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Reconstitution of lymphocyte subpopulations after hematopoietic stem cell transplantation: comparison of hematologic malignancies and donor types in event-free patients

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ABSTRACT

The reconstitution of different immunocyte subsets after hematopoietic stem cell transplantation (HSCT), follows different timelines. We prospectively investigated changes in lymphocyte subsets after HSCT and their associations with primary diagnosis, conditioning regimen, and HSCT type in event-free patients. A total of 95 patients (48 with acute myeloid leukemia, 22 with acute lymphoid leukemia, and 25 with myelodysplastic syndrome) who underwent allogeneic HSCT (34 sibling matched, 37 unrelated matched, and 24 haploidentical HSCT) but did not experience any events such as relapse or death were enrolled in this study. Lymphocyte subpopulations (T cells, helper/inducer T cells, cytotoxic/suppressor T cells, memory T cells, regulatory T cells, natural killer (NK) cells, NK-T cells, and B cells) were quantified by flow cytometry of peripheral blood from recipients 7 days before and 1, 2, 3, 6, and 12 months after HSCT. Leukocyte counts recovered within 1 month after HSCT. However, the number of T and B lymphocytes recovered at 2 months after HSCT. NK cell counts recovered shortly after haploidentical HSCT. However, T lymphocytes and their subpopulations showed delayed recovery after haploidentical HSCT. Lymphocyte subsets showed different sequential patterns according to HSCT type but no differences were seen according to primary diagnosis or conditioning regimen.

1. Introduction

Hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for hematologic malignancies, but is associated with therapy-related mortality (TRM). HSCT recipients undergo conditioning chemotherapy before HSCT, aimed at eradicating malignant cells and inducing immune suppression to prevent stem cell rejection by the recipient's immune system. However, due to an immunosuppressive effect, such therapy can lead to serious infections, which are the main contributor to TRM [1].

Immunity is impaired in the first month after HSCT, and the recovery of cell counts can take years. The reconstitution of different immune cell subsets after HSCT occurs in different timelines [2–4]. Neutrophils, monocytes, macrophages, dendritic cells, and natural killer (NK) cells contribute to innate immunity whose cells recognize and eradicate pathogens or aberrant cells without the needs for antigen presentation [4]. In contrast, T and B cells contribute to adoptive immunity, which is delayed and requires priming by the antigen, leading to long-term specific responses using unique receptor sequences [4].

The desired outcome of HSCT is the reestablishment of an effective immune system that accurately delineates self from non-self. If adequate antigen-specific immune function is present, functional immune reconstitution has the potential to decrease leukemic relapse and TRM. Clear understanding of the functional immune recovery is needed for successful HSCT. Also, understanding the

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different time courses of functional recovery of each immune cell subset can help to predict the course of disease in individual patients after HSCT.

In our present study, we prospectively investigated changes in lymphocyte subsets after HSCT and their associations with primary diagnosis, conditioning regimen, and HSCT type in the patients without any event such as relapse or death.

2. Methods

2.1. Patient characteristics

A total of 95 patients (median age, 46 (18–70) years; 48 women and 47 men) who underwent allogeneic HSCT but did not experience any events such as relapse or death were enrolled in this study. All patients underwent transplantation at a single institution between January 2008 and February 2010. Blood samples were obtained to examine lymphocyte subpopulations over a period of 12 months.

Underlying hematologic malignancies of patients included 48 cases of acute myeloid leukemia (AML), 22 of acute lymphoblastic leukemia (ALL), and 25 of myelodysplastic syndrome (MDS). The type of HSCT was classified according to donor relationship as 34 sibling matched, 37 unrelated matched, and 24 haploidentical HSCT.

The regimens for conditioning therapy for HSCT were; Bu-Cy (busulfan intravenously (iv) 3.2 mg/kg/day for 4 days plus cyclophosphamide iv 60 mg/kg/day for 2 days), Bu-Flu (busulfan intravenously (iv) 3.2 mg/kg/day for 4 days plus fludarabine iv 30 mg/m^2/day for 5 days), or Bu-Flu-ATG (busulfan iv 3.2 mg/kg/day for 2 days, fludarabine iv 30 mg/m^2/day for 6 days, plus antithymocyte globulin (Thymoglobulin) iv 3 mg/kg/day for 3–4 days). Of 34 patients who underwent sibling matched HSCT, 16 received Bu–Cy, 13 Bu–Flu–ATG, and 5 Bu–Flu conditioning, respectively. Of 37 patients who underwent unrelated matched HSCT, 30 received Bu–Flu–ATG, 6 Bu–Cy, and 1 Bu–Flu conditioning, respectively. The all remaining 24 patients who underwent haploidentical HSCT received Bu–Flu–ATG conditioning.

Post-transplant immune suppression for GVHD prophylaxis included cyclosporine 1.5 mg/kg iv every 12 h starting the day before HSCT, and subsequently switched to a 1.5–2.0-fold greater oral dose. In addition, patients received methotrexate 15 mg/m^2 iv one day and 10 mg/m^2 3, 6, and 11 days after HSCT. Starting 30 to 60 days after HSCT, the cyclosporine dose was decreased by 10% every 2–4 weeks in patients with no evidence of GVHD.

Table 1
List of lymphocyte subpopulations analyzed by flow cytometry.

Populations	Phenotype
T cell	$CD3^+$
Helper/inducer T cell	$CD3^+CD4^+$
Cytotoxic/suppressor T cell	$CD3^+CD8^+$
NK cell	$CD3^-CD16^+$ and/or $CD56^+$
NK cell subset 1	$CD56^{bright}CD16^-CD3^-$
NK cell subset 2	$CD56^{dim}CD16^+CD3^-$
NK-T cells	$CD3^+CD161^+$
Memory T cells	
Central memory T cell	$CD8^+CD45RA^-CCR7^+CD62L^+$
Effector memory T cell	$CD8^+CD45RA^-CCR7^-CD62L^-$
Regulatory T cells	$CD4^+CD25^{high}FoXP3^+$
B cells	$CD19^+$
NK cell, natural killer cell	

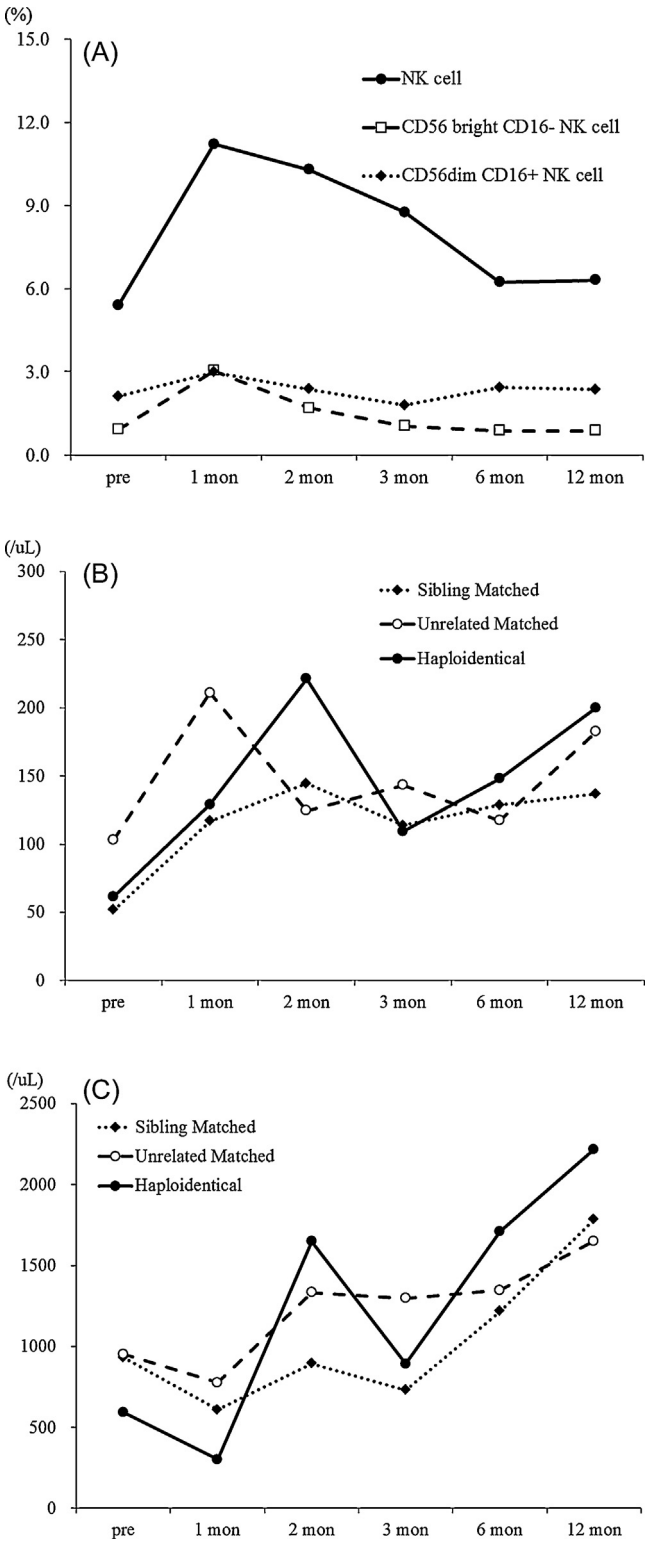

Fig. 1. Representative patterns of sequential immune recovery for cellular components, proportion of natural killer (NK) cells, and NK cell subpopulations after hematopoietic stem cell transplantation (HSCT) (A); NK cell count recovery after HSCT according to HSCT type (B); and T cell count recovery after HSCT according to HSCT count (C).