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HLA-A01-, -A03-, and -A024-binding nanomeric epitopes in polyomavirus BK large T antigen

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Abstract

Polyomavirus BK (BKV) infections are increasingly recognized. The development of immune-monitoring strategies against BKV requires definition of antigenic epitopes. Bioinformatic algorithms were used to identify 60 BKV large T-antigen(LT-Ag) peptides predicted to bind HLA class I alleles. In vitro peptide binding was used to select a subset of 19 peptides for interferon (IFN)- γ ELISPOT assays in 13 healthy subjects and 12 kidney transplant recipients. Four A01-, nine A03-, and five A24-binding immunogenic peptides were identified in 1 to 3 (14–67%) tested subjects in each group. BKV epitope sequences were identical to homologous JC virus sequences for 3 of 19 peptides and homologous SV40 sequences for 5 of 19 peptides. Homology modeling localized these epitopes to the helicase, origin of DNA binding, or J domains, respectively. In conclusion, we have identified multiple 9-mer BKV LT-Ag-derived immunogenic epitopes that bind HLA-A01, -A03, or -A24 molecules. Sequence alignments indicate that two epitopes, FLICKGVNK and RYWLFKGPI, are common to BKV, JC virus, and SV40 virus.

Keywords

BK virus; Immunity; Large T antigen; Epitopes

1. Introduction

BK polyomavirus (BKV) infects and establishes persistent asymptomatic infections in the majority of adult humans and reactivates following immunosuppression. BKV reactivation in kidney transplant (KT) recipients is evidenced by viruria or viremia. BKV is a small, double-stranded DNA virus with a 5-kb genome that shares ~70% homology with JC polyomavirus and monkey polyomavirus SV40. The sequence similarity of large T antigen (LT-Ag) is 90, 84, and 82% between BKV and JCV, BKV and SV40, and JCV and SV40, respectively [1]. Initial exposure to BKV probably occurs through the respiratory or oral route, followed by latency in the urogenital tract and hematopoietic system [2]. In the past 5–10 years, BKV has become an important cause of viral nephropathy in renal allografts [3], [4] and [5]. There is no widely accepted antiviral therapy available at this time. The mainstay of treatment is to reduce immunosuppression, thereby allowing the immune system to mount a successful antiviral defense.

The authors declare that they have no commercial or other association that might pose a conflict of interest with regard to this work.

Investigators have explored the immune response to BKV LT-Ag in healthy controls as well as KT recipients [6], [7], [8], [9] and [10]. Most studies have used peptide pools spanning the entire length of VP-1 or LT-Ag proteins and focused their attention on HLA-A02 restricted epitopes. Outside of HLA-A02, a limited number of peptides have been described that elicit interferon (IFN)- γ production in CD4-positive and CD8-positive T cells obtained from HLA-B07-, HLA-B08-, HLA-DRB1-03-, HLA-DRB1-09-, and HLA-DRB1-13-positive subjects [6]. We present data that tentatively identify several new HLA-A01-, -A03-, and -A24-binding LT-Ag epitopes that are recognized by healthy control subjects as well as immunosuppressed KT recipients.

2. Subjects and methods

2.1. Human subjects

HLA-A01-, -A03-, and -A24-positive healthy control subjects (n = 13) varied in age from 29 to 49 years old, with a median age of 40 years old, and were recruited according to institutional review board (IRB)-approved protocol (IRB No. 0608014). Serologic studies were performed as previously described [11] using BKV virus-like particle-based ELISA assays. Of 13 healthy controls, 9 were BKV seropositive. One subject was not tested because serum was unavailable (Table 1). The KT recipients (n = 12) were recruited under a separate protocol (IRB No. 000586) 7–74 months (median 13 months) posttransplant. Patient age varied from 25 to 75 years old, with a median age of 61 years old. Of 11 KT recipients tested, all were BKV seropositive. The BKV serologic status of 1 patient was not tested because serum was unavailable (Table 1). All patients received pretransplant induction with Campath (n = 9) or thymoglobulin (n = 3) and were subsequently maintained on tacrolimus immunosuppression therapy. At the time of blood draw, 2 patients had documented BK viremia and viruria, whereas 7 had viruria but no viremia and three had no detectable BKV DNA (Table 1). Peripheral blood mononuclear cells (PBMCs) were isolated from 30 ml of heparinized blood using the standard Ficoll Hypaque density gradient centrifugation technique [12].

2.2. Prediction and localization of HLA-A01, -A03, and -A24 restricted BKV LT-Ag-specific peptides

Virus-specific peptides were selected from a BKV LT-Ag sequence (GI 135280). This sequence was analyzed for the presence of peptides with predicted ability to bind to HLA-A01, -A03, and -A24 using three different algorithms: (a) SYFPEITHI (<http://www.syfpeithi.de/home.htm>), (b) BIMAS (http://www.bimas.cit.nih.gov/molbio/hla_bind/), and (c) artificial neural network (ANN) algorithm (<http://tools.immuneepitope.org>).

2.3. In vitro REVEAL major histocompatibility complex (MHC) peptide-binding assay

This commercially available assay was used to determine the ability of SYFPEITHI predicted peptides to bind to their corresponding MHC alleles (ProImmune, Inc., Bradenton, FL). The binding of each predicted peptide was compared with that of two control peptides. This assay was carried out in vitro using a 96-well plate, which contained the MHC protein and β -2 microglobulin components of the MHC molecule mixed with the peptide of interest. Binding detection was achieved by a labeled antibody that can only tag the correctly folded native conformation of the MHC–peptide complex. The signal generated by each peptide was given a score relative to the “pass/fail” control binder and compared with the positive control peptide, which was a known T-cell epitope. The score was reported as a percentage of the signal generated by the test peptide versus the positive control peptide, and the peptides were given a pass or fail result.

2.4. Bioinformatics analysis of epitope sequences

Peptides of interest were evaluated for amino acid conservancy across three different species of polyomavirus, namely BKV (strain Dunlop, UniProtKB/Swiss-Prot entry P03071), JCV (strain MAD 1, UniProtKB/Swiss-Prot entry P03072) and SV40 (strain 776, UniProtKB/Swiss-Prot entry P03070). Mapping of epitopes to functional domains was performed by a homology modeling approach. BKV LT-Ag (Swiss Prot P03071) was modeled with MODELLER9 v1 [13] using the available tridimensional structures of SV40 LT-Ag (PDB code 1 SVL) [14], respectively. The original model was refined by performing a moderate number (500, in 10 rounds of 50 steps) of adopted basis Newton–Raphson minimization steps in CHARMM [15]. The model was then analyzed with PROCHECK [16], which detected no geometrical errors.

2.5. BKV LT-Ag PepMix

A pool of peptides with >70% purity was purchased from JPT Peptide Technologies GmbH (Berlin, Germany) and used for in vitro stimulation. This PepMix contains a total of 170 15-mer peptides with 11-amino-acid overlaps spanning the 691 amino acid-long BKV LT-Ag coding sequence from BKV strain AS (UniProtKB/Swiss-Prot P14999). The peptides were chemically synthesized, purified, and analyzed by liquid chromatography–mass spectrometry (LC–MS). The PepMix was dissolved in 125 µl of DMSO (Sigma, St. Louis, MO), and 5 µl aliquots were stored at –20°C until use.

2.6. BKV LT-Ag 9-mer peptides

A total of 19 HLA-A01, -A03, and -A24 binding selected 9-mer peptides were obtained from Prospector PEPscreen (ProImmune) with purity of >90%, as analyzed by LC–MS. The peptides (10 mg/ml) were dissolved in DMSO (Cat. No. D2650, Sigma) and maintained at –20°C until use.

2.7. Preparation of BKV-infected cell lysates and mock cell lysates

BK virus-infected cell lysate and mock cell lysate were prepared using previously described methods [8]. In brief, Gardner strain, purchased from the American Type Culture Collection (ATCC VR837), was grown in human embryonic lung fibroblasts (WI-38 cells; ATCC CCL-75) using Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and L-glutamine and incubated at 37°C under 5% CO₂ for 5 weeks. Cells were harvested, subjected to three freeze–thaws, and centrifuged to obtain a soluble antigen fraction. Protein concentration was measured and equalized prior to testing.

2.8. In vitro stimulation and expansion of BKV-specific T cells

PBMCs were cultured in 24-well tissue culture plates at a density of 5 million cells/ml in RPMI 1640 medium supplemented with 2 mM L-glutamine, 10 mM HEPES, 100 IU/ml penicillin/streptomycin (Mediatech, Inc., Manassas, VA), 1 mM sodium pyruvate, 12% heat-inactivated fetal calf serum (Invitrogen, Carlsbad, CA), with and without BKV LT-Ag PepMix (at a final concentration of 1 µg/ml/peptide) or with mock- and BKV-infected cell lysate (at a final concentration of 10 µg/ml) at 37°C in a 5% CO₂ incubator. After 3 days, recombinant human interleukin (IL)-2 (Proleukin, Chiron, Emeryville, CA) was added to a final concentration of 30 IU/ml. Every 2 days thereafter, 50% of the culture medium was replaced with fresh medium containing IL-2. Incubation was continued for 13–15 days before IFN-γ ELISPOT assay.

2.9. IFN-γ ELISPOT assay

The frequency of functional IFN-γ producing BKV-specific T cells was measured by ELISPOT assay. Testing was performed in 96-well nitrocellulose-backed plates (Millipore,

Bedford, MA) precoated with anti-IFN- γ monoclonal antibody 1-D1K (10 μ g/ml, Mabtech, Cincinnati, OH). BKV LT-Ag activated PBMCs (1×10^5 /well) were added in duplicate wells, together with HLA-matched EBV-transformed B lymphoblastoid cell lines (B-LCL) expressing HLA-A01, -A03, or -A24 alleles (2×10^4 cells/well). These cells were preloaded with individual BKV peptides (10 μ g/ml), an irrelevant negative control peptide (10 μ g/ml), BKV-infected cell lysate, mock cell lysate (10 μ g/ml), or BKV LT-Ag PepMix (1 μ g/peptide/ml).

The plates were incubated for 18–24 hours at 37°C in 5% CO₂. Subsequently, the wells were washed and a second biotinylated anti-IFN- γ monoclonal antibody 7-B6-1 (2 μ g/ml, Mabtech, Cincinnati, OH) was added for an additional 2 hours at 37°C. The wells were washed and avidin–peroxidase complex (Vectastain ABC Kits, Vector Laboratories, Inc., Burlingame, CA) was added for 1 hour to develop the reaction. Individual cytokine-producing cells were detected as dark red spots after a 5-minute reaction with 3-amino-9-ethylcarbazole (Sigma, St. Louis, MO) solubilized with dimethylformamide, 50 mM acetate buffer, pH 5.0. The numbers of IFN- γ spots were counted using an automated ELISPOT plate reader (Immunospot, Cellular Technologies, Ltd., Cleveland, OH). In all experiments, subjects were designated responsive only if the observed ELISPOT counts were more than the background count plus two standard deviation. Background values were obtained from expanded T cells with irrelevant negative control peptide (which was always 0) or mock cell lysate (8 ± 2 per 105 cells) and subtracted from the values obtained with the specific peptides. In all experiments, results from ELISPOT assays are expressed as IFN- γ -secreting cells per 105 cells.

2.10. Statistical analysis

ELISPOT counts for individual peptides, PepMix, and whole lysates were tabulated, and mean and standard deviation for the reactive samples were calculated in a Microsoft Excel spreadsheet. Mean counts for each peptide were compared between healthy controls and kidney transplant patients using the Mann–Whitney rank sum test. Proportions of healthy and immunosuppressed subjects reacting to specific peptides were compared using χ^2 or Fisher's exact tests. Differences corresponding to $p < 0.05$ were considered statistically significant. All calculations were performed using SigmaStat for Windows, version 2.03 (San Rafael, CA).

3. Results

3.1. Peptide prediction by SYFPEITHI and in vitro peptide-binding assays

A total of 60 high-scoring (score = >18) BKV LT-Ag peptides for MHC class I alleles HLA-A01, -A03, and -A24 were predicted by SYFPEITHI. From these 60 peptides, 19 peptides (4, 10, and 5 peptides for HLA-A01, -A03, and -A24, respectively) were selected for ELISPOT analysis based on the results of an in vitro REVEAL MHC peptide-binding assay (Table 2). The actual MHC binding strength for each peptide is presented in Fig. 1, Fig. 2 and Fig. 3, as well as in Table 4, Table 5 and Table 6 as a percentage of relative pass/fail score. With the exception of peptides P270, P414, and P506, SYFPEITHI-predicted peptides were also successfully predicted by either the BIMAS or the ANN algorithm, and 6 peptides were predicted by all three algorithms. Homology modeling revealed that 9 of 19, 7 of 19, and 3 of 19 peptides are located at the helicase, the origin of DNA binding, and the N-terminal J domains, respectively. In addition, peptides P604 and P394 (on helicase domain) and peptide P235 (origin of the DNA-binding domain) were completely exposed on the surface of the LT-Ag molecule. Table 2 also includes a list of other BKV LT-Ag- and VP1-derived peptides that have been published to date.

Sequence alignments indicated that peptides P222 and P422 showed 100% homology with JCV and SV40 counterpart epitopes. Peptide P172 demonstrated 100% similarity to its JCV counterpart but only 78% homology with the SV40 epitope. Peptides P342, P506, P394, and P33 exhibited 100% sequence similarity to SV40, but only 78–89% homology to their JCV counterpart epitopes. The remaining 15 peptides showed 67–89 and 56–89% homology to JCV and SV40 counterpart epitopes, respectively (Table 3). Although 17 of 19 selected peptides did not have identical amino acid sequences, the anchor residues of 14 of 19 and 13 of 19 peptides, respectively, were identical to their JCV and SV40 counterparts. The auxiliary anchor residues were identical to JCV and SV40 counterparts for 10 of 14 and 11/14 BKV peptides, respectively (Table 3).

3.2. IFN- γ response to BKV LT-Ag peptides in HLA-A01 individuals

In an IFN- γ ELISPOT assay, activated T cells from all subjects reacted strongly to mitogen phorbol myristate acetate (PMA) + ionomycin calcium stimulation. Using HLA-matched Epstein–Barr virus-transformed B-LCLs pulsed with LT-Ag-derived peptides as antigen-presenting cells, we found that peptides P77 and P604 were recognized by 3 of 7 healthy subjects each. Peptide P604 was also recognized by 1 of 5 KT recipients tested. Peptide P235 was recognized by 2 of 7 healthy subjects and 2 of 5 KT recipients. Peptide P270 was recognized by 1 healthy subject and 1 KT recipient. Fig. 1 and Table 4 illustrate the positive results obtained with individual HLA-A01 binding 9-mer peptides. Only 2 of 4 patients responded to BKV-infected cell lysate but 7 of 7 healthy controls and 1 KT patient responded to BKV LT-Ag PepMix. The frequency (0 of 1 healthy subjects and 2 of 4 KT patients) with BKV-infected cell lysate was lower than the frequency (7 of 7 healthy subjects, 1 of 1 KT recipients) obtained with BKV LT-Ag PepMix, indicating that the latter preparation was a better positive control for our experiments.

3.3. IFN- γ response to BKV LT-Ag peptides in HLA-A03 individuals

One healthy subject and 2 KT patients had the phenotype HLA-A03, -A24 and were tested separately for HLA-A03 and HLA-A24 (vide infra) binding peptides. In all, a total of 3 healthy controls and 6 KT recipients (of which 2 had viruria) were tested against the predicted HLA-A03 peptides in an IFN- γ ELISPOT assay. T cells from all subjects reacted strongly to mitogen PMA + ionomycin calcium stimulation. Table 5 and Fig. 2 demonstrate the positive results obtained with individual HLA-A03 binding 9-mer peptides. Among the healthy controls, 3 of 3 responded to peptides P208 and P506, whereas 2 of 3 responded to peptides P159, P172, and P414, and 1 of 3 responded to peptide P156. In the KT patient group, 4 of 6, 3 of 6, and 2 of 6 responded to peptides P506, P156, and P159, respectively. Only 1 of 4 patients responded to peptides P172, P208, P222, P227, and P394. Peptide P414 was recognized by 1 of 6 patients tested. There were no statistically significant differences between the responder rates for KT recipients and healthy subjects for any of the HLA-A alleles analyzed.

3.4. Cell-mediated response to BKV LT-Ag peptides in HLA-A24 individuals

One healthy subject and 2 kidney transplant patients in this group were also tested for HLA-A03 binding peptides because they carried both alleles. BKV LT-Ag PepMix activated T cells from all healthy and transplanted subjects reacted strongly to mitogen PMA + ionomycin calcium stimulation. Table 6 and Fig. 3 demonstrate positive results obtained with individual HLA-A24 binding 9-mer peptides. Among healthy subjects, peptides P242, P33, and P388 stimulated response in 2 of 4, 1 of 4, and 1 of 4 subjects, respectively. In the KT group, consisting of 1 viruric and 2 nonviruric KT patients, peptides P33, P242, P287, and P388 all induced a response in 2 of 3 patients. One KT patient responded to peptide P422.

4. Discussion

The emergence of BKV infection as a significant problem in kidney transplant patients has stimulated studies on the human response to viral proteins. Initial efforts sought to measure the global immune response to virus-infected cell lysates or mixtures of peptides spanning the entire VP-1 or LT-Ag proteins. More recently, efforts have been made to dissect the overall immune response into its individual components by defining populations of circulating T cells that recognize specific viral epitopes. This approach has led to the identification of two HLA-A02-restricted epitopes on BKV VP-1 [17]. Evidence has been presented that CD8-positive T-cell-mediated immune responses to these epitopes are clinically relevant with respect to the resolution of active viral infection. Four different HLA-A02-restricted epitopes have been mapped on BKV LT-Ag protein to date [7], [8] and [10]. HLA-A02-positive individuals account for only 45.6% of the U.S. Caucasian population (data from <http://tpis.upmc.edu/tpis/HLAMatchmaker/>). To broaden our understanding of the human response to BKV infection in subjects with other HLA types, we have sought to define BKV LT-Ag epitopes with the ability to bind HLA-A01, -A03, and -A24 alleles. The frequencies of these alleles in the U.S. Caucasian population are 27, 23.8, and 12.1%, respectively.

We chose to study the BKV LT-Ag protein because it is produced early in the viral life cycle and, therefore, immune surveillance against LT-Ag epitopes is expected to maintain viral latency. Our work has identified 4 HLA -A01, 9 HLA-A03, and 5 HLA-A24 binding epitopes on BKV LT-Ag. The ELISPOT assay used in the epitope mapping did not demonstrate any IFN- γ responses in PBMCs stimulated directly after isolation (day zero). The requirement for 14-day expansion prior to peptide exposure likely reflects the low frequency of BKV-sensitized T cells in the circulation. For healthy subjects, this low frequency could, in turn, exist because exposure to BKV occurred in the remote past and led to only transient, low-level viremia before the virus assumed a stage of latency. Serologic studies confirmed that most subjects studied had prior exposure to BKV. Interestingly, 2 of 3 seronegative subjects responded to one or more BKV peptide and LT-Ag PepMix. As reported for other viruses, this may reflect a situation in which virus exposure occurred in the remote past, so that although antibody production had ceased, BKV memory cells persisted in the circulation [18] and [19]. Alternatively, one could consider these results to be the results of in vitro sensitization. Although sometimes regarded as an experimental artifact, such in vitro sensitization could also be considered analogous to the de novo sensitization that occurs in humans during their first encounter with BKV. A study of cord blood has at times been mentioned as one approach to distinguish between pre-existing virus-sensitized cells and sensitization that occurs in vitro. It is not clear whether this would be valid for BKV because in one study vertical transmission of this virus was suggested by the presence of viral DNA in 12 of 15 placental samples [20].

The magnitude of our ELISPOT response, although relatively weak, is quite comparable to that reported by other workers. Binggeli et al. reported a mean spot count of 2.8 ± 2.7 per 100,000 cells in patients with progressive BK viremia and 12.8 ± 15.7 spots per 100,000 cells in resolving viremia [9]. Ginevri et al. obtained 10–243 spots per 100,000 cells in healthy BKV-seropositive subjects [21]. In both studies, PBMCs were stimulated and screened using a mixture of overlapping peptide pools, rather than individual 9-mer peptides. Because of interindividual variation, a clear, mathematically defined positive score applicable to all subjects could not be defined by us. Hence, it became necessary to subtract subject-specific background counts to classify individuals as responders or nonresponders. Within the limitations of the number of cases analyzed, neither this study nor previously published ones indicate that BKV epitopes recognized by healthy subjects are qualitatively different from those recognized by immunosuppressed patients. Although IFN- γ ELISPOT

assays were performed on expanded PBMC rather than purified T-cell fractions, the use of nanomeric peptides as screening antigen makes it most likely that the observed responses are derived from CD8-positive T cells.

Although we have clearly shown in vitro binding of the reported peptides to the corresponding MHC molecules, determination of actual HLA restriction of these peptides has been hampered by the low frequency of subjects testing positive and the lack of complete high-resolution HLA typing data on these subjects. Indeed, several epitope candidates predicted for one HLA also show promising binding scores for other alleles (for example: P506, A*11, A*68; P156, A*03, A*01; P604, A*01, A*03; P172, A*03, A*02). Disparate MHC class I/peptide complexes can be recognized by the same T-cell receptor [22]. Li et al. have described a BKV LT-ag-derived 15-mer peptide, P613, which elicits a cell-mediated immune response in both HLA-A02 and DRB1*0901 subjects [6]. Thus, MHC restriction is not a prerequisite for the development of a successful antiviral response following natural infection or vaccination. Promiscuous binding of T-helper epitopes to MHC class II is well established. More recent studies show that HLA class I allele promiscuity is also common among viral epitopes recognized by cytotoxic T cells [23]. It is also worth noting that some epitopes relevant to mouse polyomavirus immunity are presented by MHC class Ib (rather than class Ia) molecules [24]. Because class Ib molecules exhibit very limited polymorphism, this observation raises the possibility of developing peptide-based viral vaccines with broad coverage across multiple MHC.

Sequence identity between the tested BKV peptides P222 and P422 and homologous JCV and SV40 LT-Ag sequences indicates that immunization by these BKV LT-Ag peptides will result in cross-sensitization to JCV and SV40 proteins. The validity of this concept has been demonstrated experimentally for LT-Ag peptides binding to HLA-A02 and HLA-B07/B08 [6]. Epitopes shared among BKV, JCV, and SV40 have also been demonstrated on the major capsid protein VP-1 [17], [25] and [26]. On the other hand, some BKV epitopes defined by us are not identical to their JCV and SV40 homologs, and the differences involve amino acids that affect MHC molecule anchor as well as nonanchor sites. This implies that the human immune system has the capability to display the fine specificity needed to distinguish between these three related polyomavirus species. This notion also has experimental support in work performed with SV40 LT-ag-specific cytotoxic T-cell clones [27].

The BKV epitopes defined here may find potential use in the development of peptide-based vaccines. Multiple peptides must be pooled to ensure broad coverage of human subjects encompassing all common MHC alleles. Future work planned to facilitate this goal includes characterization of the phenotype, functional repertoire, cytotoxic capacity, MHC restriction, and cross-reactivity patterns of the immune cells responding to stimulation by these peptides. Attempts will also be made to enhance the immunogenicity of the peptides by chemical modifications or use of adjuvants.

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Fig. 1.

PBMCs from healthy control (HC; n = 7) and kidney transplant patients (KT) (n = 5) were expanded with or without BKV-infected cell lysate/BKV LT-Ag PepMix for 13–15 days. Expanded T cells were harvested and tested in an IFN- γ ELISPOT assay. We used HLA-A01 binding individual BKV LT-Ag peptides as an antigen and HLA-matched allogeneic LCL as antigen-presenting cells. Positive results are expressed as the mean number of IFN- γ spots/105 cells. The numbers in brackets below every peptide represent the percentage relative to pass/fail control score obtained with in vitro peptide-binding assays. Bars without standard deviation indicate that a single positive result was obtained with some selected peptides. In HC subjects group, 3 of 7 responded to peptides P77 and P604, 2 of 7 responded to peptide P235, and 1 of 7 responded to peptide P270. In the KT recipients group, 2 of 5 responded to peptide P235, and 1 of 5 responded to peptides P270 and P604.

Fig. 2.

PBMCs from healthy controls (HC; n = 3) and kidney transplant patients (KT) (n = 6) were expanded with or without BKV-infected cell lysate/BKV LT-Ag PepMix for 13–15 days. Expanded T cells were harvested and tested in an IFN- γ ELISPOT assay. We used HLA-A03 binding individual BKV LT-Ag peptides as an antigen and HLA-matched allogeneic LCL as antigen-presenting cells. Positive results are expressed as the mean number of IFN- γ spots/105 cells. The numbers in brackets below every peptide represent the percentage relative to pass/fail control score obtained with in vitro peptide-binding assays. Bars without standard deviation indicate that a single positive result was obtained with some selected peptides. Of 3 HC subjects tested, all responded to peptides P208 and P506, 2 responded to peptides P159, P172, and P414, and only 1 responded to P156. In the KT patients group, 4 of 6 responded to peptide P506, 3 of 6 responded to peptide P156, 2 of 6 responded to peptides P159, 1 of 6 responded to peptide P414, and 1 of 4 responded to peptides P172, P208, P222, P227, and P394.

Fig. 3.

PBMCs from healthy control subjects ($n = 4$) and kidney transplant patients (KT, $n = 3$) were expanded with or without BKV-infected cell lysate/BKV LT-Ag PepMix for 13–15 days. Expanded T cells were harvested and tested in an IFN- γ ELISPOT assay. We used HLA-A24 binding individual BKV LT-Ag peptides as an antigen and HLA-matched allogeneic LCL as antigen-presenting cells. Positive results are expressed as the mean number of IFN- γ spots/105 cells. The numbers in brackets below every peptide represent the percentage relative to pass/fail control score obtained with in vitro peptide-binding assay. Bars without standard deviation indicate that a single positive result was obtained with some selected peptides. In the HC subjects group, 1 of 4 responded to peptides P33 and P388 and 2 of 4 responded to peptide P242. In the KT patients group, 4 of 5 selected peptides (P33, P242, P287, and P388) and peptide P422 stimulated response in 3 of 4 and 1 of 4 KT patients, respectively.

Table 1

HLA type, serology, and virology data on subjects studied

Healthy controls	HLA	BKV serology	KT patients	HLA	BKV serology	BKV DNA
Healthy controls (n = 13)			KT patients (n = 12)			
HC 1	A01, B08, B70	Positive	KT 1	A01, B07, B08	Positive	VM-/VU+
HC 2	A01, A24, B52, B27	Positive	KT 2	A01, A02, B08, B62	Unknown	VM-/VU+
HC 3	A01, A68, B57	Positive	KT 3	A01, A11, B18, B44	Positive	VM-/VU+
HC 4	A01, A02, B14, B41	Positive	KT 4	A01, A29, B44, B51	Positive	VM-/VU+
HC 5	A01, A02, B14, B41	Positive	KT 5	A01, B08, B18	Positive	VM-/VU+
HC 6	A01, A01, B14, B41	Positive	KT 6	A03, 31, B08, B39	Positive	VM+/VU+
HC 7	A01, A30, B38, B57	Positive	KT 7	A01, A03, B08	Positive	VM+/VU+
HC 8	A03, A25, B35, B62	Positive	KT 8	A03, B07	Positive	VM-/VU-
HC 9	A03, A29, B44, B65	Positive	KT 9	A02, A03, B45	Positive	VM-/VU+
HC 10	A03, A24, B07, B51	Unknown	KT 10	A03, A24, B62, B35,	Positive	VM-/VU+
HC 11	A2, A24, B7, B13	Negative	KT 11	A03, A24, B61, B62	Positive	VM-/VU-
HC 12	A24, A29, B44, B72	Negative	KT 12	A24, A32, B8, 39	Positive	VM-/VU-
Healthy controls	HLA	BKV serology	KT patients	HLA	BKV serology	BKV DNA
HC 13	A11, A24, B51, B60	Negative				
B K LCL	A03, A24, B35, B37	Unknown	Pat 003 LCL	A01, A02, B08, B27	Unknown	Unknown

BKV serological status of healthy controls (n = 12) and KT recipients (n = 11) were tested using BKV virus-like particle-based ELISA assays. Nine of 12 healthy controls and 11 of 11 KT patients were BKV seropositive. BKV serostatus of 1 healthy control and 1 KT patient was not tested because plasma samples were not available. Of 12 KT patients, 7 had BK viremia but no viremia, 2 had BK viremia and viremia, and 3 were negative for both. HLA alleles for which in vitro peptide binding was studied are in bold font. B K LCL and Pat 003 LCL were used as HLA-matched antigen-presenting cells in this study. HC, healthy control subject; KT, kidney transplant; LCL, lymphoblastoid cell line; VU, BK viremia; VM, BK viremia.

Table 2

Listing of putative BKV LT-Ag and VP1-derived epitopes described to date

AA position	Amino acid sequence	HLA binding	SYFPEIT HI score	Frequency in healthy control subjects	Frequency in KT patients
Peptides tested in this study					
604*	R L D S E I S M Y	HLA-A01	26	3/7	1/5
270*	V S W K L I T E Y	HLA-A01	22	1/7	1/5
235*	Y S A L T R D P Y	HLA-A01	21	2/7	2/5
77*	W S S S E V P T Y	HLA-A01	20	3/7	0/5
222*	F L I C K G V N K	HLA-A03	31	0/0	1/4
172*	I L Y K K L M E K	HLA-A03	28	2/3	1/4
342*	A V D T V L A K K	HLA-A03	27	0/0	0/4
506*	S V K V N L E K K	HLA-A03	22	3/3	4/6
156*	R T L A C F A V Y	HLA-A03	21	1/3	3/6
159*	A C F A V Y T T K	HLA-A03	21	2/3	2/6
227*	G V N K E Y L L Y	HLA-A03	19	0/0	1/4
414*	I V F N V P K R R	HLA-A03	19	2/3	1/4
208*	S A I N N F C Q K	HLA-A03	18	3/3	1/4
394*	A W L H C L L P K	HLA-A03	18	0/0	1/4
33*	A Y L R K C K E F	HLA-A24	24	1/4	2/3
242*	P Y H T I E E S I	HLA-A24	21	2/4	2/3
388*	Q Y M A G V A W L	HLA-A24	21	1/4	2/3
287*	V F L L L G M Y L	HLA-A24	20	0/4	2/3
422*	R Y W L F K G P I	HLA-A24	20	0/4	1/3
Previously published BKV LT-Ag and VP1 derived peptides					
362 ^a	M L T E R F N H I L	HLA-A02	NA	5/6[8]	5/7[8]
398 ^a	C L L P K M D S V	HLA-A02	NA	3/13[24], 4/5[7]	0/13[24]

AA position	Amino acid sequence	HLA binding	SYFPEIT HI score	Frequency in healthy control subjects	Frequency in KT patients
40 ^a	V I F D F L H C I	HLA-A02	NA	3/6[8], 4/5[7]	2/7[8]
410 ^a	F L H C I V F N V	HLA-A02	NA	3/6[8], 4/5[7], 1/13[24]	2/7[8], 0/13[24]
558 ^a	S L Q N S E F L L	HLA-A02	NA	2/5[7]	ND
579 ^a	L L L I W F R P V	HLA-A02	NA	0/6[8], 5/5[7], 10/13[24]	1/7[8], 3/10[24]
157 ^a	T L A C F A V Y T	HLA-A02	NA	1/5[7]	ND
216 ^a	K L C T F S F L I	HLA-A02	NA	1/5[7]	ND
472 ^a	V V F E D V K G T	HLA-A02	NA	1/5[7]	ND
553 ^a	I Y L R K S L Q N S E F L L E	HLA-A02	NA	Not mentioned	ND
557 ^a	K S L Q N S E F L L E K R I L	HLA-A02	NA	Not mentioned	ND
27 ^a	L P L M R K A Y L	B07 & B08	NA	1/1 and 1/2[6]	ND
57 ^a	T L Y K K M E Q D V K V A H Q	HLA-DRB1*0301	NA	2/2[6]	ND
613 ^a	T F S R M K Y N I C M G K C I	HLA-A02 & DRB1*0901	NA	3/3, 2/2[6]	ND
108 ^b	L L M W E A V T V	HLA-A02	NA	4/10[17], 2/6[24]	1/2[17]
44 ^b	A I T E V E C F L	HLA-A02	NA	3/6[24], 8/11[28]	ND

* BKV LT-Ag peptides described in this study. The number in brackets indicates the published reference. ND = not done; NA = not applicable. All studies used HLA binding algorithms for initial peptide selection, but Li et al. [6] used the overlapping peptide pool approach to directly evaluate HLA restriction. Cross-reactivity between JCV and BKV was reported for 6 peptides, not found for 3 peptides, and not evaluated for the remaining 26 peptides.

^aPreviously published BKV LT-Ag peptides.

^bPreviously published BKV VP1 peptides.

Table 3

Sequence alignment of BKV, JCV, and SV40 LT-Ag peptides

HLA-A01 binding peptides					
	P604	P270	P235	P77	
BKV	RLDSEISMY	VSWKLITEY	YSALTRDPY	WSSEVPTY	
JCV	*** <u>L</u> *****	*****V*Q*	*****C*Q**	*N*****	
SV40	***K*F*LS	*****V***	*****F	*DAT*I***	
HLA-A03 binding peptides					
	P222	P172	P342	P506	P156
BKV	FLICKGVNK	ILYKKLMEK	AVDTVLAKK	SVKVNLEKK	RTLACFAVY
JCV	*****	*****	*****A**Q	*****R*	**V*S****
SV40	*****	L*****	*****	*****	*****I*
	P159	P227	P414	P208	P394
BKV	ACFAVYTTK	GVNKEYLLY	IVFNVPKRR	SAINFCQK	AWLHCLLPK
JCV	*S*****	*****F*	**L*I**K*	*****Y***	**I*****Q
SV40	*** <u>I</u> ****	*****M*	M*Y*I**K*	*****YA**	*****
HLA-A24 binding peptides					
	P33	P242	P388	P287	P422
BKV	AYLKCKKEF	PYHIEESI	QYMAGV AWL	VFLLLGMYL	RYWLFKGPI
JCV	***** <u>L</u>	**AVV****	*****I	*****M****	*****
SV40	*****	*FSV*** <u>L</u>	EW*****	*L*****	*****

Sequence alignments of HLA-A01, -A03, and -A24 binding peptides indicate the homology with JCV and SV40 counterpart peptides.

* Sequence identity. Predicted anchor amino acids are in boldface and predicted auxiliary amino acids are underlined.

Table 4

BKV LT-Ag-specific T cells by IFN- γ ELISPOT assay in HLA-A01 subjects

HLA-A01 peptides	% relative pass/fail score	Healthy subjects	SFU/100,000 cells	KT patients	SFU/100,000 T cells
PMA	—	7/7	631 $\pm$ 344	5/5	895 $\pm$ 547
P77	132.7	3/7	12 $\pm$ 4	0/5	0
P235	184	2/7	25 $\pm$ 19	2/5	20 $\pm$ 6
P270	152.83	1/7	23	1/5	17
P604	139.97	3/7	14 $\pm$ 3	1/5	33
BKV-infected lysate	—	0/1	0	2/4	44 $\pm$ 11
BKV LT-Ag PepMix	—	7/7	205 $\pm$ 119	1/1	124

A total of 4 HLA-A01 binding peptides were selected out of 10 predicted peptides after in vitro REVEAL MHC peptide-binding assays. The percentage relative pass/fail score for each selected peptide was more than the pass/fail control binder and was compared with positive control peptide YSEHPFTTSQY, which is a known HLA-A01 restricted T-cell epitope tested in this assay.

Table 5

BKV LT-Ag-specific T cells by IFN- γ ELISPOT assay in HLA-A03 subjects

HLA-A03 peptides	% relative pass/fail score	Healthy subjects	SFU/100,000 cells	KT patients	SFU/100,000 cells
PMA	—	3/3	914 $\pm$ 297	6/6	870 $\pm$ 281
P156	96.83	1/3	14	3/6	30 $\pm$ 27
P159	127.53	2/3	43 $\pm$ 25	2/6	18 $\pm$ 7
P172	133.84	2/3	58 $\pm$ 54	1/4	145
P208	137.01	3/3	25 $\pm$ 17	1/4	116
P222	112.35	NA	NA	1/4	36
P227	103.49	NA	NA	1/4	24
P342	140.69	NA	NA	0/4	0
P394	109.53	NA	NA	1/4	109
P414	148.34	2/3	27 $\pm$ 12	1/6	47
P506	139.67	3/3	28 $\pm$ 18	4/6	21 $\pm$ 11
BKV-infected lysate	—	1/1	23	2/4	55 $\pm$ 38
BKB LT-Ag PepMix	—	3/3	240 $\pm$ 53	2/2	151 $\pm$ 194

A total of 10 HLA-A03 binding peptides were selected out of 43 predicted peptides after in vitro REVEAL MHC peptide binding assays. The percentage relative pass/fail score for each selected peptide was more than the pass/fail control binder and was compared with positive control peptide ILRGSVAHK, which is a known HLA-A03 restricted T-cell epitope tested in this assay.

Table 6

BKV LT-Ag-specific T cells by IFN- γ ELISPOT assay in HLA-A24 subjects

HLA-A24 peptides	% relative pass/fail score	Healthy subjects	SFU/100,000 cells	KT patients	SFU/100,000 cells
PMA	—	4/4	888 $\pm$ 245	3/3	1063 $\pm$ 70
P33	224.27	1/4	27	2/3	21 $\pm$ 8
P242	130.9	2/4	31 $\pm$ 1	2/3	49 $\pm$ 5
P287	128.56	0/3	0	2/3	38 $\pm$ 12
P388	195.61	1/4	25	2/3	17 $\pm$ 4
P422	130.25	0/4	0	1/3	32
BKV-infected lysate	—	1/1	28	0/1	0
BKB LT-Ag PepMix	—	1/3	481	2/2	152 $\pm$ 195

A total of five HLA-A24 binding peptides were selected out of seven predicted peptides after in vitro REVEAL MHC peptide binding assays. The percentage relative pass/fail score for each selected peptide was more than the pass/fail control binder and was compared with positive control peptide AFLPWHLRF, which is a known HLA-A24 restricted T-cell epitope tested in this assay.