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Graft Monocytic Myeloid-Derived Suppressor Cell Content Predicts the Risk of Acute Graft-versus-Host Disease after Allogeneic Transplantation of Granulocyte Colony-Stimulating Factor–Mobilized Peripheral Blood Stem Cells

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A B S T R A C T

Myeloid-derived suppressor cells (MDSCs) are powerful immunomodulatory cells that in mice play a role in infectious and inflammatory disorders, including acute graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation. Their relevance in clinical acute GVHD is poorly known. We analyzed whether granulocyte colony-stimulating factor (G-CSF) administration, used to mobilize hematopoietic stem cells, affected the frequency of MDSCs in the peripheral blood stem cell grafts of 60 unrelated donors. In addition, we evaluated whether the MDSC content in the peripheral blood stem cell grafts affected the occurrence of acute GVHD in patients undergoing unrelated donor allogeneic stem cell transplantation. Systemic treatment with G-CSF induces an expansion of myeloid cells displaying the phenotype of monocytic MDSCs ($Lin^{low/neg}HLA\text{-}DR^{-}CD11b^{+}CD33^{+}CD14^{+}$) with the ability to suppress alloreactive T cells in vitro, therefore meeting the definition of MDSCs. Monocytic MDSC dose was the only graft parameter to predict acute GVHD. The cumulative incidence of acute GVHD at 180 days after transplantation for recipients receiving monocytic MDSC doses below and above the median was 63% and 22%, respectively ($P = .02$). The number of monocytic MDSCs infused did not impact the relapse rate or the transplant-related mortality rate ($P > .05$). Although further prospective studies involving larger sample size are needed to validate the exact monocytic MDSC graft dose that protects from acute GVHD, our results strongly suggest the modulation of G-CSF might be used to affect monocytic MDSCs graft cell doses for prevention of acute GVHD.

INTRODUCTION

Myeloid-derived suppressor cells (MDSCs) consist of a group of morphologically and functionally heterogeneous population of myeloid progenitor cells, dendritic cells, and immature myeloid cells that suppress immune responses in vivo and in vitro [1]. In mice, the expression of the Gr-1 antigen (Ly6C/Ly6G) and cellular morphology have been used to characterize 2 major populations of MDSCs: granulocytic MDSCs expressing $CD11b^{+}Ly6G^{+}Ly6C^{int}CD115^{low}$ and monocytic MDSCs (M-MDSCs) expressing $CD11b^{+}Ly6G^{-}Ly6C^{+}CD115^{+}$ [2,3].

Human MDSC phenotype is less defined. Both granulocytic and monocytic human MDSCs express CD33, CD11b, and low/negative levels of HLA-DR with variable expression of CD15 (granulocyte MDSC) and CD14 (M-MDSC). These populations are able to inhibit alloreactive responses mediated by T lymphocytes and natural killer (NK) cells through a variety of mechanisms that include L-arginine depletion by arginase 1 and the inducible nitric oxidase, generation of reactive oxygen species, release of transforming growth factor-β and IL-10, cysteine sequestration, and regulatory T cell (Treg) induction [1,4]. In physiological situations, immature myeloid cells mature into dendritic cells, macrophages, and neutrophils/granulocytes upon entering the peripheral blood. In pathological conditions, growth factors boost immature myeloid cell expansion and interfere with their normal differentiation, inducing the MDSC phenotype [1].

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Table 1
Patient, Graft, and Transplant Characteristics

Characteristic	Value
Number of patients	60
Number of donors	60
Median patient age at transplant, yr (range)	44 (18-67)
Median donor age, yr (range)	34 (20-43)
Median follow-up, mo (range)	17 (13 days to 53 mo)
Diagnosis	
Acute myeloid leukemia	6 (10%)
Non-Hodgkin lymphoma	21 (35%)
Multiple myeloma	12 (20%)
Hodgkin disease	17 (28%)
Chronic lymphocytic leukemia	4 (6%)
Male/female patients	39/21 (65%/35%)
Male/female donors	46/14 (77%/23%)
HLA disparity	
10/10 alleles	30 (50%)
9/10 alleles	17 (28%)
8/10 alleles	13 (22%)
Conditioning regimen	
Cyclophosphamide (100 mg/kg) + thiotepa (10 mg/kg)	19 (32%)
Cyclophosphamide (60 mg/kg) thiotepa (10 mg/kg) + fludarabine (60 mg/m^2)	17 (28%)
Cyclophosphamide (60 mg/kg) + thiotepa (10 mg/kg) + fludarabine (120 mg/m^2) + total body irradiation (2 Gy)	2 (3%)
Fludarabine (90 mg/m^2) + melphalan (100-140 mg/m^2)	15 (25%)
Fludarabine (90 mg/m^2) + total body irradiation (2 Gy)	4 (7%)
Fludarabine (150 mg/m^2) + busulfan (.8 mg/kg)	3 (5%)
GVHD prophylaxis	
Methotrexate with cyclosporine	52 (87%)
Mycophenolate mofetil with cyclosporine	8 (13%)

Although MDSCs have been primarily studied in cancer patients in which they are responsible of immune escape phenomena [5,6], there has been growing interest in understanding their role in infectious and inflammatory disorders including acute graft-versus-host disease (aGVHD) after allogeneic hematopoietic stem cell transplantation (allo-HSCT). GVHD represents 1 of the most frequent complications of allo-HSCT and remains a major cause of morbidity and mortality related to this therapy. Advances in aGVHD prevention and treatment, new conditioning regimens, and better donor selection have improved the outcomes of patients undergoing allo-HSCT [7].

In preclinical mouse models, cotransplantation of functional MDSCs in a fully MHC-mismatched mouse model of HSCT led to a decrease in aGVHD severity and mortality without abrogating the graft-versus-tumor (GVT) effect [8]. These cells were obtained in vitro through exposure of bone marrow cells to granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and IL-13. Additional evidence to support the involvement of MDSCs in aGVHD was shown in patients in which a specific subset of MDSC expressing the monocytic CD14 marker and low levels or lack of the antigen presenting HLA-DR molecules (CD14$^+$HLA-DR$^{low/neg}$ cells) accumulate after allo-HSCT [9]. These cells suppressed the proliferation of autologous T cells and their frequency correlated significantly with the serum levels of G-CSF, which in fact has been demonstrated to induce T cell–suppressive CD14$^+$ myeloid cells [10].

Based on these findings, the aim of the present study was first to investigate the effect of G-CSF administration, used to mobilize hematopoietic stem cells, on the frequency of CD14$^+$ M-MDSCs (Lin$^{low/neg}$HLA-DR$^-$CD11b$^+$CD33$^+$CD14$^+$) in the peripheral blood stem cell (PBSC) grafts of 60 unrelated donors and second to search for a correlation between the number of M-MDSCs infused with the graft and the incidence of aGVHD.

METHODS

Patients and Donors

All patients were transplanted from their respective unrelated donors at the Fondazione IRCCS Istituto Nazionale dei Tumori, Milan. Written informed consent was obtained, and the institutional review board approved the study (Comitato Etico Indipendente, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, protocol number INT45/12; 4/24/2012). Patients, donors, and transplant characteristics are described in Table 1. Stem cell mobilization, collection, treatment, and storage were done according to the standard operating procedures approved by the Joint Accreditation Committee of International Society for Cellular Therapy Europe and the European Group for Blood and Marrow Transplantation.

Donors were mainly young men (46 men versus 14 women; median age 34 [range, 20 to 43]) treated with G-CSF [filgrastim] 10 μg/kg/day for 5 days before leukoapheresis. All patients received allo-HSCT for hematologic malignancies (Table 1). Three patients (5%) received fludarabine (150 mg/m^2) and busulfan (.8 mg/kg), 4 patients (7%) received fludarabine (90 mg/m^2) combined with low-dose total body irradiation (2 Gy), and all other patients received a conditioning regimen based either on fludarabine (90 mg/m^2) and melphalan (100 to 140 mg/m^2; 15 patients [25%]) or thiotepa (10 mg/kg) and cyclophosphamide (60 to 100 mg/m^2; 38 patients [63%]) with or without fludarabine (60 to 120 mg/m^2) (Table 1). GVHD prophylaxis consisted of cyclosporine and short-course methotrexate (n = 52 [87%]) or mycophenolate mofetil (n = 8 [13%]). Diagnosis and clinical grading of aGVHD were performed using the international standard criteria [7]. Sixty age-matched healthy control subjects were also included in the study.

Chimerism Analysis

Chimerism analysis was carried out as previously described [11] on peripheral blood collected monthly after transplantation.

Flow Cytometry and Graft Content Analysis

Sixty PBSC grafts collected by apheresis procedure were analyzed by flow cytometry at the moment of transplantation, and the number of CD34$^+$ hematopoietic stem cells, total CD3$^+$ lymphocytes, CD4$^+$ helper and CD8$^+$ cytotoxic T cells, CD19$^+$ B lymphocytes, and CD16$^+$-CD56$^+$ NK cells was determined using Trucount tubes containing fluorescent beads as an internal standard (BD Biosciences, San Jose, CA) [12] and the appropriate monoclonal antibodies (Supplemental Table 1). M-MDSCs (defined as Lin$^{low/neg}$HLA-DR$^-$CD11b$^+$CD33$^+$CD14$^+$), Tregs (CD4$^+$CD25$^+$CD127$^-$FoxP3$^+$), and invariant NK T cells (iNKT; CD3$^+$TCRVα24-Jα18$^+$TCRVβ11$^+$CD4$^+$ and CD3$^+$TCRVα24-Jα18$^+$TCRVβ11$^+$CD4$^-$) were analyzed by flow cytometry retrospectively on frozen samples [6,13] using the listed fluorochrome-coupled monoclonal antibodies (Supplementary Table 1). Staining of cells was performed at 4°C for 20 minutes in the dark in fluorescent activated cell sorter staining buffer (1× PBS supplemented with 2% FBS). For intracellular FoxP3 staining, after extracellular staining, cells were permeabilized and stained according to manufacturer instructions (FoxP3 Staining Kit; Miltenyi Biotec, Bergisch-Gladbach, Germany). Cell acquisition and analysis were performed on a FacsCalibur cytometer using CellQuest software (BD Biosciences) or on a MACS Quant cytometer using MACS Quantify Software (Miltenyi Biotec). A minimum of 5 × 10^5 events were collected for accurate data acquisition.

Immunomagnetic Cell Separation

Immunomagnetic separations were performed using Automacs Pro Separator (Miltenyi Biotec). For CD3$^+$ lymphocyte isolation, peripheral blood mononuclear cells isolated by Ficoll density gradient (Lymphoprep; Axis-Shield, Oslo, Norway) were suspended in separation buffer (PBS supplemented with .5% BSA) and incubated with anti-CD3 microbeads (Miltenyi Biotec) for 15 minutes at 4°C. After washing, cells were separated through a double-column positive selection. For M-MDSC enrichment, PBSCs were first incubated with anti-HLA-DR microbeads (Miltenyi Biotec) to deplete HLA-DR positive cells. Thereafter, CD33$^+$ cells were separated using anti-CD33 microbeads (Miltenyi Biotec). Separations were performed according to manufacturer instructions. Purity of the selected populations was evaluated by flow cytometry, demonstrating an efficiency of separation above 90% in all experiments.