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Phenotypic and functional comparison of mobilized peripheral blood versus umbilical cord blood megakaryocyte populations

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Abstract

Background aims. Hematopoietic stem cell transplantation of mobilized peripheral blood progenitor cell (PBPC) products results in rapid platelet engraftment, whereas the use of cord blood (CB) shows significant delays. The difference in the quality and number of megakaryocyte (MK) progenitors that may be responsible for the delay in platelet engraftment has not been fully defined. The objective of this study was to quantify the cells of the MK lineage in PBPC and CB products to determine whether potential differences exist. *Methods.* We examined PBPC or CB for differences in surface markers and subpopulations as well as polyploidization status within the MK lineage. Colony-forming assays were used to determine whether differences exist in the clonogenic MK progenitor cell. Finally, we transplanted PBPC and CB mononuclear cells into NOD/SCID/IL2Rγ−/− (NSG) mice to study platelet engraftment rates. *Results.* Equivalent MK populations and polyploidization was observed in PBPCs and CB. MK progenitors were present only in CD34+ cells and had little difference in colony growth between PBPC and CB. Additionally, MK subpopulations were similar in either product with a slightly more progenitor-enriched phenotype in CB. Finally, when PBPC or CB was transplanted at similar doses, equivalent platelet engraftment rates were observed. *Conclusions.* PBPC and CB contain similar frequencies of MK populations, and, when transplanted in comparable doses, CB is as effective as PBPCs in producing platelet engraftment *in vivo*. Understanding the differences in MK populations between PBPC and CB could help generate protocols to improve platelet engraftment after CB transplantation.

Key Words: *cord blood, megakaryocyte, peripheral blood progenitor cell, platelet engraftment*

Introduction

Allogeneic hematopoietic stem cell (HSC) transplantation is used to effectively treat a wide variety of hematologic malignancies. Bone marrow (BM) was first used as the source for hematopoietic support, becoming successful on understanding the role the human leukocyte antigens (HLA) and proper matching of donor BM cells with the patients. Transplantation with unrelated BM after myeloablative therapy results in recovery from neutropenia in a median of 18 days, whereas recovery from thrombocytopenia occurs in a median of 32 days [1]. Comparison studies of autologous or allogeneic BM demonstrate similar times to neutrophil and platelet recovery [2,3]. However, the invasiveness of BM harvest prompted investigators to find alternative cellular sources for transplantation. Circulating peripheral HSCs had limited success in early clinical transplantations, but technological advances in apheresis and cryopreservation enabled investigators to concentrate numbers of peripheral blood (PB) HSCs and improvements in time to engraftment were observed [4,5]. The use of granulocyte colony-stimulating factor (G-CSF)-mobilized PB progenitor cells (PBPCs) promoted more rapid platelet recovery in a median of 15 days when compared with BM (median, 39 days), whereas the time to neutrophil engraftment was 9 days with PBPC and 10 days with BM recovery [6–8].

Although the use of PBPC has improved the success of HSCT, a significant number of patients needing an allotransplant lack a suitable HLA-matched adult donor. The use of cord blood (CB) as a cellular source for transplantation has provided an alternative approach for such patients [9]. Compared with allogeneic BM, CB transplantation is associated with reduced graft-versus-host disease and similar rates of survival but results in delayed

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engraftment of neutrophils (26 days with CB, 18 days with BM) and platelets (44 days with CB, 24 days with BM) [10]. In clinical studies, double CB transplantation was established to increase the cell doses available for larger patients. With double CB transplantation, however, the time to engraftment remained delayed with 26 days to neutrophil and 53 days to platelet recovery [11].

It is unclear why such discrepancies exist between PBPC and CB transplantation with regard to platelet recovery. The differences between PBPC and CB transplantations might be due to the quality of the engrafting population responsible for platelets or simply to the lower cell dose in CB grafts. On the basis of clinical evidence, CB units might have lower doses or completely lack unique MK populations responsible for rapid platelet recovery that are contained within BM and PBPC grafts. In the present study, we compared the MK lineage-specific phenotypic surface expression including known MK subpopulations as well as the polyploidization status of the MKs to identify these potential differences. Additionally, we performed transplantation studies with increasing doses of CB and PBPCs in NOD/SCID/IL2Rγ−/− (NSG) mice to effectively compare the two sources in promoting fast engraftment of total hematopoietic cells and platelets.

Methods

CB and mobilized PBPCs

CB products and G-CSF–mobilized PBPCs were obtained under MD Anderson Cancer Center Institutional Review Board–approved protocols with informed consent. Blood was layered over Histopaque (Sigma), and mononuclear cells (MNCs) were collected from the buffy coat.

Flow cytometry phenotypic analysis

Cell surface phenotyping of MKs was performed with the use of anti-CD45 peridinin chlorophyll, anti-CD61 allophycocyanin, anti-CD34 allophycocyanin-e780, anti-CD41a V500 or anti-CD42b phycoerythrin/Pecy5 monoclonal antibodies. Antibodies were obtained from either eBioscience or BD Biosciences. Cells were acquired on a LSR Fortessa (BD Biosciences) and analyzed with the use of FlowJo (Tree Star) software. A minimum of 50,000 events were acquired.

Megakaryocyte polyploid analysis

Cells were stained with cell surface markers (CD61, CD41a, CD42b, CD45 and CD34) and subsequently washed. Cells were then fixed with Foxp3 Perm Buffer set (eBioscience) according to the manufacturer's instructions. After fixation, cells were incubated with 100 ng/test RNAse for 15 min at room temperature in darkness before staining with 400 μL propidium iodide (Sigma) (40 μg/mL). Cells were acquired by means of the LSR Fortessa and analyzed with the use of FlowJo software. A minimum of 500,000 events were acquired.

CD34+ isolation

CD34+ cells were isolated by means of MACS (Miltenyi Biotech) magnetic column separation. CB or PBPC MNCs were stained with CD34 Microbeads (Miltenyi Biotec) and selected according to the manufacturer's instructions. CD34− cells were also retained from the eluted fractions.

Colony-forming unit–megakaryocyte assay

Graded cell doses were plated into a collagen-based Megacult-C megakaryocyte colony-forming assay (Stem Cell Technologies, Vancouver, British Columbia, Canada). The assay was performed according to the manufacturer's instructions. The assay was cultured in a 150-mm Petri dish with an open 35-mm sterile water dish and placed in a 37°C, 5% CO_2 incubator for 14 days. Slides were fixed and stained with anti-CD41a antibody. Images of colony-forming unit–megakaryocyte (CFU-MK) colonies on microscope slides were taken with the use of an Olympus DP-10 camera (Olympus Imaging).

Transplantation

NSG mice were sublethally irradiated with 300 rad, 24 h before transplantation. PBPC and CB MNC samples were prepared in phosphate-buffered saline and transplanted into mice by tail vein injection. Mice were bled twice weekly by retro-orbital vein collection. Samples were split into two, with half used to perform a CBC/DIFF count with the use of an ADVIA120 Hematology Analyzer (Siemens Corporation). The other half was red blood cell (RBC)-lysed with RBC Lysis 1× (Biolegend) for 5 min and washed twice. Cells were first stained with anti-CD41a fluorescein isothiocyanate and anti-CD45 phycoerythrin murine monoclonal antibodies for 30 min and washed. Cells were subsequently stained for anti-CD61, anti-CD41a and anti-CD45 human monoclonal antibodies for 30 min and washed. Before acquiring cells on the flow cytometer, 25 μL of counting beads (Spherotech) was added to