Quantification of Bone Marrow Plasmacytoid Dendritic Cells after Allogeneic Hematopoietic Stem Cell Transplantation by Flow Cytometry

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INTRODUCTION

The immune system is orchestrated by dendritic cells (DCs), a heterogeneous group of professional antigen-presenting cells. In contrast to conventional dendritic cells (cDCs), plasmacytoid dendritic cells (pDCs) show weak or no immunostimulatory expression. They promote tolerance following solid organ transplant and hematopoietic stem cell transplantation (HSCT) through several tolerogenic immune-modulation pathways. Like cDCs, pDCs originate from CD34+ hematopoietic progenitors in the bone marrow (BM), entering the blood as precursor pDCs and further differentiate into pDCs with expression of CD45, CD123^{bright} HLA-DR and CD4^{low}. After allogeneic hematopoietic stem cell transplantation (allo-HSCT), reconstitution of pDC numbers tends to be slow, both cDC and pDC counts recover to pre-HSCT levels within 2 months. However, return to normal levels takes 1 year for cDCs and even longer for pDCs. Here we report immune reconstitution of pDCs in the BM of 48 patients with leukemia or other malignancies following allo-HSCT and its correlation with the transplantation outcome.

Table 1 Patient Characteristics and Details of Stem Cell Transplants (n = 48)

Characteristics	n (%) median (range)
Patients	
Males	25 (52%)
Age	(years) 50 (20-69)
Diagnosis	
Acute myelogenous leukemia	28 (58%)
Acute lymphoblastic leukemia	6 (13%)
Chronic myelogenous leukemia	2(4%)
Myelodysplastic syndrome	6 (13%)
Non-Hodgkin lymphoma	5 (10%)
Aplastic anemia	1 (2%)
Status at the time of transplant	
CR	26 (54%)
PR	8 (17%)
NR	5 (10%)
Relapse	2 (4%)
Not applicable*	7 (15%)
Conditioning regimen	
Myeloablative	26 (54%)
Bu(4)/Flu	19 (40%)
Cy/TBI	7 (14%)
Reduced intensity	22 (46%)
Bu(2)/Flu	16 (33%)
Flu/Mel	2 (4%)
Mel	1 (2%)
TLI/ATG/Zev	2 (4%)
Flu/Cy/ATG	1 (2%)
Type of stem cell transplant (SCT)	
Peripheral Blood SCT	44 (92%)
Bone Marrow SCT	4 (8%)

ATG: antithymocyte globulin; Bu:busulfan; Cy: cyclophosphamide; Flu: fludarabine; Mel: melphalan; TBI: total body irradiation; TLI: totoal lymphoid irradiation; ZEV: Zevalin

*Not applicable include aplastic anemia, Myelodysplastic syndrome and non-Hodgkin lymphoma

PATIENT SAMPLES AND METHODS

Following approval from the institutional review board (IRB) the electronic medical record database was searched to identify cases of leukemia and patients with other hematological malignancies who received stem cell transplantation. We defined pDCs as CD45+CD123^{bright}HLA-DR+CD4^{low} cells. Multi-parameter flow cytometry analysis was used to detect CD123^{bright}HLA-DR+CD4^{low} population in the CD45+ BM regenerating cells 6 months (median) after allo-HSCT in 48 patients with either leukemia or hematological malignancies. Patient characteristics and details of stem cell transplants are summarized in Table 1. pDCs levels (%) in the BM detected at 6 months (median) along with patients' stem cell engraftment level, severity of acute GVHD, event-free survival, relapse and death are analyzed at different follow up duration. This study was approved by the local ethics committee (University of California at Davis). Treatment of patients was not affected by this study and no additional procedures were performed.

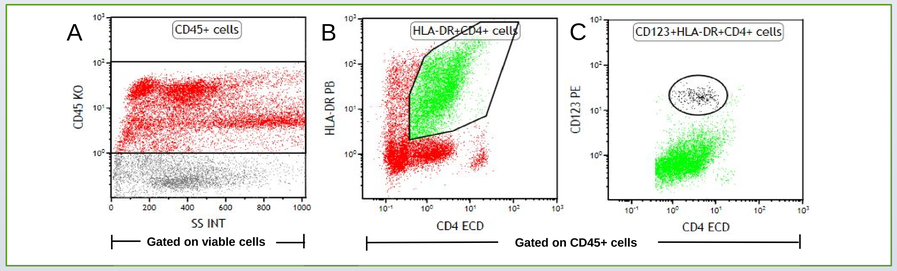

Figure 1. Representative flow cytometry diagram shows sequential gating strategy for detection of pDCs (CD45+CD123^{bright}HLA-DR+CD4^{low}) in the BM aspirate after allo-HSCT. CD45+ cells were gated on viable cells (A); HLA-DR+CD4^{low} were gated on CD45+ population (B); the expression of CD123 was shown in (C), gated on CD45+HLA-DR+CD4^{low} cells. The pDC population (CD45+CD123^{bright}HLA-DR+CD4^{low}) was shown as black dots in (C). Median detection time was 6 months.

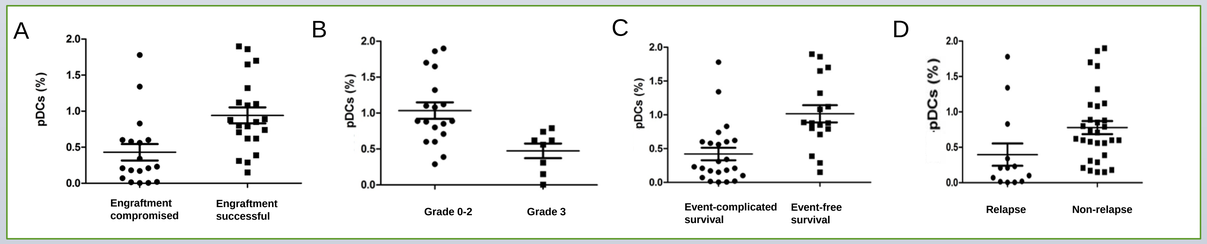

Figure 2. Significant association between the levels of pDC population and transplantation outcomes after allo-HSCT. Higher percentages of pDCs were seen in patients with engraftment success compared to those engraftment compromised (A) (P = 0.001), acute GVHD Grade 0 - 2 compared to those with Grade 3 (B) (P < 0.001), event-free survival compared to those event-complicated (C) (P < 0.001), non-relapse compared to those relapsed (D) (P < 0.05). Each dot represents pre-pDCs percentage in the BM aspirate from each patient; error bars represent SEM. Engraftment success is defined as 98-100% chimerism; "engraftment compromised" includes those with partial chimerism or no engraftment. Median follow up time was 16 months.

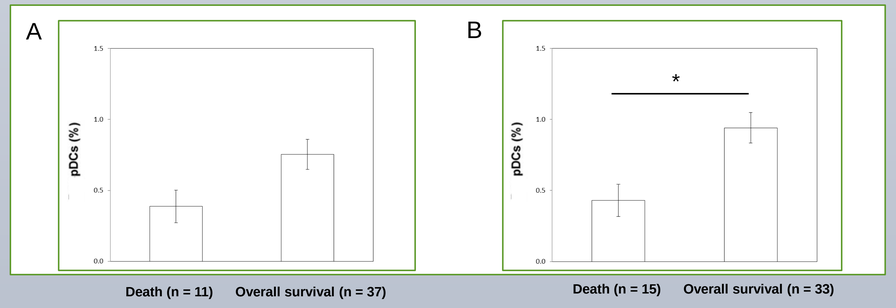

Figure 3. Association between the levels of pDC population and overall survival. Higher percentages of pDC were seen in survived patients compared to those succumbed to their diseases at 16 months (median follow up duration) (A) and 30 months (median follow up duration) (B) after allo-HSCT (*P < 0.05). Data are presented as mean ± SEM.

RESULTS & DISCUSSIONS

To investigate the immune reconstitution of pDCs and its association with the clinical outcome of the patients after allo-HSCT, flow cytometry was used to quantitate the percentages of CD45+CD123^{bright}HLA-DR+CD4^{low} pDCs from the BM (Figure 1A-C). Our data have shown that higher level of the pDCs was significantly higher in cohorts with engraftment success (P=0.001, Figure 2A), less severity of acute GVHD (P<0.001, Figure 2B); event-free survival (P<0.001, Figure 2C) and non-relapse status (P<0.05, Figure 2D). Consistently, higher levels of pDCs were seen in the BM from the survived patients compared to those who succumbed to their diseases (P<0.05, Figure 3A-B).

Reddy et al showed that a low DC recovery (including pDCs) at the time of engraftment is associated with a worsened clinical outcome in HSCT patients who received standardized-intensity myeloablative conditionings. Soon after that, Mohty et al showed a similar phenomenon in patients received reduced-intensity myeloablative conditioning and demonstrated that pDCs in the peripheral blood (PB) correlate favorable outcome. ELze et al [3] analyzed different types of DCs including pDCs in pediatric patients. Their data showed that the time-trend of pDCs in the peripheral blood for days 0-200 is a significant predictor of relapse-free survival.

It has been known that pDCs originate from CD34+ hematopoietic progenitors in the BM, entering the peripheral circulation as precursor pDCs. Pre-pDCs further differentiate into pDCs in BM and PB. [4] It has been shown that both the frequencies and absolute numbers of BM-pDCs were significantly greater than those of PB-pDCs [5, 6] and they were less mature than PB-pDCs.[6] pDCs in the BM is potentially superior to that from PB to be a sensitive indicator to predict pDC recovery speed, functional status and the level of a homeostatic set-point.

In summary, our data have shown that higher levels of pDCs reconstituted from HSCT facilitate successful engraftment and associate with event-free survival, less severity of GVHD and lower incidence of relapse and death. Therefore, quantification of pDCs in the BM using flow cytometry in early stage of HSCT is a rapid method to predict HSCT engraftment and the overall survival.

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