher than 3/10 (30%) KTx/PVN ($p<0.01$), and 4/9 (44%) HIV+/PML group ($p=0.02$) (Fisher's exact test, 2 tail). These data suggest that in immunocompromised patients, the cellular immune response against T-ag is lower than in HI regardless of the antigenic stimulation associated with an elevated BKV viral load in KTx/PVN and JCV viral load in the HIV+/PML group.

We then performed ^{51}Cr lysis assays using the same peptide-stimulated peripheral blood mononuclear cells (PBMC) from HI and KTx/PVN. The results were negative in all cases, commensurate with the low percentages of tetramer staining cells after *in vitro* stimulation. However, an enriched T_{p579}-specific T-cell line from a HI (Fig 2, panel b), used as effectors, could lyse a T_{p579}-pulsed T2 cell line, which expresses the A*0201 molecule only, but not the M02 BLCL that is totally mismatched for MHC class-I alleles(Figure 2, panel c). These results demonstrate that T_{p579}-specific $CD8^+$ T-cells are functionally active CTL that are A*0201-restricted in their target cell recognition.

The three KTx/PVN with positive BKV T_{p579} tetramer staining had BK DNA viral load that was undetectable in serum and their viral load in urine was undetectable, 1.23×10^5/ml and 1.5×10^5/ml, respectively, as measured by quantitative PCR(Chen et al., 2006). These patients were tested at 90, 170 and 172 weeks after PVN diagnosis. Conversely, there was a trend for the viral load of eight patients with a negative BKV T_{p579} tetramer staining to be higher and ranged from undetectable to 5.83×10^5 copies/ml serum (mean:1.43×10^5) and from undetectable to 5.29×10^9 copies/ml urine (mean:1.25×10^9)($p=0.1462$, one way Anova). These patients were tested between 4 and 70 weeks after KTx/PVN diagnosis (mean: 17 weeks).

To explore further potential cross-reactivities between BKV and JCV CTL epitopes, we constructed A*0201 tetramers with the JCV T_{p397} and T_{p409} which have 2 and 3 amino acids different from the corresponding BKV T_{p398} and T_{p410}, respectively. No detectable tetramer staining was found in PBMC of 9 PML/HIV patients and 8 HI tested which suggest that these JCV peptides are not CTL epitopes.

Discussion

Our results confirm that T-ag is a target of the cellular immune response (Binggeli et al., 2007, Comoli et al., 2004, Koralnik et al., 2001, Li et al., 2006, Provenzano et al., 2006, Randhawa et al., 2006, Tong, 2006). Provenzano, et al. have also identified the BKV T_{p579} epitope in $A*0201^+$ healthy BKV-seropositive individuals and shown that a T_{p579}-specific cell line could lyse a human melanoma cell line (HBL) transfected with BKV Tag despite low CTL precursor frequencies, estimated to be around 1/20,000 by limiting dilution analysis. This result suggests that BKV T_{p579}/JCV T_{p578} can be naturally processed and presented to the cell surface, where it can be recognized by p579/p578-specific CTL. That low frequency of CTL is consistent with our present data showing that T_{p579}-specific $CD8^+$ T-cells were undetectable by tetramer staining *ex vivo* in fresh blood samples of all our study subjects, including KTx/PVN. Furthermore, a CTL response against this epitope could only be detected by Provenzano

and colleagues after 3 rounds of *in vitro* stimulation of PBMC with peptide-loaded autologous dendritic cells over 21 days, and using a T_{p579}-specific $CD8^+$ T-cell line in our study (Fig 2C).

Our data suggest that while A*0201-restricted CTL epitopes are present in BKV and JCV T-ag, they do not elicit a stronger CTL response compared to previously identified epitopes of the major capsid protein, VP1 (Chen et al., 2006, Du Pasquier et al., 2003, Koralnik et al., 2002, Koralnik et al., 2001). This finding has also been reported by a recent study by Binggeli (Binggeli et al., 2007) who examined the global T cell response to BKV T and VP1 proteins using libraries of overlapping peptides, and found lower responses against Tag compared to VP1. Interestingly, the most frequently recognized epitope, BKV T_{p579}, is identical to the corresponding epitope of JCV T_{p578}. This cross-recognition of BKV and JCV epitopes by CTL was already demonstrated in the VP1 protein (Chen et al., 2006, Krymskaya et al., 2005, Sharma et al., 2006). Since most adults are seropositive for both viruses, a correct interpretation of the CTL response depends on the baseline condition of the patients. In PML, the importance of the CTL response is indicated by the correlation of detectable cellular immunity and outcome of PML, indicating that the functional significance of this response is directed against JCV (Du Pasquier et al., 2004). In KTx/PVN, a strong anti-polyomavirus CTL response is associated with a lower BK viral load and antibody titers, suggesting that these cells are directed against BKV (Chen et al., 2006). Finally, it is safe to assume that dually JC and BK-infected individuals have responses against antigens present in both viruses.

In KTx/PVN, the T_{p579}–specific CTL could only be detected more than a year after PVN diagnosis. A longitudinal study of KTx/PVN, which is outside of the scope of the present study, will be needed to determine whether BKV T_{p579}-specific CTL may be part of a chronic, rather than an acute, response against BKV, and to understand how they participate in the control BKV replication and disease outcome.

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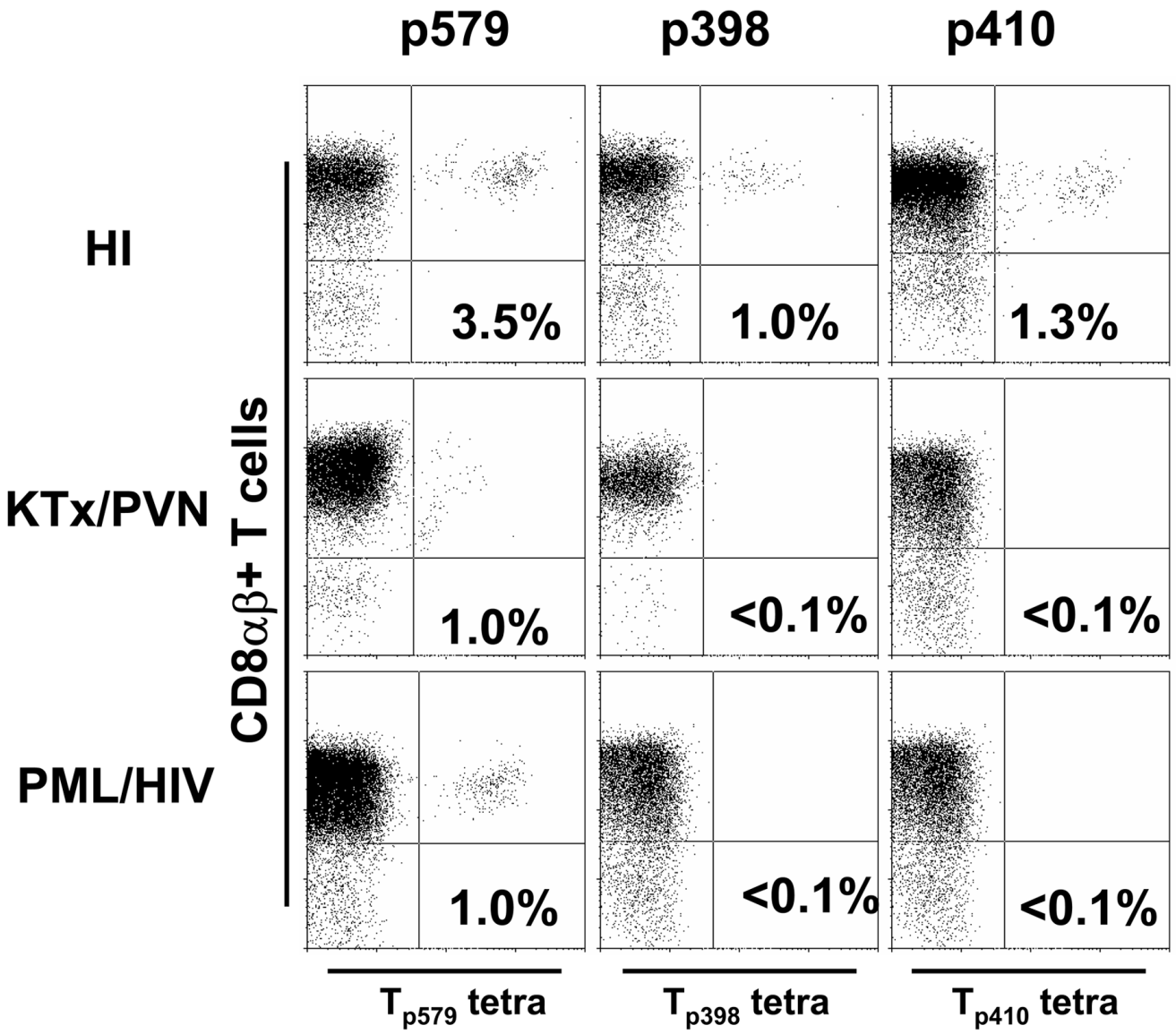

Figure 1.

Staining of PBMC from an HLA A*0201^{+} healthy individual, a PML patient and a KTx/PVN patient with tetrameric HLA-A*0201/BKV $VP1_{p579,}$ $VP1_{p410}$ and $VP1_{p398}$ complexes after *in vitro* stimulation with the respective peptides for 10–14 days. The percentages of CD8αβ^{+} T cells that bind the tetramers (dots in right upper quadrant of each panel) are indicated. Results were considered positive if the percentage of tetramer staining cells was equal or greater to 0.1% of CD8αβ^{+} T cells and formed a distinct population of cells on the dot plot.

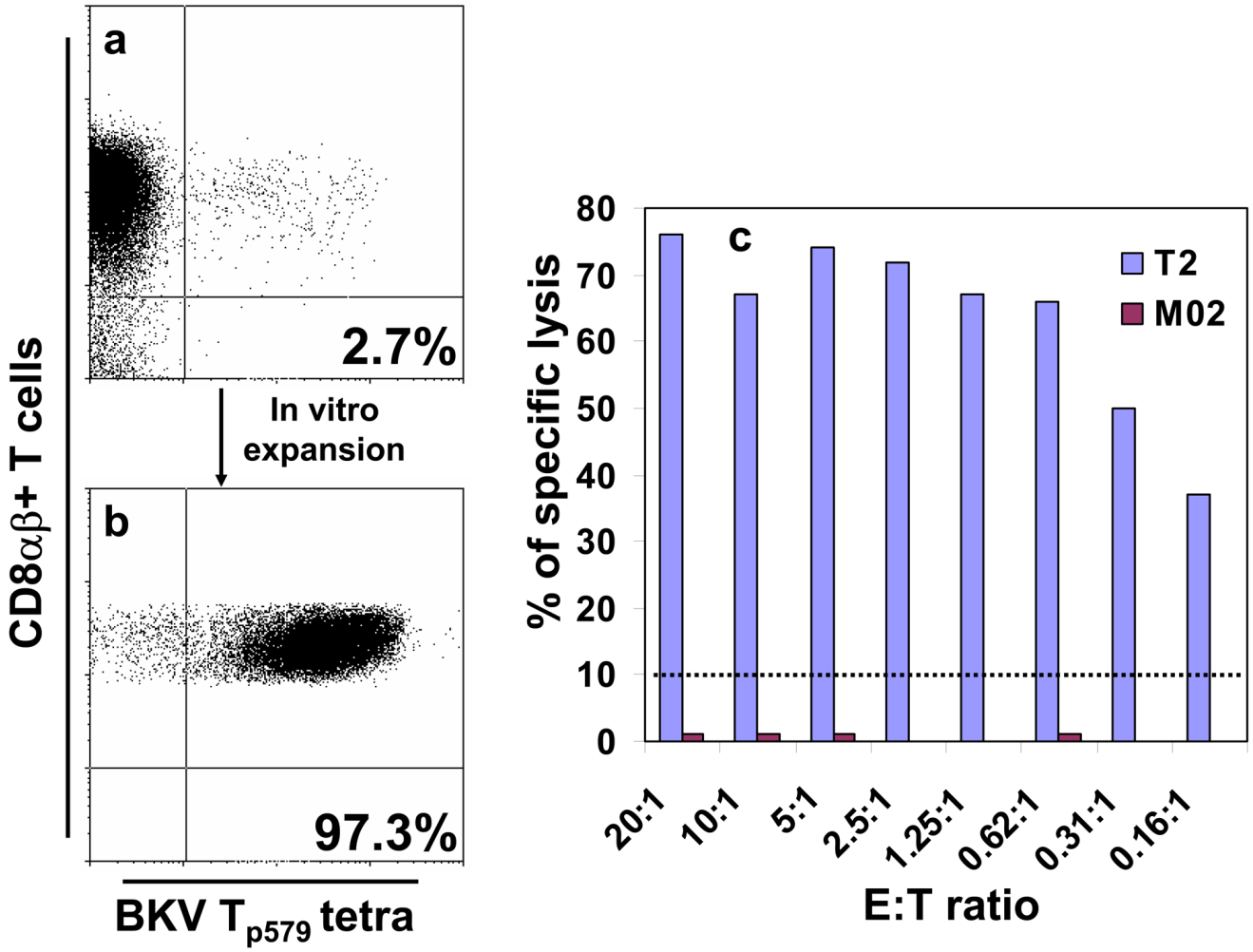

Figure 2.

Effector cell recognition of the BKV T_{p579} peptide is HLA-A*0201-restricted. BKV T_{p579}-stimulated PBMC from an HLA A*0201^+ healthy individual were stained with the respective tetramer, gating on CD8αβ$^+$ T cells. A small population comprising 2.7% of tetramer-binding $CD8^+$ T cells (panel a, right upper quadrant) was sorted by flow cytometry and propagated in vitro for 2 months. A 97.3% enriched BKV T_{p579}-specific CTL cell line (panel b, right upper quadrant) could lyse the p579-pulsed T2 cell line, expressing the A*0201 class I molecule only, but not the p579-pulsed M02 BLCL (totally mismatched for HLA class I alleles with the p579 CTL line) (panel c)

Table 1

Immunological, virological and serological data in 32 study subjects

Subjects		T-ag[a]				VL (copies/ml)		HAI titer		Weeks after diagnosis
		p579	p410	p398	p570	Urine	Plasma	BKV	JCV	
						(BKV)				
HI	#1	−	−	−	−	−	NA	1/512	1/32	
	#2	0.8%	−	−	−	−	NA	1/1024	1/32	
	#3	3.5%	1.3%	0.4%	−	−	NA	1/512	1/32	
	#4	0.2%	−	−	−	−	NA	1/256	1/32	
	#5	0.5%	−	−	−	+	NA	1/512	1/32	
	#6	0.3%	−	−	−	−	NA	1/256	1/32	
	#7	0.5%	−	−	−	−	NA	1/2048	1/16	
	#8	1.2%	−	−	−	−	NA	1/512	1/64	
	#9	−	−	1.0%	−	+	NA	1/256	1/64	
	#10[b]	0.8%	−	−	−	NA	NA	NA	NA	
	#11	0.2%	−	−	−	−	NA	1/1024	1/64	
	#12	0.4%	−	−	−	NA	NA	1/2048	1/128	
	#13	−	−	0.2%	−	NA	NA	1/256	1/64	
Total		77%	8%	23%	0%					
						(BKV)				
KTx/PVN	#1	−	−	−	−	−	−	1/16384	1/1024	6
	#2	0.7%	−	−	−	−	−	1/16384	1/64	90
	#3	1.3%	−	−	−	1.23×10^5	−	1/1024	1/256	172
	#4	−	−	−	−	4.51×10^5	1.21×10^5	1/131072	1/256	11
	#5	−	−	−	−	5.29×10^9	5.83×10^5	1/16384	1/64	5
	#6	−	−	−	−	3.60×10^5	5.69×10^3	1/262144	1/256	7
	#7	−	−	−	−	5.08×10^5	−	1/131072	1/256	16
	#8	−	−	−	−	2.22×10^9	1.31×10^3	1/65536	1/32	4
	#9	−	−	−	−	4.67×10^7	7.94×10^3	1/32768	1/128	70
	#10	1.0%	−	−	−	1.95×10^5	−	1/131072	1/128	170

Subjects		T-ag[a]				VL (copies/ml)		HAI titer		Weeks after diagnosis
		p579	p410	p398	p570	Urine	Plasma	BKV	JCV	
Total		30%	0%	0%	0%					
						(JCV)				
HIV+/PML	#1[c]	0.3%	−	−	−	−	−	1/512	1/2048	40
	#2[d]	−	−	−	−	−	−	1/1024	1/2048	728
	#3[c]	0.3%	−	−	−	7.5×10^{3}	−	1/512	1/512	12
HIV−/PML	#1[d]	−	−	−	−	−	−	1/64	1/256	780
HIV+	#1	1.0%	−	−	−	2.7×10^{7}	−	1/64	1/128	
	#2	0.2%	−	−	−	−	−	1/512	1/32	
	#3	−	−	−	−	2.27×10^{8}	−	1/128	1/64	
	#4	−	−	−	−	1.08×10^{7}	−	1/256	1/64	
	#5	−	−	−	−	−	−	1/256	1/16	
Total		44%	0%	0%	0%					

T-ag: BKV large T antigen, VL: BKV or JCV viral load, NA: not available, +: positive using qualitative PCR, −: undetectable.

[a]: percentage of tetramer positive $CD8^{+}$ T-cells,

[b]: serology couldn't be performed for technical reason.

[c]: JCV CSF PCR-proven PML.

[d]: biopsy-proven PML