ELSEVIER

Journal of Clinical Virology 42 (2008) 198–202

JOURNAL OF CLINICAL VIROLOGY

www.elsevier.com/locate/jcv

Short communication

# 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

Yiping Chen [a], Jennifer Trofe [b,1], Jennifer Gordon [c], Patrick Autissier [a], E. Steve Woodle [b], Igor J. Koralnik [a,d,*]

[a] *Division of Viral Pathogenesis, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States*
[b] *Division of Transplantation, University of Cincinati, United States*
[c] *Department of Neuroscience, Center for Neurovirology, Temple University School of Medicine, Philadelphia, PA, United States*
[d] *Department of Neurology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, United States*

Received 23 July 2007; received in revised form 2 January 2008; accepted 3 January 2008

**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 suggest 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 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

## 1. 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

* Corresponding author at: Division of viral Pathogenesis, Beth Israel Deaconess Medical Center, Harvard Medical School, RE 213C, 330 Brookline Avenue, Boston, MA 02215, United States. Tel.: +1 617 667 1568; fax: +1 617 667 8210.
*E-mail address:* ikoralni@bidmc.harvard.edu (I.J. Koralnik).
[1] Present address: Department of Pharmacy Service, Hospital of the University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104-4322, United States.

1386-6532/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jcv.2008.01.005

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.

## 2. Methods

We studied 32 HLA-A*$0201^+$ individuals, including 13 HI, 10 biopsy-proven KTx/PVN, and a group of nine subjects (group PML/HIV), including four with PML (biopsy-proven or with CSF PCR positive for JCV: three $HIV^+$ and one $HIV^-$), and five $HIV^+$ 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 two and three 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).

## 3. 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% (Fig. 1).

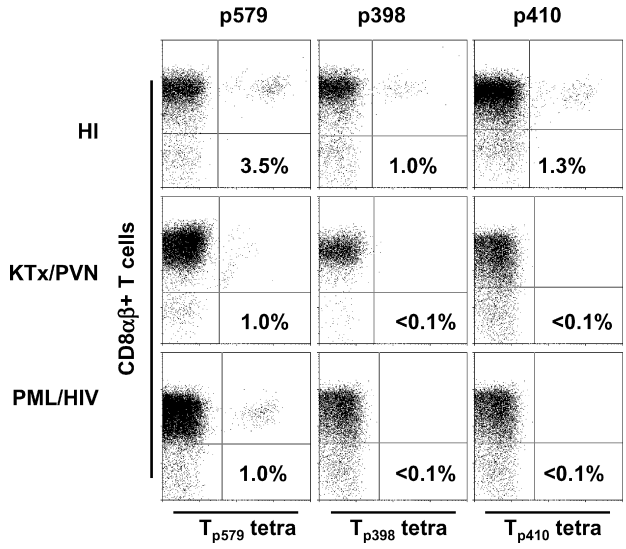


Fig. 1. Staining of PBMC from an HLA A*$0201^+$ healthy individual, a PML patient and a KTx/PVN patient with tetrameric HLA-A*0201/BKV $VP1_{p579}$, $VP1_{p410}$ and $VP1_{p398}$ complexes after *in vitro* stimulation with the respective peptides for 10–14 days. The percentages of $CD8\alpha\beta^+$ 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 $CD8\alpha\beta^+$ T cells and formed a distinct population of cells on the dot plot.

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 (Fig. 2,