Number of megakaryocytic progenitors and adhesion molecule expression of stem cells predict platelet engraftment after allogeneic hematopoietic stem cell transplantation

KS Woo[1], RY Goh[1], SH Kim[2], HC Kwon[2], HJ Kim[2], YH Lee[3] and JY Han[1]

Departments of [1]Laboratory Medicine and [2]Internal Medicine, Dong-A University College of Medicine, Busan, Korea, and [3]Department of Pediatrics, Hanyang University College of Medicine, Seoul, Korea

Background

The mechanism of platelet recovery after hematopoietic stem cell transplantation and the factors that influence its time–course are not fully understood. Rapid hematopoietic recovery results in a reduction of transplantation-related complications. In the present study, we questioned and analyzed whether there were important factors predicting the speed of platelet engraftment.

Methods

Thirty-seven patients with various hematologic diseases transplanted with allogeneic BM between January 2002 and December 2005 were included. We investigated the differences in mononuclear cell counts (MNC), numbers of infused $CD34^+$, $CD34^+$ $CD41^+$ and $CD34^+$ $CD61^+$ cells and phenotypic analysis of homing-associated cell adhesion molecules (CXCR4, CD49d and CD49e). The number of megakaryocytes formed in vitro (colony-forming unit-megakaryocytes; CFU-Mk) was also measured.

Results

Median days of ANC $\geq 0.5 \times 10^9/L$ and platelet count $\geq 20 \times 10^9/L$ were 14.8 and 17.3, respectively. The number of infused $CD34^+$ $CD41^+$ and $CD34^+$ $CD61^+$ cells correlated much better with the time to platelet engraftment than that of infused $CD34^+$ cells ($P < 0.05$ each). Rapid platelet recovery also occurred in patients receiving both higher homing-associated cell adhesion molecule doses and CFU-Mk ($P < 0.05$ each).

Discussion

Rapid platelet recovery has several advantages, including reducing the cost of supportive therapy and reducing the risk of fatal bleeding as a result of severe thrombocytopenia. Our findings suggest that phenotypic and clonogenic assessment of infused progenitor cells can identify patients in whom platelet engraftment is likely to be significantly delayed, and new strategies to overcome related problems might be employed in the very near future.

Keywords

adhesion molecules, allogeneic stem cell transplantation, megakaryocytic progenitors, platelet engraftment, stem cells.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT), employing BM, mobilized PBSC or cord blood, is the only curative therapy for a variety of hematologic, genetic and autoimmune diseases. The time to blood cell recovery is critical for the degree of risk, and delayed platelet engraftment, which manifests as prolonged thrombocytopenia, is one of the major clinical problems after HSCT [1–3].

The mechanism of platelet recovery after HSCT and the factors influencing its time–course and magnitude are not fully understood. Regarding platelet engraftment, few studies have been reported. Recently, specific markers for megakaryocytic progenitors, such as $CD34^+$ $CD41^+$,

CD34$^+$ CD61$^+$ and colony-forming unit-megakaryocytes (CFU-Mk), have been shown to be able to predict platelet engraftment in HSCT [1,4,5]. In addition, the process of engraftment after HSCT is dependent on successful trafficking to the marrow microenvironment and is thought to involve sequential utilization of a number of adhesion receptors [6]. For CD34$^+$ cells, a role in homing has been described for the adhesion molecules CXC chemokine receptor 4 (CXCR4), very late antigen (VLA)-4 (CD49d), VLA-5 (CD49e), lymphocyte function-associated antigen (LFA)-1 and CD44. Previously, investigators had reported that adhesion receptor expression by patient progenitor cells correlates with time to platelet recovery after autologous SCT [7].

Based on these considerations, we analyzed the expression of different platelet glycoproteins and adhesion molecules on CD34$^+$ cells by flow cytometry. We also measured the number of CFU-Mk in grafts. Finally, we examined the correlation between these parameters and platelet recovery to find a practical parameter to predict the capacity for platelet engraftment after HSCT.

Methods
Patient characteristics

Thirty-seven patients with various hematologic diseases who had undergone HSCT between January 2002 and December 2005 were included in this prospective study. The indications for HSCT were AML (15 patients), severe aplastic anemia (SAA; seven patients), ALL (five patients), CML (five patients) and myelodysplastic syndrome (MDS; five patients). All patients received single CMV-seropositive BM transplants from HLA-identical sibling donors ($n = 32$) and unrelated donors ($n = 5$), respectively. HLA was fully matched at intermediate to high resolution. Myeloablative therapy consisted of intravenous busulfan (0.8 mg/kg) and CY (60 mg/kg) in 30 leukemia and MDS patients. Anti-thymocyte globulins and CY were given to seven SAA patients. Before transplantation, all patients were in complete remission status, including hematologic remission in SAA. Patient characteristics and HSCT details are shown in Table 1. All samples were collected after informed consent was obtained within the guidelines of the institutional review board on human subjects. After HSCT, platelet engraftment was defined as platelet count remaining $\geq 20 \times 10^9$/L without platelet transfusions. The

Table 1. Patient characteristics at transplantation

No. of patients	37
Sex (female/male)	14/23
Median age (years)	24 (3–45)
Diagnosis	
AML	15
SAA	7
ALL	5
CML	5
MDS	5
Conditioning regimen	Busulfan and CY ($n = 30$)/ anti-thymocyte globulins and CY ($n = 7$)[a]
GvHD prophylaxis	CYA and methotrexate ($n = 37$)
Source of stem cells	HLA-identical BM ($n = 37$)
Type of transplant	Related ($n = 32$)/unrelated ($n = 5$)

[a]*These include seven SAA patients.*

day of leukocyte recovery was defined as the first of 3 consecutive days where ANC exceeded 0.5×10^9/L. CYA and short-term methotrexate were given to prevent graft rejection and for GvHD prophylaxis. To accelerate leukocyte engraftment, all patients received human G-CSF, starting on day 5.

Flow cytometry analysis

Mononuclear cell counts (MNC) of collected marrow products were measured using Sysmex XE-2100 (Sysmex Corp., Kobe, Japan). The number of cells expressing CD34, CD41, CD61, CXCR4, CD49d and CD49e was determined by direct double-color immunofluorescence. After lysis of erythrocytes, the cell aliquots were washed twice with PBS containing 1% BSA (Sigma, St Louis, MO, USA). Cells were then stained with MAb CD34 (Pharmingen, San Diego, CA, USA) and analyzed further for the expression of megakaryocytic markers (CD41 and CD61; Pharmingen) and homing-associated cell adhesion molecules (CXCR4, CD49d, and CD49e; Pharmingen). Isotype-matched mouse Ig served as controls. Flow cytometry analysis was performed with a FACSort flow cytometer (Becton Dickinson, San Jose, CA, USA). Absolute numbers of CD34$^+$ CD41$^+$, CD34$^+$ CD61$^+$, CD34$^+$ CXCR4$^+$, CD34$^+$ CD49d$^+$ and CD34$^+$ CD49e$^+$ cells were calculated by multiplication of MNC with the percentage of each fraction (Figure 1).