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Functional Myeloid-Derived Suppressor Cell Subsets Recover Rapidly after Allogeneic Hematopoietic Stem/Progenitor Cell Transplantation

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A B S T R A C T

Myeloid-derived suppressor cells (MDSCs) are regulatory cell populations that have the ability to suppress effector T cell responses and promote the development of regulatory T cells (Tregs). They are a heterogeneous population of immature myeloid progenitors that include monocytic and granulocytic subsets. We postulated that given the rapid expansion of myeloid cells post-transplant, these members of the innate immune system may be important contributors to the early immune environment post-transplant. To evaluate the kinetics of recovery and function of MDSCs after allogeneic hematopoietic stem cell transplant (HSCT), 26 patients undergoing allogeneic HSCT were studied at 6 time points in the first 3 months after HSCT. Both MDSC subsets recovered between 2 and 4 weeks, well before the recovery of T and B lymphocytes. MDSC subset recovery positively correlated with T, B, and/or double-negative T cell numbers after HSCT. MDSCs isolated from patients post-transplant were functional in that they suppressed third-party $CD4^+$ T cell proliferation and Th1 differentiation and promoted Treg development. In conclusion, functional MDSC are present early after HSCT and likely contribute to the regulatory cell population post-transplant.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) involves rapid expansion of the hematopoietic compartment after infusion of hematopoietic stem cell containing products that have been either harvested from marrow/cord blood or collected by growth factor mobilization. All populations contain large numbers of immature myeloid cells that contain a potent immune regulatory population known as myeloid-derived suppressor cells (MDSCs).

MDSCs are a heterogeneous population of activated immature myeloid progenitor cells; precursors to macrophages, granulocytes, and dendritic cells that have been prevented from fully differentiating into mature cells [1,2]. MDSCs are potent suppressors of T cells through direct cell contact, increased production of arginase 1 (Arg-1), inducible nitric oxide synthase (iNOS), indoleamine 2,3-dioxygenase (IDO) and reactive oxygen species, and induction of regulatory T cells (Tregs) [1,2].

The expansion and functional importance of MDSCs in cancer and noncancer pathogenic conditions has been recognized. MDSC numbers are increased, and they act as immune regulatory cells in autoimmune diseases [3], solid organ transplantation [4,5], and allergic asthma [6]. We have reported that the numbers of MDSCs are positively correlated with the severity of murine colitis and that adoptive transfer of MDSCs isolated from murine colitis models or generated in vitro ameliorate murine intestinal inflammation [7].

Allogeneic HSCT transplantation is associated with a massive expansion of myeloid cell compartment, both in the donor with granulocyte colony-stimulating factor (G-CSF) mobilized hematopoietic stem cell collection and in the recipient early post-transplant. Both G-CSF and granulocyte-macrophage (GM)-CSF have been shown to expand MDSCs [8]. Cytokines prominent in the early post-transplant period,

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such as tumor necrosis factor (TNF)-α, IFN-γ, and IL-6, are known to activate or license MDSCs to become potent suppressors of multiple aspects of the immune system [9]. Small series have suggested that MDSCs are present in the grafts and recipients early after HSCT [10-12].

MDSC are divided into 2 main subsets, monocytic (M-MDSC) and granulocytic (G-MDSC), based on expression of monocytic or granulocytic cell surface markers [1,9]. Each subset may have different functions under varying inflammatory conditions. In a murine asthma model, M-MDSC inhibited whereas G-MDSC exacerbated airway inflammation [6]. Furthermore, timing appears to be important. In a sepsis model, MDSCs exaggerated inflammation in the early stage but suppressed inflammation in the later stage of sepsis [13].

In murine GVHD transplant models, MDSC accumulation in recipient mice post-transplantation was positively correlated with the severity of graft-versus-host disease (GVHD) [14]. The infusion of embryonic stem cell– or bone marrow–derived MDSCs before transplantation provided protection from lethal acute GVHD, leading to long-term survival in 37% [15] or 82% [16] of recipient mice. Adoptive transfer of MDSCs preserved graft-versus-leukemic (GVL) effects of allogeneic host T cells in a GVL model [15]. Mougiakakos et al. [10] found that $CD14^+HLA\text{-}DR^{-/low}IDO^+$ monocytic MDSCs in peripheral blood mononuclear cells (PBMCs) are significantly increased in patients with acute GVHD and that these MDSCs could suppress the proliferation of lymphocytes through IDO in vitro. More recently, another study showed extracorporeal photopheresis treatment in patients with GVHD rapidly increased the percentage of circulating G-MDSCs in PBMCs, which efficiently suppressed effector CD4 T cell responses [17]. G-MDSCs may be underappreciated in previous studies of leukocytes post-transplant because they sediment with red blood cells in conventional density gradient cell preparations (eg, Ficol) and do not survive freeze–thaw well.

To date, reconstitution of MDSC subsets after allogeneic HSCT and the relation of MDSC subsets to acute GVHD have not been well defined. We were interested in understanding the kinetics of MDSC recovery post-HSCT, their functionality, and whether they were associated with acute GVHD.

METHODS

Patients

Patients undergoing allogeneic HSCT were enrolled on a trial evaluating early immune recovery after allogeneic HSCT. This study was approved by the ethics committee of the University of Manitoba. All patients and/or their parents signed informed consent. Patient demographics and transplant outcomes are presented in Table 1.

There was an equal representation of myeloablative (busulfan/fludarabine or total body irradiation based) and nonmyeloablative conditioning regimens. Aplastic anemia patients received fludarabine/cyclophosphamide with adjusted cyclophosphamide dosing for the patient with Fanconi anemia. GVHD prophylaxis was tacrolimus and mini-methotrexate (5 mg/m^2 on days +1, +3, and +6, with an additional dose on day +11 for unrelated donors). Antithymocyte globulin was used in the aplastic anemia patients and if there was less than 10/10 HLA match. Most patients received G-CSF (filgrastim)–mobilized grafts from family or unrelated donors with a target cell dose of 5×10^6 CD34 cells/kg. Filgrastim (5 μg/kg/day) was administered daily starting on day +1 in the cord blood recipient until neutrophil recovery above 1.5×10^9/L but was otherwise not used in the other subjects unless there was delayed count recovery.

All recipients had documentation of donor engraftment. Neutrophil engraftment day was defined as the first day of 3 consecutive days with absolute neutrophil count greater than $.5 \times 10^9$/L. Platelet engraftment day was defined as the first of 7 consecutive days with platelet count greater than 20×10^9/L without transfusion support for at least 7 days. Acute GVHD was graded according to the modified Glucksberg criteria [18]. In the first 100 days post-transplant, 2 patients died (primary disease) and 6 patients had progression or relapse of malignancy.

Table 1
Patient Demographics and Transplant Outcomes (N = 26)

Characteristic	Value
Sex (female/male)	8/18
Disease	
Acute myelogenous leukemia	11
Non-Hodgkin lymphoma	4
Myelodysplastic/fibrotic syndrome	4
Acute lymphoblastic leukemia	3
Chronic lymphocytic leukemia	1
Severe aplastic anemia/Fanconi anemia	2
Common variable immunodeficiency	1
Median age at transplant, yr (range)	43 (13-62)
Donor source	
G-CSF mobilized PBSCs (16 unrelated, 6 related)	22
Bone marrow (2 unrelated, 1 related)	3
Cord blood (1 unrelated)	1
Conditioning regimen	
Myeloablative	13
Nonmyeloablative	13
Acute GVHD	
None or GVHD grade I	13
GVHD grades II-IV	13
Median days to achieve ANC 500/μL (range)	18 (12-28)
Median days of platelet recovery to 20×10^9/L* (range)	21 (13-47)
Median $CD34^+$ stem cells transplanted, $\times 10^6$/kg (range)	
PBSCs	5.4 (2.0-8.0)
Bone marrow	2.7 (1.5-3.8)
Cord blood	.21

PBSC indicates peripheral blood stem cell; ANC, absolute neutrophil count.
* Six patients did not have a nadir of platelets below 20×10^9/L.

Isolation of WBCs

Twenty-four milliliters of peripheral blood was drawn on the day before the start of the conditioning regimen, with 1 additional sample drawn each between days +4 and +5, +7 to +9, +14 to +16, +21 to +23, +27 to +29, and +80 to +100 after HSCT, for a total of 7 samples per subject. WBCs were isolated from peripheral blood using HetaSep (Stem Cell Technologies, Vancouver, Canada) according to the manufacturer protocol. All blood samples were processed and analyzed 24 hours after drawing.

Antibodies and Flow Cytometry

WBCs were stained with fluorescence-labeled antibodies against surface molecules for MDSC subsets (HLA-DR, CD45, CD33, CD15, CD66b, and CD14), B cells (CD19), and T cells (CD3, CD4, and CD8) (eBioscience, San Diego, CA; Biolegend, San Diego, CA) according to manufacturer recommendations. After staining, cells were acquired and analyzed using flow cytometry (FACS Canto II; BD Biosciences, San Jose, CA) and FlowJo software (TreeStar, San Carlos, CA) [10,17]. For intracellular staining of Arg-1 and iNOS, cells were first stained with fluorescence-labeled antibodies against HLA-DR, CD33, CD15, and CD14 and then fixed and permeabilized using eBioscience intracellular fixation/permeabilization buffer. Cells were then stained with Percp-Cy5.5–labeled anti-iNOS (Santa Cruz Biotechnology, Dallas, TX) and PE-labeled anti–Arg-1 (R&D systems, Minneapolis, MN), and analyzed by flow cytometry (FACS Canto II). The FACS Canto II was maintained by daily running BD 7-color microbeads to ensure the accuracy and consistency of the subpopulation analysis. Single staining tubes and isotype control staining tubes were used to do the compensation for all runs.

T Cell Proliferation and Differentiation Assay

To evaluate the function of the MDSC subsets, M-MDSCs and G-MDSCs were enriched by positive selection from the peripheral blood using $CD14^+$ selection kit and $CD15^+$ selection kit (Stem Cell Technologies) according to the manufacturer protocol. The purity of M-MDSC and G-MDSC cells was confirmed by flow cytometry. The control population was $CD14^-CD15^-$ cells that contained all lymphocytes. The suppressive effect of MDSC subsets on lymphocyte proliferation and T cell differentiation was evaluated as described previously [10]. Briefly, third-party PBMCs were isolated from healthy donors and labeled with carboxyfluorescein succinimidyl ester (CFSE). The 1×10^5 CFSE-labeled PBMCs were then stimulated with anti-CD3/CD28 microbeads