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Low Counts of Plasmacytoid Dendritic Cells after Engraftment Are Associated with High Early Mortality after Allogeneic Stem Cell Transplantation

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A B S T R A C T

Dendritic cells (DCs) are antigen-presenting cells that drive immune responses and tolerance and are divided in different subsets: myeloid DCs (mDCs: lineage−; HLA-DR+, 11c+), plasmacytoid dendritic cells (pDCs: HLA-DR+, CD123+), and monocyte-derived DCs (moDC: lineage−, 11c+, 16+). After hematopoietic stem cell transplantation (HSCT), low DC counts in the recipients' peripheral blood (PB) have been associated with worse outcomes, but the relevance of DC graft content remains unclear, and there are few data in the setting of unrelated donor HSCT. We evaluated the DC graft content and monitored DC recovery in PB from 111 HSCT recipients (median age, 17 years; range 1 to 74), who received bone marrow (46%), umbilical cord blood (32%), or PB (22%) from unrelated (81%) or related donors (19%). In 86 patients with sustained allogeneic recovery, patients with higher counts of all DC subsets (pDC, mDC, and moDC) 3 weeks after engraftment had lower incidence of nonrelapse mortality (NMR) and acute graft-versus-host disease (aGVHD) and better survival. pDC counts were associated with more striking results: patients with higher pDC counts had much lower incidences of NRM (3% versus 47%, $P < .0001$), lower incidence of aGVHD (24% versus 67%, $P < .0001$), and better overall survival (92% versus 45%, $P < .0001$). In contrast, higher pDC counts in the graft was associated with an increased risk of aGVHD (55% versus 26%, $P = .02$). Our results indicate that DC counts are closely correlated with HSCT outcomes and warrant further prospective evaluation and possible early therapeutic interventions to ameliorate severe aGVHD and decrease mortality.

INTRODUCTION

In recent years, hematopoietic stem cell transplantation (HSCT) has undergone significant improvement. However, the incidence of graft-versus-host disease (GVHD), infection, relapse, and mortality are still relatively high [1]. After transplantation, a cascade of complex and poorly understood events occur, which involves interactions of transferred and newly formed cells of donor origin with host cells. The type and intensity of these interactions will ultimately lead to different grades of acute GVHD (aGVHD), graft-versus-tumor (GVT) effects, and risk of infections, directly contributing to the success of the treatment [1].

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Dendritic cells (DC) are major antigen-presenting cells that act mainly through the activation or inhibition of T cells, leading to activation of Immune responses or tolerance [2-5], depending on their subtype and maturation status [6-8]. DCs represent a heterogeneous population of cells consisting of distinct subsets: myeloid (or conventional) DCs (mDC) and plasmacytoid DCs (pDC). Although mDCs exhibit proinflammatory responses leading to proliferation and activation of $TCD8^+$ cells to become cytotoxic, pDCs are involved on peripheral tolerance, viral host defense, and interferon production [2,3,9,10]. Circulating macrophages may also differentiate into DCs, being a potent allostimulatory cell subtype, referred to as monocyte-derived DCs, 16+/14− monocytes (moDC), or inflammatory DCs [11].

After HSCT, DC recovery in peripheral blood (PB) is relatively slow, and normal counts are only achieved after 3 months or later, depending on the recipients' clinical condition [12-14]. At such early phases (first 3 months), studies have shown that lower DC counts are associated with a higher presence of aGVHD and a poorer overall survival (OS), although their impact on disease relapse remains controversial [15-17]. Besides, some studies addressed the clinical relevance of DC content in the graft with conflicting results [15,18-20]. To date, only a few studies have simultaneously analyzed the pDC graft content, the kinetics of DC recovery, and the HSCT outcomes in the same patient population. In addition, most of these studies only included patients receiving grafts from matched related donors and determined DC counts in fixed time points, independent of day of engraftment or stem cell source.

In the present study, we analyzed DC recovery after transplantation and DC content in the graft in patients receiving allogeneic HSCT, mainly from unrelated donors. Using a novel strategy, considering the day of engraftment as baseline for DC recovery analyses, we investigated the association between the kinetics of DC recovery and the main HSCT outcomes.

PATIENTS AND METHODS

Patients from 4 transplantation centers were included. Each individual provided written informed consent to participate according to the Declaration of Helsinki. The study was approved by each center's local ethics committee.

PB samples were obtained at predefined time points as follows: before the conditioning regimen was administered, at engraftment, at days +3, +7, +14 and +21 after engraftment, and at days +60, +100 and +180 after transplantation. This strategy was used to normalize pDC recovery considering the engraftment date as the baseline in all patients, as engraftment is known to be delayed in umbilical cord blood (UCB) recipients compared with bone marrow (BM) and PB recipients. Graft samples were also analyzed when available (30 BM, 17 UCB, 13 PB).

Cells Populations Identification by Flow Cytometry

T CD4 lymphocytes, T CD8 lymphocytes, pDC (lineage−, HLA-DR high, CD123 high), mDCs (lineage−, CD11c+, CD16−), and moDCs (lineage−, CD11c+, CD16+) were quantified by 4-color flow cytometry. Briefly, fresh EDTA-anticoagulated PB or graft samples (mobilized PB, BM, or UCB) were stained with a combination of fluorochrome-conjugated monoclonal antibodies (CD3 APC, CD4 FITC, CD8 PE, CD11c APC, CD34 APC, CD45 Per-CP, CD123 PE HLA-DR FITC, [Becton Dickinson, San Jose, CA], CD16 PE [Immunotech] and Dendritic exclusion kit [Cytognos, Salamanca, Spain]). Samples were processed within 24 hours, and data were acquired for $\geq 10^5$ leukocytes/tube using the FACSCalibur flow cytometer (BD Biosciences). The Infinicity software (Cytognos) was used for data analysis. All analyses were performed in a central laboratory at UNIFESP by 2 independent investigators (Figure 1).

Statistical Analyses

For the analyses of the main clinical outcomes, patients were divided into 2 groups on the basis of different absolute cell count cutoffs for each time point. Using the Mann-Whitney test, we analyzed all quartiles of cell counts at each time point, and the median values of the absolute counts were used to separate 2 groups (high and low counts), as they showed the best correlation with the studied outcomes: aGVHD and chronic GVHD (cGVHD), diagnosed and graded according to published criteria [21,22], relapse or progression, nonrelapse-related mortality (NRM), progression-free survival (PFS), and OS.

Probabilities of PFS and OS were calculated using the Kaplan-Meier estimator and compared using the log-rank test, and cumulative incidence rates were calculated for aGVHD and cGVHD as well as relapse, with death being considered a competing event. Ninety-five percent confidence intervals (CIs) were estimated using the Greenwood formula. Adjusted probabilities for outcomes after transplantation were estimated using the Cox proportional hazards method. The association between cells counts and HSCT outcomes was investigated in the final multivariate models adjusting for patient-, disease-, and transplantation-related variables with an impact ($P < .01$) in the univariate analyses or if they had been reported to be clinically relevant. First-order interactions between DC counts and each variable of interest were examined. The results are presented as relative risks of failure (adverse prognostic factors versus good prognostic factors), with the 95% CIs and 2-sided P values. SPSS, version 20.0 (SPSS Inc., Chicago, IL), was used for all statistical analyses except for the cumulative incidence analyses, which were performed using S-PLUS software (TIBCO Software Inc., Palo Alto, CA).

RESULTS

Between May 2010 and November 2012, 111 patients (65% male; median age, 17 years; range, 1 to 74) from 4 transplantation centers were included. Chimerism data were evaluated during the first 3 months after HSCT. *Full donor chimerism* was defined as the presence of more than 95% of cells of donor origin. Patients who did not achieve neutrophil recovery or had neutrophil recovery but not full donor chimerism (n = 13), who died 1 week or earlier after engraftment (n = 10), or who were lost from follow-up (n = 2) were excluded from the analyses. There were no significant differences between the included and excluded patients groups regarding any of the clinical features (data not shown). Analyzed patient characteristics are presented in Table 1. Conditioning regimen, graft source, GVHD prophylaxis, time to transplantation, and all other clinical decisions were made according to each center's guidelines.

The most common underlying diagnosis was acute leukemia (70%). Patients received stem cells from BM (48%), UCB (29%), or PB (23%), derived from related (19%) or unrelated donors (81%). Most patients received a myeloablative conditioning regimen (62%), and one half of them (51%) received total body irradiation. T cell depletion with antithymocyte globulin was used in 37% of patients. Median follow-up was 24 months (range, 4 to 47).

Kinetics of DC Recovery

As expected, the median total nucleated cell (TNC) counts were almost 10-fold higher in BM and PB samples (4.43×10^8 TNC/kg) compared with UCB samples ($.56 \times 10^8$ TNC/kg). Similarly, DC counts were also approximately 10-fold higher in BM/PB, although the percentages were comparable among all stem cell sources (.19% in BM/PB versus .20% in UCB). However, no significant differences were noted regarding the kinetics of pDC, mDC, or moDC recovery after HSCT according to the type of stem cell sources used, except for slightly lower pDC counts at engraftment and at day +3 after engraftment and lower mDCs counts during the first 2 weeks in UCB recipients compared with BM/PB recipients. pDC and mDC counts remained relatively stable during the first 3 weeks after engraftment and started to increase at day +60 until day +100 in both patient groups, whereas the moDC count was stable and comparable to normal ranges very soon after engraftment, at day +3.