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Immune Reconstitution after Allogeneic Hematopoietic Stem Cell Transplantation Is Associated with Selective Control of JC Virus Reactivation

Chen Sabrina Tan [1,2,3,*], Thomas A. Broge Jr. [1,3], Long Ngo [4], Sarah Gheuens [1,3,†], Raphael Viscidi [5], Evelyn Bord [1,3], Jacalyn Rosenblatt [6], Michael Wong [2,7,‡], David Avigan [6], Igor J. Koralnik [1,3]

[1] *Center of Virology and Vaccine Research, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts*
[2] *Division of Infectious Diseases, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts*
[3] *Division of NeuroVirology, Department of Neurology, Beth Israel Deaconess Medical Center, Boston, Massachusetts*
[4] *Division of General Medicine, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts*
[5] *Department of Pediatrics, Johns Hopkins Medical Center, Baltimore, Maryland*
[6] *Division of Hematology Oncology, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts*
[7] *The Transplant Center, Beth Israel Deaconess Medical Center, Boston, Massachusetts*

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JC virus (JCV) causes progressive multifocal leukoencephalopathy (PML) in immunocompromised patients. The mechanism of JCV reactivation and immunity in a transplanted immune system remains unclear. We prospectively studied 30 patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) and collected blood and urine samples before HSCT and 3, 6, and 12 to 18 months after HSCT. Before HSCT, JCV DNA was detected in 7 of 30 urine, 5 of 30 peripheral blood mononuclear cells (PBMC) and 6 of 30 plasma samples. Although JC viruria remained stable after HSCT with detection in 5 of 21 samples, viremia was detected in only 1 of 22 plasma and none of 22 PBMC samples 12 to 18 months after HSCT. Prevalence of anti-JCV IgG was 83% before HSCT and decreased to 72% at 12 to 18 months. Anti-JCV IgM was rarely detected. JCV-specific $CD4^+$ and $CD8^+$ T cell responses increased 12 to 18 months after HSCT. Although JC viruria correlated directly with detection of anti-JCV IgG, the cellular immune response to JCV measured by ELISpot was inversely correlated with anti-JCV IgG response. The diagnosis of acute myelogenous leukemia and age group were 2 independent patient factors associated with significantly reduced cellular immune responses to JCV. This prospective study in HSCT patients provides a model of interactions between the host immune response and viral activation in multiple compartments during the recovery of the immune system.

INTRODUCTION

JC virus (JCV) causes progressive multifocal leukoencephalopathy (PML) in immunocompromised patients [1,2]. Up to 80% of the general populations is seropositive for JCV and both the humoral and cellular immune responses are necessary for containment of viral proliferation [3,4]. Thus, immunocompromised patients, including those with hematological malignancies requiring allogeneic hematopoietic stem cell transplantation (HSCT), are at increased risk for developing PML. Indeed, PML was initially described in 3 patients with hematological malignancies in 1958 [5]. Currently, many more patients survive HSCT because of, in part, improved long-term immunosuppression treatment they receive after transplantation. Among all published reports of transplant recipients with PML, HSCT patients make up the largest group; up to 8% of PML patients have hematological cancers [6,7]. The incidence rate of PML in patients with HSCT was estimated at 35.4 in 100,000 person-years [8]. Furthermore, PML can develop as early as 1.5 months or as late as years after transplantation and is associated with the myeloablative conditioning regimen used to wipe out the HSCT recipient cells in preparation for transplantation [7,9]. The median survival time for HSCT recipient with PML is less than 2 years [7]. Thus, PML is devastating in HSCT patients as there is no effective therapy for this disease.

Although studies have examined the host immune responses to JCV in patients with PML, little is known of the host-viral interactions before PML onset [10-12]. Of importance, a better understanding of how the host immune responses control viral proliferation is crucial to preventing the development of PML. Even though the cellular immune system cannot eradicate chronic viruses, immune surveillance prevents active infection under normal immune conditions.

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* Correspondence and reprint requests: Chen Sabrina Tan, MD, Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, E/CLS 1011, 330 Brookline Ave., Boston, MA 02215.
E-mail address: ctan@bidmc.harvard.edu (C.S. Tan).
† Current address: Sarah Gheuens, Biogen Idec, Cambridge, Massachusetts.
‡ Current address: Michael Wong, Sarepta Therapeutics, Cambridge, Massachusetts.

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Reactivation of chronically latent viruses remains a major complication after HSCT [13]. It is unclear when JCV reactivation occurs or, in HSCT, how the transplanted immune system interacts with JCV in the infected host to maintain viral latency. Thus, we designed a prospective study to analyze host immune responses to JCV before HSCT and to examine the dynamic changes as the transplanted immune system reconstitutes and expands its antiviral armamentarium.

METHODS

Study Subjects and Samples

This study was approved by the Dana Farber Harvard Cancer Center Institutional Review Board. Adult patients were enrolled consecutively from April 2008 to July 2010 as they presented for allogeneic HSCT at Beth Israel Deaconess Medical Center. Thirty healthy volunteers were also enrolled. All subjects consented to the study. Blood and urine samples were obtained before HSCT and 3 months, 6 months, and 12 to 18 months after HSCT. Plasma and peripheral blood mononuclear cells (PBMC) were isolated as previously reported [12]. Aliquots of PBMC, plasma, and urine were stored at -80°C for DNA extraction.

DNA Extraction and Quantitative PCR for JCV

Total DNA was extracted from PBMC using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) and from plasma and urine samples using the Qiagen MinElute kit, following the manufacturers' instructions. JCV DNA was detected and quantified by quantitative PCR (qPCR) using standard TaqMan assay conditions and large T primers (TaqMan, Thermo Fisher Scientific, Waltham, MA) as previously described [14]. Each sample was run in triplicate on an ABI 7300 Real-time PCR System (Thermo Fisher, Waltham, MA). JCV DNA viral load in PBMC were expressed as copies per μg of DNA used for qPCR. DNA viral load in plasma and urine were expressed as copies per mL of the samples. A sample was considered positive if at least 2 of 3 replicate wells showed positive amplification with a limit of detection of 188 copies per mL for urine and plasma and 10 copies per μg for PBMC.

Cellular Immune Response to JCV

Intracellular cytokine staining

After 10 to 14 days in culture with JCV VP1 peptides, 1×10^6 lymphocytes were incubated in RPMI 1640 with 12% FBS medium, with a vasopeptide VP1 peptide pool (2 μg/mL), or with phorbol 12-myristate 13-acetate (PMA) and ionomycin (1 μg/mL and 5 μg/mL, respectively) at 37°C for 6 hours. After the first hour, all samples received monensin (GolgiStop; BD Bioscience, Franklin Lakes, NJ). Cells were stained with fluorescently conjugated antibodies specific for human CD4 (clone L200) and CD8 (clone SK1), then fixed, permeabilized (BD Cytofix/Cytoperm), and stained with antibodies specific for IFN-γ (clone B27) and CD3 (clone SK7). All antibodies were obtained from BD Biosciences. Data were acquired on a FACS Calibur flow cytometer (BD Biosciences) and analyzed with FlowJo software (Treestar, Ashland, OR). The intracellular cytokine staining (ICS) result was considered positive when the percentage of IFN-γ–producing $CD4^+$ or $CD8^+$ T cells was equal to or greater than 2 times the baseline value. The ICS results were reported after subtraction of baseline value as previously described [12].

ELISpot

In this assay, 1×10^5 lymphocytes were incubated on 96-well MultiScreen HTS plates (Millipore, Billerica, MA) coated with anti-human IFN-γ (5 μg/mL) in RPMI 1640 with 12% FBS medium, with a VP1 peptide pool (2 μg/mL), or with phytohemagglutinin (10 μg/mL) at 37°C overnight. After washing, the plates were incubated with antihuman IFN-γ biotin (U-Cytech, Utrecht, Netherlands) for 2 hours at room temperature, washed, and incubated with streptavidin (Southern Biotechnology, Birmingham, AL). The plates were developed with nitroblue tetrazolium-5-bromo-4-chloro-3-indolylphosphatechromogen (Pierce, Rockford, IL) and analyzed on an Immunospot Analyzer (Cellular Technology, Shaker Heights, OH). Results were considered positive when the number of spot forming units was greater than 50 per 10^6 cells after subtraction of baseline, and greater than 3 times the baseline value. Results were reported after subtraction of baseline values.

Tetramer staining

Tetramer stainings were performed in human leukocyte antigen (HLA)-A*0201-positive study subjects. Lymphocytes were stimulated with the HLA-A*0201-restricted JCV VP1 epitopes, $VP1_{p36}$ or $VP1_{p100}$, and stained with a fluorescently conjugated tetramer specific to HLA-A*0201/$VP1_{p36}$ or HLA-A*0201/$VP1_{p100}$ as previously described [15]. Data were acquired on a FACS Calibur flow cytometer (BD Biosciences) and analyzed with FlowJo software.

Humoral Immune Response to JCV

Anti-JCV IgM, IgG, and IgA were quantified by ELISA as previously described [16]. All samples were run in duplicate and the mean values are reported.

Statistical Analysis

Because of the longitudinal nature of the data, which were collected at 4 time intervals of interest (before HSCT and 3 months, 6 months, and 12 to 18 months after HSCT), we first used a smoothing cubic spline function to graphically examine the underlying trend of the dependent variables (eg, percentages of JCV-specific $CD4^+$ T lymphocytes) through time. Potential nonlinear effect of time was visualized especially at the last time interval. The 4 different categorical study time points were used to model the time effect. The within-subject correlation in the data was taken into account in the modeling using linear mixed-effects model. We used compound symmetry or autoregression as the variance-covariance structure. We also adjusted for the potential confounders with the 6 covariates (transplantation type, conditioning regimen, graft-versus-host disease, presence of concurrent viremia, age, and diagnosis of acute myelogenous leukemia) in the linear mixed-effects models. We found that the dependent variables were largely non-normal and the covariates were mostly statistically nonsignificant; therefore, we also used a nonparametric test, Kruskal-Wallis, to assess the effect of time. The inference on the time effect was similar in both the parametric (with linear mixed-effects model) and nonparametric analyses. We chose to report the results from the nonparametric tests. We also used Spearman correlation coefficients to assess the association between JC viremia, viruria, and cellular and humoral immune response parameters. We used the SAS software version 9.12 for all of the analyses.

RESULTS

Subjects Characteristics

We enrolled a total of 30 patients undergoing allogeneic HSCT at Beth Israel Deaconess Medical Center. Patients were followed up to 18 months after HSCT. Two patients died in the initial 100 days after transplantation, and 5 other patients passed away before reaching the last study time point. No patient developed PML. Two thirds of these patients were male and the most frequent indication for HSCT was acute myelogenous leukemia (Table 1). Although 6 patients had umbilical cord transplantation, others had

Table 1
Study Subjects Characteristics

Characteristic	n
Indication for transplantation	
Acute myelogenous leukemia	13
Multiple myeloma	6
Chronic lymphoid leukemia	3
Chronic myeloid leukemia	2
Non Hodgkin's lymphoma	2
Acute lymphoid leukemia	1
Others	3
Gender	
Male	20
Female	10
Types of transplantation	
Matched unrelated donor	13
Matched related donor	11
Umbilical cord blood	6
Conditioning regimens	
Myeloablative	22
Reduced intensity	8
GVHD	
GVHD during study period	11
No GVHD during study period	19
Concurrent viremia*	
Viremia during study period	11
No viremia during study period	19

GVHD indicates graft-versus-host disease.

* Study subjects with diagnosis of another viral infection during the study period, including herpes simplex, Epstein Barr virus, cytomegalovirus, and parainfluenza.