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Abnormalities of the Bone Marrow Immune Microenvironment in Patients with Prolonged Isolated Thrombocytopenia after Allogeneic Hematopoietic Stem Cell Transplantation

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A B S T R A C T

Prolonged isolated thrombocytopenia (PT) is a serious complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Whether abnormalities of the bone marrow (BM) immune microenvironment are involved in the pathogenesis of PT remains unknown, however. Twenty patients with PT, 40 matched patients with good graft function (GGF) after allo-HSCT, and 20 healthy donors (HD) were enrolled in this nested case-control study. Th1, Th2, Tc1, Tc2, Th17, and Treg cells were analyzed by flow cytometry, and IFN-γ, IL-4, IL-17, IL-6, IL-21, and thrombopoietin levels in BM plasma were evaluated with a cytometric bead assay and ELISA. Relative to GGF patients and HD controls, PT patients had significantly higher proportions of Th1 and Tc1 cells, resulting in higher Th1/Th2 and Tc1/Tc2 ratios in the BM microenvironment. In addition, the excessive polarization of Th17 was observed in patients with PT. Changes in BM plasma cytokines were consistent with our cellular findings. These results suggest that dysregulated T cell responses in the BM microenvironment might play an important role in the pathogenesis of PT.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is considered an effective treatment for patients with hematologic malignancies, whereas prolonged isolated thrombocytopenia (PT) is a frequent complication after allo-HSCT [1,2]. PT is defined as the engraftment of all peripheral blood cell lines other than a platelet (PLT) count $<20 \times 10^9$/L or dependence on PLT transfusions for more than 60 days after allo-HSCT. Previous reports have identified a low PLT count for 60 days or 100 days after allo-HSCT as an independent risk factor for poor prognosis [3,4]. Several risk factors may influence PT after allo-HSCT, including the dose of infused $CD34^+$ cells, disease status, graft-versus-host disease (GVHD), cytomegalovirus (CMV) infection, and certain drugs [5-8]. In addition to the related factors mentioned above, the exact pathogenesis of PT remains unclear.

PLT production is a consecutive process beginning with the commitment of hematopoietic stem cells to the megakaryocyte (MK) lineage and ending with the fragmentation of MKs [9,10]. This process occurs within a complex bone marrow (BM) microenvironment. Many cell types, including osteoblasts, perivascular cells, endothelial cells, mesenchymal stem cells, and various mature immune cells, contribute to the BM microenvironment. Our previous studies have shown that patients with PT have defective perivascular cells and endothelial progenitor cells in the BM microenvironment, which may be involved in the occurrence of PT [11]; however, less is known about the BM immune microenvironment in patients with PT.

The BM immune microenvironment can influence the process of thrombopoiesis. It has been reported that activated T lymphocytes augment the proliferation of human BM MK progenitor cells in vitro [12]. Another in vitro study suggested that both BM T lymphocytes and adherent cells of the monocyte-macrophage lineage may suppress megakaryocytopoiesis in patients with acquired amegakaryocytic thrombocytopenic purpura [13]. In the

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BM of patients with immune thrombocytopenia (ITP), $CD8^+$ T cells have been shown to significantly inhibit the apoptosis of BM MKs, thereby inhibiting PLT production [14]. In our previous study, we found a dysregulated BM immune microenvironment, including excessive polarization of Th1, Tc1, and Th17 cells and a remarkable decrease in Treg cells, in patients with ITP [15]. Moreover, we found an increased type 1 immune response in the BM microenvironment of patients with poor graft function after allo-HSCT [16]. Therefore, we hypothesize that the occurrence of PT also may be associated with dysregulated immune responses in the BM microenvironment.

To confirm this hypothesis, we conducted a prospective nested case-control study to evaluate whether the levels of T cell subtypes and related cytokines in the BM microenvironment of allo-HSCT recipients with PT differed from those in patients with good graft function (GGF) or in health donor (HD) controls. Our aim was to provide new insights into the pathogenesis underlying PT after allo-HSCT.

MATERIALS AND METHODS

Patients and Controls

A prospective nested case-control study was designed. Cases were identified from patients who underwent allo-HSCT between September 1, 2015, and September 18, 2016 at Peking University Institute of Hematology. A total of 20 patients who had developed PT after allo-HSCT were included. For each case, 2 matched controls with GGF were selected at random from the same cohort at the time when PT occurred. These control cases were matched based on published criteria [11] as follows: age at allo-HSCT (±1 year), underlying disease, pre-HSCT cycles of chemotherapy (±1 cycle), and disease status at HSCT ("risk-set sampling"). The characteristics of the PT patients and GGF controls are summarized in Table 1.

Definitions of PT and GGF

PT was defined as the engraftment of all peripheral blood cell lines (an absolute neutrophil count [ANC] $>.5 \times 10^9$/L and a hemoglobin [Hb] level >70 g/L without transfusion support) other than a PLT count $<20 \times 10^9$/L or the dependence on PLT transfusions for more than 60 days after allo-HSCT in the presence of complete donor chimerism. GGF [16-18] was defined as persistent successful engraftment (ANC $>.5 \times 10^9$/L for 3 consecutive days, PLT count $>20 \times 10^9$/L for 7 consecutive days, and Hb level >70 g/L without transfusion support) beyond 28 days after allo-HSCT. Patients with evidence of poor graft function [16,17,19,20] or severe GVHD, including grade

Table 1
Characteristics of Allo-HSCT Recipients with PT and GGF

Characteristics	PT* (N = 20)	GGF* (N = 40)	*P* Value†
BM evaluated time (post-HSCT days), median (range)	60 (57-65)	61 (56-66)	.89
BM myeloid:erythroid ratio when BM evaluated, mean ± SEM	(2.33 ± .42):1	(2.89 ± .22):1	.64
Blood cell count, median (range)			
WBC ($\times 10^9$/L)	2.89 (1.69-6.38)	3.78 (2.12-6.20)	.02
ANC ($\times 10^9$/L)	2.12 (.75-5.35)	2.95 (.90-5.52)	.03
Hb, g/L	86 (72-115)	106 (75-125)	<.0001
PLT ($\times 10^9$/L)	12 (8-19)	123 (50-230)	<.0001
Age at HSCT, yr, median (range)	41 (18-55)	42 (18-56)	.78
Sex, male/female, n	14/6	25/15	.77
Underlying disease, n			
AML	8	16	1.00
ALL	9	18	1.00
MDS	3	6	1.00
Status at HSCT, n			.78
Standard-risk	8	14	
High-risk	12	26	
Stem cell source, n			1.00
BM and peripheral blood	20	40	
Transplanted total nucleated cell dose ($\times 10^8$/kg), median (range)	7.22 (4.05-9.60)	7.15 (3.80-9.52)	.85
Transplanted $CD34^+$ cell dose ($\times 10^6$/kg), median (range)	2.46 (1.55-5.20)	2.40 (1.26-5.16)	.86
Transplanted lymphocytes cell dose ($\times 10^8$/kg), median (range)	3.06 (1.50-6.90)	3.13 (1.23-7.50)	.86
Donor match, n			1.00
HLA-identical sibling donor	4	7	
HLA-partially matched related donor	16	33	
Sex mismatch, n			
Female to male	5	6	.48
Female to female	1	3	1.00
Male to female	4	9	1.00
Male to male	10	22	.79
ABO mismatch, n			
No	7	12	.77
Minor	9	18	1.00
Major	4	10	.76
Pre-HSCT cycles of chemotherapy, median (range)	3 (0-6)	4 (0-7)	.72
Conditioning, n			1.00
BU/CY	4	7	
BU/CY+ATG	16	33	
History of GVHD, n	14	24	.57
Onset of acute GVHD, d, median (range)	24 (18-56)	22 (15-53)	.82
History of CMV reactivation, n	17	23	.04
Onset of CMV reactivation, d, median (range)	29 (21-52)	28 (20-50)	.82
CMV reactivation treated with ganciclovir, n	13	17	.17
History of EBV reactivation, n	5	7	.51

AML indicates acute myelogenous leukemia; ALL, acute lymphocytic leukemia; MDS, myelodysplastic syndrome;

* Group matching criteria included age at HSCT (±1 years), pre-HSCT cycles of chemotherapy (±1 cycle), disease status at HSCT and BM microenvionment evaluated time after HSCT (±5 days). For each case, 2 GGF controls were randomly selected from the same cohort at which the PT occurred ("risk-set sampling").

† The continuous variables were compared using the Mann-Whitney *U* test, and the differences in frequency between the 2 groups were compared using the chi-square test. The criterion for statistical significance was $P < .05$.