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Early Cytomegalovirus Reactivation Leaves a Specific and Dynamic Imprint on the Reconstituting T Cell Compartment Long-Term after Hematopoietic Stem Cell Transplantation

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Human cytomegalovirus (CMV) reactivation frequently occurs during the early phase of immune recovery after allogeneic hematopoietic stem cell transplantation (HSCT). Whereas the recovery of virus-specific immunity in the early phase after HSCT is extensively studied, the impact of CMV on the reconstitution and composition of the T cell compartment long-term after HSCT is unknown. We analyzed T cell reconstitution 1 to 2 years after HSCT in 131 pediatric patients. One year after HSCT, patients with early CMV reactivation (n = 46) had 3-fold higher $CD8^+$ T cell numbers (median, 1323 versus 424 cells/μL; $P < .0001$) compared with patients without CMV reactivation (n = 85). This effect, caused by a major expansion of $CD8^+$ effector memory (EM) and end-stage effector (EMRA) T cells, was independent of pretransplantation donor and recipient CMV serostatus and not seen after Epstein-Barr virus or adenovirus reactivations. At 1 and 2 years after HSCT, the absolute numbers of $CD8^+$ naive and central memory T cells, as well as $CD4^+$ naive, CM, EM, and EMRA T cells, did not differ between patients with or without CMV reactivation. In the second year after HSCT, a significant contraction of the initially expanded $CD8^+$ EM and EMRA T cell compartments was observed in patients with early CMV reactivation. In conclusion, CMV reactivation early after pediatric HSCT leaves a specific and dynamic imprint on the size and composition of the $CD8^+$ T cell compartment without compromising the reconstitution of $CD8^+$ and $CD4^+$ naive and central memory T cells pivotal in the response to neo and recall antigens.

INTRODUCTION

In immunocompetent individuals, CMV infection generally causes mild symptoms, after which latency is established and asymptomatic reactivations can occur sporadically throughout life [1]. In contrast, CMV reactivation occurs frequently during the transient period of immune deficiency after allogeneic hematopoietic stem cell transplantation (HSCT) in the case of a CMV seropositive donor and/or recipient. This donor- or recipient-derived CMV infection or reactivation, hereafter called *reactivation*, can lead to disseminated infections, causing interstitial pneumonitis, colitis, and hepatitis. Despite the pre-emptive use of antiviral medication, CMV infections remain a major cause of morbidity and mortality after HSCT [2,3]. Reconstitution of $CD4^+$ and $CD8^+$ T cell immunity is pivotal to provide protection against and achieve sustained control of CMV reactivations [4-6]. In the first months after HSCT, the repopulation of the T cell compartment is facilitated by cytokine- and antigen-driven homeostatic peripheral expansion of T cells that were transplanted with the graft [7,8]. The thymic-dependent T cell reconstitution of naive and central memory cells, essential for a balanced immune system with a broad specificity against neo- and recall antigens, is delayed for many months to years [7].

The differentiation stages of human T cells can be discerned based on the expression of cell surface markers CD45RA and CCR7. Naive ($CD45RA^+$ $CCR7^+$) cells differentiate upon antigen exposure into central memory (CM, $CD45RA^-$ $CCR7^+$) and effector memory (EM, $CD45RA^-$ $CCR7^-$) cells and eventually regain CD45RA when differentiating into end-stage effector (EMRA, $CCR7^-$ $CD45RA^+$) cells [9]. Whereas the expression of costimulatory molecules and chemokine receptors varies between the differentiation stages, ex vivo cytolytic capacity increases during differentiation and telomere length shortens [9,10]. With increasing age, a continuous accumulation of CMV-specific $CD8^+$ T cells, also called *memory inflation*, has been described in healthy CMV-seropositive individuals. These cells mainly represent EM and EMRA T cells [11-13]. A durable expansion of these late differentiated T cells has also been observed in infants with congenital and postnatal CMV infections [14-17] and after primary CMV infection or reactivation in patients continuously receiving immunosuppressive medication after solid organ transplantation (SOT) [12,18,19].

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In recent years, the interaction between viral reactivations and early immune reconstitution after HSCT has been the studied extensively [4-6,20,21]. However, apart from an early report in 1985, the influence of viral reactivations occurring during the early phase of immune reconstitution on the composition of the T cell compartment in steady state conditions after HSCT is largely unknown [22]. In this study, we report the impact of early CMV reactivations on the reconstitution and composition of the T cell compartment 1 and 2 years after HSCT in a large cohort of pediatric HSCT recipients.

METHODS

Ethics Statement

All transplantations were performed according to European Society for Blood and Marrow Transplantation guidelines. Blood samples were routinely obtained and analyzed after approval by the institutional review board (protocol P01.028). Informed consent was provided by the patient and/or a parent or guardian.

Patients and Blood Sampling

Between January 1, 2002 and December 31, 2011, 227 children received a bone marrow or peripheral blood stem cell transplantation after myeloablative conditioning for malignant and nonmalignant hematological disorders in the Leiden University Medical Center. A total of 59 patients died in the first year and 11 were lost to follow-up (Figure S1). Of the 157 remaining patients, 26 were excluded, resulting in a cohort of 131 patients. Exclusion criteria were relapse of malignant disease (n = 8) or autologous reinfusion (n = 1) in the first year after HSCT, systemic immunosuppressive drugs at 1 year after HSCT (n = 15), or clinically relevant CMV infection after day +250 (n = 2). Patients who received 2 transplantations (n = 14) were analyzed only after their second transplantation. Peripheral blood samples were obtained for routine analysis at different time points after HSCT and the sample drawn closest to 1 year (median, 363 days; range 302 to 478 days) after HSCT was analyzed in this study. In 76 patients, this sample could be compared with a sample obtained 2 years (median, 729; range, 498 to 849 days) after HSCT and at least 180 days after the first sample.

Monitoring and Treatment of Viral Reactivations

After HSCT, plasma was routinely screened for Epstein-Barr virus (EBV), CMV, and human adenovirus (HAdV) DNA by real time quantitative PCR [23-25]. The limit of detection in these assays was 50 (1.7 log) viral DNA copies/mL. Reactivation of CMV, EBV, and HAdV was defined as detection of viral DNA in plasma at least once, whereas pre-emptive treatment with ganciclovir, rituximab, or cidofovir, respectively, was initiated upon detection of 1000 (3 log) or more viral DNA copies/mL at 2 or more consecutive time points [26-28].

Flow Cytometric Analysis

To determine the size of individual lymphocyte populations and subsets, peripheral blood mononuclear cells (PBMC) were analyzed by flow cytometry. PBMC were separated using ficoll-isopaque density gradient centrifugation (Leiden University Medical Center Pharmacy, Leiden, Netherlands). PBMC were stained with CD45, CD14, CD33, CD235a, CD3, CD19, CD56, CD4, CD8, and TCR-γδ antibodies, listed in Table S1. Four-color flow cytometry was performed on a BD FACS Calibur II flow cytometer (Becton Dickinson Biosciences [BD], Franklin Lakes, NJ) and data were analyzed using BD Cellquest software. Lymphocytes were defined as $CD45^+$ CD33/CD235a/$CD14^-$ cells within the forward/sideward scatter lymphocyte gate and absolute cell numbers per μL of peripheral blood were calculated. In a representative subcohort of 53 consecutive patients who underwent transplantation between 2008 and 2011, T cell differentiation was also analyzed based on CD45RA and CCR7 expression.

Statistical Analysis

Statistical analysis was performed using SPSS Statistics 20 (IBM SPSS Inc, Chicago, IL). GraphPad Prism software (version 6.00; GraphPad Software, San Diego, CA) was used to construct figures. Because cell numbers did not follow Gaussian distribution, Mann-Whitney U test (2 groups), Kruskall-Wallis test (> 2 groups), and Wilcoxon matched-pairs signed ranks test (paired analysis) were used for the univariate analysis of numerical parameters. Pearson's correlation test was performed on log-transformed data for analysis of univariate correlations. Fisher exact test (2 groups) and Pearson's chi-square test (> 2 groups) were used for univariate analysis of categorical parameters. Logistic regression analysis and linear regression analysis on log-transformed data were performed for multivariate analysis of parameters with P values $\leq$ .10 in univariate analysis.

RESULTS

Incidence of CMV Reactivations and Description of Patients

In the study cohort of 131 pediatric stem cell transplantation recipients who were available for follow-up 1 year after HSCT, 46 patients (35%) experienced a CMV reactivation in the first 100 days after HSCT. The median duration of CMV viremia was 36 (range, 1 to 185) days, starting 5 to 62 (median, 26) days after HSCT. The criteria for pre-emptive ganciclovir treatment were met in 33 of 46 patients (Table 1). At the time of transplantation, patients with CMV reactivation after HSCT were more often CMV seropositive (83 versus 31%, $P < .0001$) and were older (median, 13.0 versus 7.9 year; $P = .001$) than patients without CMV reactivation. Also, patients receiving a graft from a CMV-seropositive donor more often experienced a CMV reactivation (78 versus 31%, $P < .0001$). As shown in Table 1, no significant differences were observed in other HSCT-related parameters. In a logistic regression analysis of parameters with P values < .10 in univariate analysis, pretransplantation CMV serostatus of recipient and donor, as well as patient age, differed significantly between children with and without CMV reactivation after HSCT ($P < .0001$, $P < .0001$, and $P = .017$, respectively).

Patients with Early CMV Reactivation Had Higher $CD8^+$ T Cell Numbers One Year after HSCT

Compared with 85 patients without CMV reactivation, 46 patients with early CMV reactivation had significantly higher lymphocyte numbers 1 year after HSCT. This could be attributed to an increase of $CD3^+$ T cells (median, 2083 versus 1257 cells/μL; $P < .0001$), whereas NK and B cell numbers did not differ (Figure 1A). Within the T cell compartment, absolute $CD8^+$ T cell numbers were 3-fold higher (median, 1323 versus 424 cells/μL; $P < .0001$) in patients with CMV reactivation (Figure 1B). Patients with a low level CMV reactivation (n = 13), ie, CMV plasma DNA load above the limit of detection, but not at 2 consecutive time points above 3.0 log copies/mL, already had significantly higher $CD8^+$ T cell numbers 1 year after HSCT compared with patients without CMV reactivation (median, 709 versus 424 cells/μL; $P = .0001$) (Figure 1C). The highest measured plasma CMV DNA load and the time between CMV clearance and analysis at 1 year after HSCT did not influence the number of $CD8^+$ T cells at 1 year after HSCT (Figure S2). Also, the impact of early CMV reactivation on the $CD8^+$ T cell compartment did not differ between patients who received a stem cell graft from an HLA-identical related donor, matched unrelated donor, or haplo-identical donor (data not shown).

$CD4^+$ T cell numbers were comparable in patients with and without CMV reactivation (median, 702 versus 684 cells/μL, $P = .79$) (Figure 1B). Although γδ T cells only represented a small proportion (median, 4%) of all T cells, γδ T cell levels were 2-fold higher in patients with an early CMV reactivation (median, 104 versus 47 cells/μL; $P = .0005$) (Figure 1B).

The Expansion of the $CD8^+$ T Cell Subset was Independent of Pretransplantation CMV Serostatus and Not Seen after EBV and HAdV Reactivation

In univariate analysis, $CD8^+$ T cell numbers—but not $CD4^+$ T cell numbers (data not shown)—were significantly