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Enumeration of bone marrow plasmacytoid dendritic cells by multiparameter flow cytometry as a prognostic marker following allogeneic hematopoietic stem cell transplantation

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ABSTRACT

Plasmacytoid dendritic cells (pDCs) promote tolerance in solid organ transplants and hematopoietic stem cell transplantation (HSCT). pDCs originate from $CD34^+$ hematopoietic progenitors. Following allogeneic hematopoietic stem cell transplant (allo-HSCT), pDC reconstitution in the BM and PB gradually attain levels similar to those in healthy individuals. We have investigated the recovery of pDC following allo-HSCT as a means to predict successful marrow engraftment. We retrospectively studied immune reconstitution of pDC in the BM of 48 patients following allo-HSCT for initial diagnoses of leukemia or other malignancies. Multi-parameter flow cytometry was used to detect the $CD45^+$ $CD123^{bright}$ HLA-DR^+ $CD4^{low}$ pDCs in BM aspirates at 2–14 months (median 6 months) post allo-HSCT. Percentages of pDCs were analyzed along with engraftment, acute graft-versus-host disease (aGVHD), event-free survival, relapse and death over a period of up to 39 months (median 30) following HSCT. We report that higher levels of pDCs in the BM post-HSCT are associated with successful engraftment, less severity of aGVHD, lower relapse rate, higher event-free survival and overall survival (P value < 0.05 for all). pDC levels detected at a shorter time interval 2–8 months (median 5 months) following HSCT also showed similar results. We conclude that pDC numbers are associated with HSCT engraftment and overall survival. Flow cytometry offers rapid quantification of pDCs as an early predictor of outcome following HSCT.

1. Introduction

Little is known about the function of resident bone marrow (BM) plasmacytoid dendritic cells (pDCs). BM engraftment after hematopoietic stem cell transplantation (HSCT) entails successful stem cell homing and repopulation in the recipient BM, and re-establishment of effective hematopoiesis. Early engraftment after HSCT is manifested by the release of neutrophils and monocytes into the blood. Numerous approaches have been used to identify reliable biomarkers to predict early engraftment that precede a rise in absolute neutrophil count (ANC > 500/μL) [1,2]. Though it has been established that the appearance of monocytes precedes granulocytes, platelets, and natural killer cells during engraftment, monocytes are not used as an engraftment marker, because the number of monocytes, even under normal conditions, is affected by many clinical conditions [3].

The functional immune system is partly regulated by dendritic cells (DCs), a heterogeneous group of professional antigen-presenting cells (APCs). There is considerable evidence that pDCs express little or no immunostimulatory molecules, which distinguish them from conventional dendritic cells (cDCs) [4]. They have an ability to promote tolerance following solid organ transplants and HSCT. Like cDCs, pDCs originate from $CD34^+$ hematopoietic progenitors in the BM, enter the blood as precursor pDCs and further differentiate into pDCs [5]. After allo-HSCT, both cDC and pDC counts gradually recover after 1–2 months [6]. However, returning to normal levels takes up to one year for cDCs and even longer for pDCs [6].

pDCs in adult BM show a continuous maturation spectrum. They consistently express CD45, $CD123^{bight}$, HLA-DR, $CD4^{low}$, CD303 and CD304; they express CD34 during their early stage but lose this as they mature. They do not express “lineage” (Lin), CD3 and CD11c [5,6,7,8]. Precursor-pDCs further differentiate into pDCs in the BM and are then released into the circulation, thus pDCs have been identified in both BM and peripheral blood (PB), with numbers of BM-pDCs being greater than PB-pDCs [9]. For example, Szabolcs et al. have reported five-fold greater numbers of pDCs in human BM than in PB [10].

BM-pDCs consistently have lower levels of HLA-DR expression,

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measured as mean fluorescence intensity (MFI), than PB-pDCs, suggesting that BM-pDCs are more immature, and therefore more prone to be tolerogenic than PB-pDCs. Therefore, pDC number in BM is potentially superior to that from PB as a sensitive indicator of pDC recovery status.

DCs are potent APCs and pDCs play an important role in linking the innate and adaptive immune systems. Although their importance in the healthy immune system and a variety of diseases is well established, the role of each category of DCs, especially pDC, in reconstituting the cellular immune repertoire after allo-HSCT for leukemia and other malignancies has not yet been fully elucidated. In this study, we use flow cytometry to quantify pDCs in the BM and investigate their roles following HSCT. We suggest that BM-pDCs can serve as a useful supplementary marker for prediction of engraftment after HSCT.

2. Materials and methods

2.1. Patients

In this retrospective study we recorded pDC recovery (see Flow cytometry analysis section), stem cell engraftment, acute GVHD complications, event-free survival, relapse and death following allo-HSCT in 48 patients with an initial diagnosis of leukemia or other malignancy. Patient demographic information is summarized in Table 1. All patients were followed up after transplantation with defined endpoints of relapse or death. Median follow-up time for patients who did not experience relapse or death was 30 months.

The study was approved by the local IRB (University of California at Davis). Treatment of patients was not affected by this study and no additional procedures were performed.

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[a]	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: total lymphoid irradiation; ZEV: Zevalin.

[a] Not applicable include aplastic anemia, Myelodysplastic syndrome and non-Hodgkin lymphoma.

Engraftment was defined as an absolute ANC > 500 for 3 consecutive days together with evidence of full donor chimerism using non-HLA DNA assays to identify unique donor and recipient alleles (sensitivity of 1–5%) [11]. We define “successful engraftment” as patients with 98–100% donor chimerism. “Event-free survival” refers to patients without subsequent hospitalization due to severe infection or CBC abnormality that requires transfusion.

2.2. Flow cytometry analysis

Two to fourteen months (Median 6) after allo-HSCT, BM aspirate specimens were collected in EDTA and labeled within 24 h of collection with a panel of antibodies. The antibodies, devices, gating strategy, quality control methods and method for the enumeration of cell population were previously described [12,13]. Specimens were analyzed using a Beckman Coulter Gallios™ 10 color flow cytometer for the following markers: CD2, CD4, CD5, CD7, CD11b, CD13, CD15, CD22, CD33, CD34, CD36, CD38, CD45, CD56, CD64, CD117, CD123, CD303, and HLA-DR. Data analysis was performed using Kaluza analysis software [14].

In this study, at least 20,000 events were acquired for each sample. For analysis, cell populations were gated according to characteristic forward and side scatter properties, in conjunction with antigen backgating. In the forward/side scatter (FSC/SSC) plot, pDC are found directly below monocytes in the two-dimensional scattergram (same size, less SSC). In the CD45/SSC plot, pDC had dimmer CD45 + than monocytes and less SSC. All pDC were $CD22^{dim+}$, $HLA\text{-}DR^{+}$, $CD303^{+}$, $CD123^{+}$, $CD45^{+}$, $CD38^{+}$, $CD36^{+}$, $CD117^{-}$, $CD64^{-}$, $CD56^{-}$, $CD34^{-}$, $CD15^{-}$, $CD13^{-}$, $CD11b^{-}$, and $CD5^{-}$. Interesting patterns of $CD34^{+}$/$CD45^{+}$ were noted in BM pDC. No non-monocytic $CD4^{+}/CD3^{-}$ clusters were identifiable in cases without pDC. Percentages of $CD45^{+}$ $CD123^{bright}HLA\text{-}DR^{+}$ $CD4^{dim}$ cells were quantified following the sequential gating strategy shown in Fig. 1A-C.

2.3. Statistical analysis

Data were analyzed and graphed using GRAPHPAD PRISM version 5.0 (GraphPad Software Inc., San Diego, CA, USA). Results were expressed as mean ± SEM. The Mann-Whitney U test was used to compare the statistical difference between groups. Fisher's exact test was used to compare specifically the proportions of MRD between groups. A P-value < 0.05 was considered statistically significant.

3. Results and discussion

The most widely used marker of myeloid engraftment following transplantation is the time, from nadir, to when the absolute neutrophil count (ANC) reaches 500/μL. This is attained, on average, around 14 days post-transplant, but is occasionally delayed up till 1 month post-transplant. Extensive supportive care is very important during the neutropenic period to prevent opportunistic infections or other complications before engraftment is complete. Alternative metrics for engraftment, including studies of DCs have been hampered by their low frequencies in the marrow and by the lack of specific DC markers.

The clinical characteristics of 48 patients recruited in this study are shown in Table 1. The most frequent indication for transplantation was acute myelocytic leukemia (AML, 28 patients), followed by acute lymphocytic leukemia (ALL, 6 patients), myelodysplastic syndrome (MDS, 6 patients), Non-Hodgkin lymphoma (NHL, 5 patients), chronic myelocytic leukemia (CML, 2 patients), and aplastic anemia (AA, 1 patient). All patient information was recorded for at least 2 years after transplantation or until death.

To investigate the immune reconstitution of pre-pDCs and its association with the clinical outcome of patients following allo-HSCT, flow cytometry was used to quantify the percentages of $CD45^{+}$ $CD123^{bright}$ $HLA\text{-}DR^{+}$ $CD4^{dim}$ pDCs in BM (Fig. 1A–C). We report that higher levels