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Quantitative analysis of BKV-specific CD4 + T cells before and after kidney transplantation☆

Esvet Mutlu [a,d,1], Sadi Köksoy [a,d,*,1], Derya Mutlu [a,2], Vural T. Yılmaz [b,d,2], Hüseyin Koçak [b,d,2], Ayhan Dinçkan [c,d,2], Gültekin Süleymanlar [b,d], Meral Gültekin [a,3]

[a] Department of Medical Microbiology, Division of Immunology, Akdeniz University School of Medicine, Antalya, Turkey
[b] Department of Internal Medicine, Division of Nephrology, Akdeniz University School of Medicine, Antalya, Turkey
[c] Department of General Surgery, Akdeniz University School of Medicine, Antalya, Turkey
[d] Transplantation Institute, Akdeniz University Medical Faculty, Antalya, Turkey

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ABSTRACT

Background: BK virus (BKV) is the main infectious cause of renal allograft dysfunction. Although recent studies showed an inverse correlation between BKV-specific T-cell responses and viral load after transplantation, the importance of pre-transplant response in the process of virus reactivation has only been studied once. In this study, we aimed to determine whether pre-transplant CD4 + T-cell response can be used for prediction of BKV reactivation and BKV nephropathy (BKVN), by a method that can practically be used in routine patient monitoring.
Methods: BKV-specific CD4 + T-cell responses of 31 kidney recipients (all from live donors) were measured by an IFN-γ-enzyme-linked-immunospot (ELISPOT) method using mixture of peptides, at day 0 and +1, +3, and +6 months posttransplant. Additionally, seven other reactivation patients as another group were also analyzed. BKV viral loads in plasma were measured by real-time polymerase chain reaction (PCR). Responses of 10 healthy people were also included as controls in the analysis.
Results: All but one patient and all of the controls had detectable CD4 + T-cell responses. Reactivation occurred in 8 out of 31 patients. There was no significant association between pretransplant BKV-specific CD4 + T-cell responses and BKV reactivation and between BKV DNA levels and CD4 + T-cell responses. In the additional group consisting of reactivation patients, four patients who had BKVN showed negative correlation between BKV-DNA levels and BKV-specific CD4 + T-cell responses ($p < 0.05$). One patient who developed BKVN, however, was not able to mount a similar CD4 + T-cell response to viral reactivation despite immunosuppressive reduction.
Conclusion: Even though our cohort is small, our results may suggest that pre-transplant measurement of BKV specific CD4 + T-cell response may not be necessary, and that post-transplant monitoring, particularly during reactivation, may be more helpful in the management of the infection.

1. Introduction

BKV has emerged as the most important infectious agent which occurs mostly within the first year after renal transplantation and is thought to be because of potent immunosuppressive therapies [1–3]. Reactivation of BKV can result in viruria in 30–50% and viremia in 13–22% of kidney transplant recipients [4]. Furthermore, BKVN might develop in 1–10% of these patients and lead to graft loss in 10 to >80% of them [5,6]. However, all latent infections do not seem to reactivate or progress to BKVN. There is no effective prophylaxis for BKV and the current management of BKV replication is reduction or modification of immunosuppressive regimen to restore or improve BKV-specific immunity [7–11]. Most transplant centers use virologic monitoring in plasma or urine to monitor renal transplant recipients for BKV replication [1,12–14]. However, it does not give any information on the host response to the virus thus making prediction of individual courses difficult.

Abbreviations: BKV, BK virus; BKVN, BK virus nephropathy; ELISPOT, enzyme linked immunospot; IFN-γ, interferon gamma; PBMCs, peripheral blood mononuclear cells; SFU, spot forming units.

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* Corresponding author at: Department of Medical Microbiology, Akdeniz University School of Medicine, Antalya 07070, Turkey.
E-mail addresses: dresvetmutlu@yahoo.com (E. Mutlu), sadikoksoy@akdeniz.edu.tr (S. Köksoy), drderyamutlu@yahoo.com (D. Mutlu), vuraltaneryl@yahoo.com (V.T. Yılmaz), hkocakautf@gmail.com (H. Koçak), adinckan@akdeniz.edu.tr (A. Dinçkan), gsuleymanlar@akdeniz.edu.tr (G. Süleymanlar), mgultekin@akdeniz.edu.tr (M. Gültekin).

[1] Participated in research design, performance of the research, and writing of the paper.
[2] Participated in the performance of the research.
[3] Participated in research design and writing of the paper.

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Today, BKV-specific T-cell immunity is proven to be very important in the control of BKV replication [15–18]. Previous studies showed association between the recovery of BKV-specific T-cell response and viral clearance in renal transplant recipients [1,15,16,19–22]. Therefore, in association with viral load determination, measuring BKV-specific T-cell response might be a useful tool in the management of BKV replication and assessment of response to therapy.

It is suggested that IFN-γ-ELISPOT assay is a simple and reliable method for measuring BKV-specific T-cell response [23]. In two previous studies, BKV-specific T-cell responses of renal transplant recipients were monitored by the ELISPOT method using five BKV antigens individually for stimulation of PBMCs [20,21]. A mixture of overlapping peptide pools including all five BKV antigens was first used for flow cytometric analysis of BKV-specific T-cells by Trydzenskaya et al. and this method was suggested to be a fast approach allowing a comprehensive assessment of total BKV-specific T-cell response [24].

2. Objective

We aimed to learn if we could determine renal transplant recipients at risk for BKV reactivation before transplantation by measuring antiviral CD4 + T-cell response. We also questioned if monitoring of BKV-specific CD4 + T-cell response prospectively posttransplantation might be helpful in determining the clinical outcome in patients with viral reactivation. In order to find answers to these questions, we measured BKV-specific CD4 + T-cell response in kidney recipients before and after transplantation by a modified ELISPOT method using a mixture of peptide pools.

3. Materials and methods

3.1. Patients

Thirty one kidney transplant recipients who had renal transplantation between 17 April 2012 and 27 September 2013 in Akdeniz University Transplantation Institute were enrolled in this study. All of these patients had living donors. Additionally, 7 other patients diagnosed with viral reactivation during follow-up were separately studied. Ten healthy donors were enrolled as the control group. The study was approved by the local Ethical Committee of Akdeniz University and all participants signed informed consent prior to enrollment into the study.

Kidney transplant recipients were divided into two groups according to BKV replication during follow-up. Group 1 included patients without BKV replication while group 2 included patients with viral reactivation. The 7 patients who participated later in the study formed group 3. Detailed information about patients and donors (age, sex, type of donor, donor age, donor sex, HLA mismatch, immune suppression protocol, induction therapy, cold ischemia time, delayed graft function) is shown in Table 1. All patients received calcineurin inhibitor + mycophenolic acid + corticosteroid combination therapy for immunosuppression. Fourteen patients received no induction therapy, while 17 were treated with antithymocyte globulin (ATG) or basiliximab.

3.2. Collection and storage of samples

Blood samples from 31 renal transplant patients were collected before and after (+1, +3 and +6 months) transplantation. Blood samples from 10 controls were collected once. From the 7 reactivation patients, who were later included in the study, blood samples were drawn at the time reactivation was first detected and then bi-weekly or monthly later on. From these patients, blood was collected at multiple times as follows: 2 patients were followed for 5 months, 2 patients were followed for 4 months and 3 patients were followed for 3 months after diagnosis.

Peripheral blood mononuclear cells (PBMCs) were isolated from 5–10 ml of heparinized blood using a Standard Ficoll-Hypaque density gradient technique and cryopreserved using RPMI 1640 medium supplemented with 1% L-Glutamine (Invitrogen, GIBCO, Paisley, UK), 20% Fetal Bovine Serum (FBS), 10% dimethylsulfoxide (DMSO) and Gentamicin.

3.3. Preparation of mixture of peptide pools

Peptide pools (15 amino acids in length with overlaps of 11 amino acids) of BKV early (LT and st) and late (VP1, VP2, VP3) antigens were purchased from JPT (Berlin, Germany) and stored at −20 °C as lyophilized powder until used. Peptides were dissolved in DMSO (Sigma, Steinheim, Germany) and aliquoted. In the ELISPOT assay, all five antigens were mixed, diluted with PBS and used at a final concentration of 1 μg/ml for each single protein (5 μg/ml in total).

3.4. ELISPOT assay

PBMCs were thawed and allowed to recover in medium for 1–2 h and checked for viability by trypan blue staining. PBMCs having a viability of 80% or more were plated into the ELISPOT assay. IFN-γ antibody coated 96 well multitest plates (MTP), washing buffer, alkaline phosphatase labeled secondary antibody and substrate solution containing BCIP/NBT were provided in the kit and the ELISPOT assay was performed according to the manufacturer's instructions (GenID GmbH, Germany). RPMI 1640 with 1% L-Glutamine (Invitrogen, GIBCO, Paisley, UK) supplemented with heat inactivated FBS 10% was used as assay medium. 2×10^5 PBMCs per patient per well were added in 100 μl RPMI medium in duplicate wells with a mixture of peptide pools. When the number of PBMCs was not enough, cells were plated at the maximum concentration allowed by postthaw recovery, keeping the number of cells per well constant for a given sample. Negative control (PBMCs plus medium alone) and Pokeweed mitogen (GenID GmbH, Germany) as positive control were always run for each sample in duplicate. Plates were incubated for 20 h at 37 °C in a 5% CO_2 incubator. After washing the wells 6 times with washing buffer, 100 μl detection antibody was added to each well and plates were incubated for 2 h at room temperature in a humid chamber. Washing was repeated and 100 μl of substrate solution was added and plates were incubated for 15 min for spots to be visualized. After development of spots, reaction was stopped by the addition of distilled water to all wells. Plates were then air dried and spots were counted using an automated plate reader, AID EliSpot (AID, Strassberg, Germany) with AID ELISPOT software version 7.0.

Samples with spot count numbers greater than 10 in the negative control wells and less than 50 in the positive control wells for 2×10^5 cells were excluded. Mean values obtained from the negative control wells were subtracted from the mean values of wells stimulated with the peptide pool mixture. The number of spots (spot forming units-SFU)/10^6 PBMCs was calculated for each sample.

3.5. BKV DNA PCR

BKV DNA loads in plasma were measured by real time PCR in a molecular diagnostics laboratory when these tests were ordered as part of routine screening by the physician. DNA extractions from plasma samples were made with an EZ1 Advanced XL using QIAamp EZ1 Virus Mini Kit (Qiagen, Hilden, Germany). Standard curves for the quantification of BKV were constructed using five, ten-fold serial dilutions of a plasmid containing the entire linearized genome of the BK virus Dunlop strain inserted into the Bam H1 restriction site of the pBR322 plasmid (pBKV, ATCC 45025). PCR primers for the BKV VP1 gene and dual labeled probe at the 5′ end with 6-carboxyfluorescein (FAM) and the 3′ end with 6-carboxytetramethylrhodamine (TAMRA), as described previously [25], were used for the amplification reactions. To ensure that negative results were not due to nonspecific inhibition of the PCR, each PCR reaction was spiked with a plasmid containing the glyceraldehyde 3 phosphate dehydrogenase gene and specific primers and dual