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조혈모세포이식시 조혈모세포의 체외 증폭능과 생착 소요시간간의 역상관관계

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Inverse Tendency between Ex Vivo Expansion Potential of Hematopoietic Progenitors and Time to Engraftment after Hematopoietic Stem Cell Transplantation

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Background : The $CD34^+$ cell dose and infused number of committed progenitor cells in transplantation are important factors in hematologic engraftment. However, the relationship between expansion potential of progenitor cells and hematologic engraftment remains controversial. We evaluated whether expansion potential of progenitor cells is a predictive factor of post-transplantation hematologic engraftment.

Methods : Mononuclear cells isolated from mobilized peripheral blood and bone marrow were cultured with cytokine cocktail for 7 days. Progenitor cells and committed progenitors were analyzed using stem cell markers (CD34 and CD133) and lineage specific markers. Hematologic engraftment was defined as neutrophil counts over 500/μL and platelet counts over 20,000/μL without transfusion. Acute and chronic graft-versus-host disease (GVHD) were investigated.

Results : There was inverse tendency between the number and fold expansion of progenitor cells or committed (granulocytic or megakaryocytic) progenitors and time to engraftment. Especially, fold expansion of $CD34^+/CD33^+$ cells was significantly correlated with time to neutrophil engraftment in bone marrow transplantation (r=-0.56, *P*=0.04). The infused number and fold expansion of lymphoid progenitors were not related to the occurrence of acute or chronic GVHD.

Conclusions : We could not prove that expansion potential of progenitor cells and committed progenitor cells is correlated to hematologic engraftment although there is a correlation between $CD34^+/CD33^+$ cells and time to neutrophil engraftment. But, a further study on the value of expansion potential is required because there is an inverse tendency. ***(Korean J Lab Med 2006;26:385-92)***

Key Words : *Hematopoietic progenitor cells, Ex vivo expansion, Engraftment, Hematopoietic stem cell transplantation*

INTRODUCTION

Hematopoietic stem cells (HSCs) are cells isolated from the blood or bone marrow, which can renew themselves and differentiate to a variety of specialized cells. HSCs generate intermediate cells called committed progenitor cells before they give rise to differentiated cells. This differenti-

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ation is defined by the expression pattern of surface markers. Antibodies to CD34 isolate the HSCs[1]. Recently, a new stem cell marker CD133 (AC133) was discovered as a strong candidate for primitive hematopoietic cells with repopulating activity[2].

Hematopoietic stem cell transplantations are now a routine treatment modality in patients with various malignancies and disorders of the blood and immune system. But protracted severe neutropenia and thrombocytopenia until complete engraftment lead to serious bacterial or fungal infection and bleeding tendency which are major limitations of stem cell transplantation.

Two important components, HSCs and committed hematopoietic progenitor cells, are necessary for a successful transplantation of HSCs. These are required for long term engraftment and overcoming cytopenia after myeloablation. The number of $CD34^+$ cells infused is an crucial factor predicting the rate of neutrophil and platelet recovery after stem cell transplantation[3, 4]. For optimal engraftment, current transplantation protocols call for $\geq 2.5 \times 10^6$ $CD34^+$ cells/kg of patient body weight[5]. Weaver et al.[6] reported that a dose of $CD34^+$ cells equal to or more than 5×10^6/kg can ensure neutrophil recovery within 7 to 10 days of infusion in autologous peripheral blood stem cell transplantation (PBSCT). In addition, a number of studies have shown that infused granulocyte-monocyte colony forming units (CFU-GM) are significantly associated with neutrophil engraftment and that there is a correlation between platelet engraftment and the number of megakaryocytic progenitor cells[7, 8].

The purpose of this study was to evaluate the infused number and fold expansion of HSCs and committed progenitor cells which might be a predictive factor for hematologic recovery.

MATERIALS AND METHODS

1. Specimen collection and processing

Thirteen patients (non-Hodgkin's lymphoma in 6 and multiple myeloma in 7) who underwent autologous PBSCT and 14 allogeneic bone marrow transplantation (BMT) were included in this study. Homogenous patients with lymphoma and multiple myeloma for PBSCT were chosen in order to minimize differences in the disease state, mobilization, and transplantation protocols. Mobilized peripheral blood cells (mPB) were collected by leukapheresis using a Fenwal CS3000 plus (Baxter Healthcare, Deerfield, IL, USA) after mobilization with granulocyte-colony stimulating factor and cyclophosphamide. Two to three leukapheresis procedures were performed in each patient of autologous PBSCT. The indications for BMT were AML in 5 patients, ALL in 3, CML in 3, and MDS in 3. Seven patients received from HLA-identical siblings, 6 from HLA-identical unrelated donors, and 1 from 2 allele mismatched sibling. A non-myeloablative conditioning regimen of Bu-Fludara-ATG (busulphan 4 mg/kg/day orally on d -7 and d -6, fludarabine 30 mg/m^2/day intravenously on d -7 to d -2, antithymocyte globulin 20 mg/kg/day intravenously on d -5 to d -2) and methylprednisolone (2 mg/kg/day intravenously on d -5 to d -2) was given. None of the hematopoietic cell grafts was T-cell depleted. All patients received cyclosporine for GVHD prophylaxis. Cyclosporine 1.5 mg/kg was given intravenously every 12 hr from d -1 and switched to an oral dose when oral intake became feasible. G-CSF 450 μg was given intravenously once daily starting on d 0 or d 5 until peripheral blood absolute neutrophil count (ANC) was over 3,000/μL. The study protocol was approved by the Institutional Review Board of Asan Medical Center.

2. Ex vivo expansion using liquid culture

Low density mononuclear cells (MNCs) (<1.077 g/mL) were separated by density gradient centrifugation (1,500 rpm, 30 min) using Histopaque®-1077 (Sigma, Milwaukee, WI, USA) and then washed twice with phosphate buffered saline (PBS). Separated MNCs were suspended in Iscove's modified Dulbecco's medium (IMDM) (GIBCO, Grand Island, NY, USA) supplemented with 30% human AB type serum, 2 mM glutamine (Sigma), 100 μ/mL penicillin (GIBCO), 100 μg/mL streptomycin (GIBCO), and growth factors such as 50 ng/mL recombinant human stem cell factor, 20 ng/mL thrombopoietin, 50 ng/mL FLT3/FLK2, 20 ng/mL recombinant human granulocyte-macrophage colony stimulating factor, 20 ng/mL interleukin-3, and 3 u/mL erythropoietin (all growth factors were purchased from R & D systems Inc, Minneapolis, MN, USA). Optimal concentrations of cytokines were chosen based on a previous experiment[9].

The 5 mL of culture mixture (1×10^5 cells/mL) was

seeded in culture flasks (GIBCO) and was subsequently maintained at 37℃ in 5% CO_2 atmosphere in a humidified incubator. After 7 days of incubation, the expanded cells were harvested from the flasks.

Nucleated cell count in the pre-culture specimen was measured by an automated cell counter (XE-2100, Sysmex, Kobe, Japan). The number of MNCs was determined by multiplying the number of nucleated cells by the sum of the percentage of mononuclear cells. The number of MNCs in the post-culture specimen was obtained by counting the number of viable cells using trypan blue exclusion in a hemocytometer.

3. Flow cytometry

The numbers of total MNCs, progenitor cells ($CD34^+$ or $CD133^+$) and committed progenitor cells ($CD34^+$ and lineage specific $antigen^+$) in all the specimens before and after 7-day culture were measured. The quantities of progenitor cells and committed progenitor cells were measured by flow cytometer (FACScan, Becton-Dickinson, San Jose, CA, USA) using monoclonal antibodies (Becton-Dickinson). Progenitor cells were enumerated by 3-color immunofluorescence staining using PerCP-conjugated CD45, fluorescein isothiocynate (FITC)-conjugated CD34, and phycoerythrin (PE)-conjugated CD133 antibodies[10].

Committed progenitor cells were analyzed using anti CD45-perCP, anti CD34-FITC, and anti lineage specific marker-PE (Fig. 1). The lineage specific markers used were CD13 and CD33 for granulocytic cells, CD19 for B lymphoid cells, CD7 for T lymphoid cells, CD61 for megakaryocytic cells, and glycophorin A for erythroid cells. Granulocytic and megakaryocytic progenitor cells were analyzed in mPB and bone marrow (BM) specimens, but lymphoid and erythroid progenitor cells were only analyzed in bone marrow specimens. Each pre-and post-culture cell suspension of 100 μL was mixed with 10 μL of each antibody and incubated at room temperature for 20 min in the dark. And then the cells were washed twice with PBS and fixed with 1% paraformaldehyde until analysis.

Immunophenotypic analysis was performed by a flow cytometer equipped with CELL-Quest software (Becton-Dickinson). Absolute number of progenitor cells and committed progenitor cells were calculated by multiplying MNCs number by the percentage of each progenitor subset determined by flow cytometry.

4. Patient monitoring after hematopoietic stem cell transplantation

Complete blood cell counts, including reticulocyte counts, were determined daily in all patients from the start of conditioning therapy for hematopoietic stem cell transplantation. The day of neutrophil recovery was defined as the first of 2 consecutive days with an ANC over 500/μL following post-transplant nadir. The day of platelet recov-

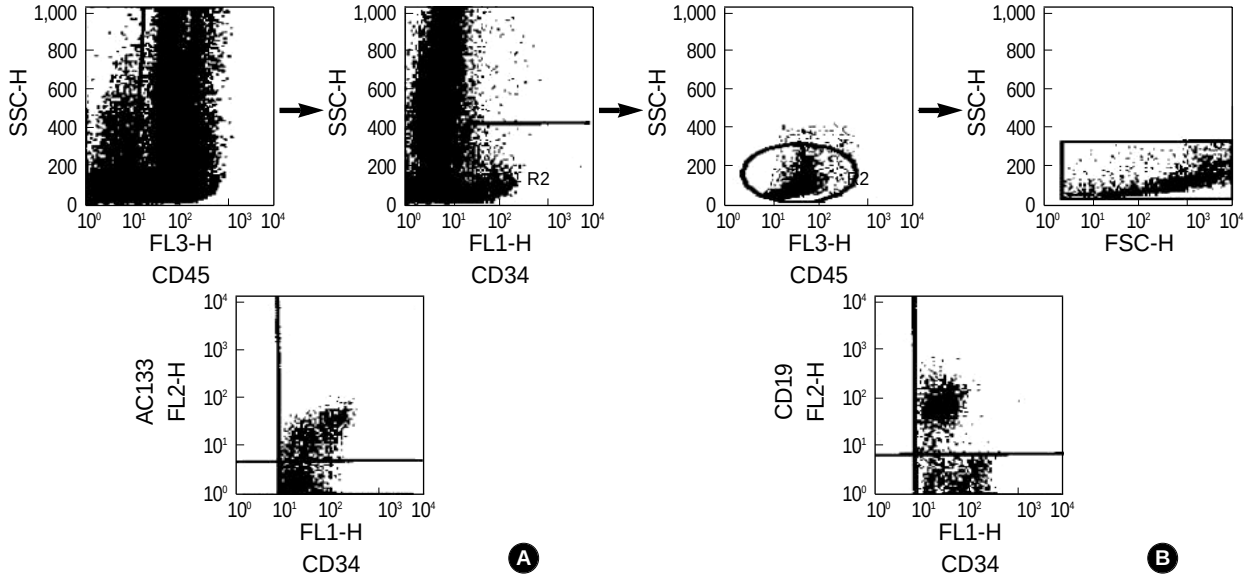

Fig. 1. Three-color flow cytogram for the measurement of hematopoietic progenitor cells. (A) Measurement of $CD45^+$, $CD34^+$ or $CD133^+$($AC133^+$) cells. (B) Measurement of $CD45^+$, $CD34^+$ and $CD19^+$ cells.

ery was defined as the first of 7 consecutive days with a platelet count over 20,000/μL without platelet transfusion. The first of 3 consecutive days showing reticulocyte counts of 1.0% or more was recorded for red blood cell recovery after BMT. Acute GVHD was diagnosed and graded according to the standard criteria[11]. The diagnosis of chronic GVHD was made in the basis of clinical symptoms, laboratory tests and, histopathologic findings of the skin, oral mucosa and gastrointestinal tract. And the chronic GVHD was graded as either limited or extensive[12, 13].

5. Statistical analysis

Fold expansion was calculated by dividing the number of viable progenitor cells and committed progenitor cells harvested on day 7 by the number of those cells at the culture initiation. Linear regression analysis was used to assess the effect of infused cell dose and expansion potential to neutrophil or platelet engraftment. The difference in the number of infused lymphoid progenitor cells or fold expansion between the group with GVHD and one without GVHD was analyzed by Mann-Whitney U test. P value below 0.05 was considered to be statistically significant.

RESULTS

1. Frequency and expansion potential of progenitor cells

After 7-day culture, the number of MNCs and progenitor cells were increased. Fold expansion of MNCs and progenitor cells was highly variable among individuals and showed no difference between BM and mPB. The number and proportion (%) of progenitor cells ($CD34^+$ or $CD133^+$) before and after culture were similar between mPB and BM (Table 1).

2. Number of infused progenitor or committed cells and hematologic recovery after hematopoietic stem cell transplantation

Patients who underwent autologous PBSCT received a median of 7.2×10^6/kg (range, 1.13-33.1) progenitor cells. Median times to ANC over 500/μL and platelets over 20,000/μL were 13 days (range, 10-23) and 18 days (range, 13-25) after transplantation, respectively. The number of infused progenitor cells influenced the time to neutrophil ($r=-0.48$, $P=0.08$) and platelet ($r=-0.41$, $P=0.14$) recovery, but there was no significant relation.

A median of 5.0×10^6/kg (range, 1.14-17.0) progenitor cells from allogeneic donors was infused to 14 recipients who underwent allogeneic BMT. Median times to ANC over 500/μL and platelets over 20,000/μL were 21 days (range, 11-28) and 26 days (range, 19-88) after transplantation, respectively. The number of infused progenitor cells was correlated with time to neutrophil ($r=-0.44$, $P=0.10$) and platelet ($r=-0.48$, $P=0.07$) recovery, but was not statistically significant.

The number of infused myeloid progenitor cells ($CD34^+$/$CD13^+$ cells or $CD34^+$/$CD33^+$ cells) was not related to neutrophil recovery, and the number of infused megakaryocytic progenitor cells ($CD34^+$/$CD61^+$ cells) was not associated with platelet recovery either.

3. Expansion potential of progenitor or committed cells and hematologic recovery after hematopoietic stem cell transplantation

Fold expansion of progenitor cells and committed cells

Table 1. Number and fold expansion of mononuclear cells and progenitor cells before and after 7-day culture

	N	Fold expansion of MNCs	Progenitor cells ($CD34^+$ or $CD133^+$)				Fold expansion of progenitor cells
			Pre		Post		
			N ($\times10^3$/mL)	%	N ($\times10^3$/mL)	%	
mPB	13	4.5 (3.1-5.3)	4.9 (3.8-15.7)	1.94 (0.97-3.14)	19.2 (10.5-42.3)	1.17 (0.94-1.44)	2.2 (1.3-4.7)
BM	14	5.9 (3.1-11.8)	10.6 (5.1-15.1)	2.23 (1.50-3.33)	26.1 (11.7-44.0)	1.40 (1.04-1.96)	3.1 (2.1-5.2)
P-value		0.21	0.36	0.77	0.87	0.36	0.88

The data are expressed as median (interquartile range).
Abbreviations: N, number; MNCs, mononuclear cells; mPB, mobilized peripheral blood; BM, bone marrow.

was demonstrated in Table 2. Median fold expansion of $CD34^+/CD13^+$ cells and $CD34^+/CD33^+$ cells was slightly higher in BM than mPB, but was not statistically significant ($P=0.06$, $P=0.14$).

With mPB, the days to neutrophil ($r=-0.31$, $P=0.30$) or platelet recovery ($r=-0.39$, $P=0.18$) were shorten in the group with more expansion potential of progenitor cells, but there was not a significant correlation. The days to neutrophil recovery was shorten in patients with a higher fold expansion of $CD34^+/CD13^+$ ($r=-0.31$, $P=0.31$) and $CD34^+/CD33^+$ cells ($r=-0.35$, $P=0.20$), and the days to platelet recovery was shorten in patients with a higher fold expansion of $CD34^+/CD61^+$ cells ($r=-0.23$, $P=0.39$). But these relationship were not statistically significant.

With BM, the days to neutrophil ($r=-36$, $P=0.19$) and platelet recovery ($r=-50$, $P=0.07$) were shorten in the group with a greater fold expansion of progenitor cells. Fold expansion of $CD34^+/CD33^+$ showed a significant correlation with the days to neutrophil recovery ($r=-0.56$, $P=0.04$) (Fig. 2). But, other expansion potential of $CD34^+/CD33^+$ ($r=-37$, $P=0.19$) and $CD34^+/CD61^+$ cells ($r=-26$, $P=0.34$) was not significantly correlated with the days to neutrophil and platelet recovery, respectively.

Table 2. Expansion potential of progenitor cells in mPB and BM

Subset of progenitor cells	Fold expansion		*P*-value
	mPB (N=13)	BM (N=14)	
$CD34^+$ or $CD133^+$	2.2 (1.3-4.7)	3.1 (2.1-5.2)	0.88
$CD34^+/CD13^+$	1.4 (1.0-2.1)	3.6 (1.7-6.0)	0.06
$CD34^+/CD33^+$	1.9 (1.0-6.2)	4.5 (1.8-7.9)	0.14
$CD34^+/CD61^+$	18.1 (7.9-50.2)	6.9 (3.0-13.2)	0.55

The data are expressed as median (interquartile range).
Abbreviations: mPB, mobilized peripheral blood; BM, bone marrow.

4. Expansion potential of erythroid progenitor cells and red blood cell recovery after BMT

With BM, median fold expansion of $CD34^+$/glycophorin A^+ cells was 7.2 (range, 0.3-41.0). Median time to reticulocyte over 1.0% was 24 days (range, 17-49) after transplantation. There was no correlation between red blood cell recovery and fold expansion of erythroid progenitor cells ($r=-0.15$, $P=0.60$).

5. Lymphoid progenitor cells and the occurrence of acute and chronic GVHD after BMT

Median infused numbers of $CD34^+/CD19^+$ cells and $CD34^+/CD7^+$ cells were 0.81×10^6/kg (range, 0.11-6.42) and 0.26×10^6/kg (range, 0.04-4.10), respectively. Median fold expansions of $CD34^+/CD19^+$ cells and $CD34^+/CD7^+$ cells were 1.3 (range, 0.1-11.6) and 2.8 (range, 0.2-18.4), respectively. Acute GVHD developed in 57.1% (8/14) and grade 2-4 incidence was 28.6% (4/14). The number of infused lymphoid progenitor cells or fold expansion showed no difference between the group with grade 2-4 acute GVHD and one with grade 1 acute GVHD or without acute GVHD (Table 3). Chronic GVHD could be evaluated in 11 patients and incidence was 54.5% (6/11). At the onset of chronic GVHD, 5 patients had a limited disease and 1 patient had an extensive disease. The number of infused lymphoid progenitor cells was not related to the occurrence of chronic GVHD. Compared to the group

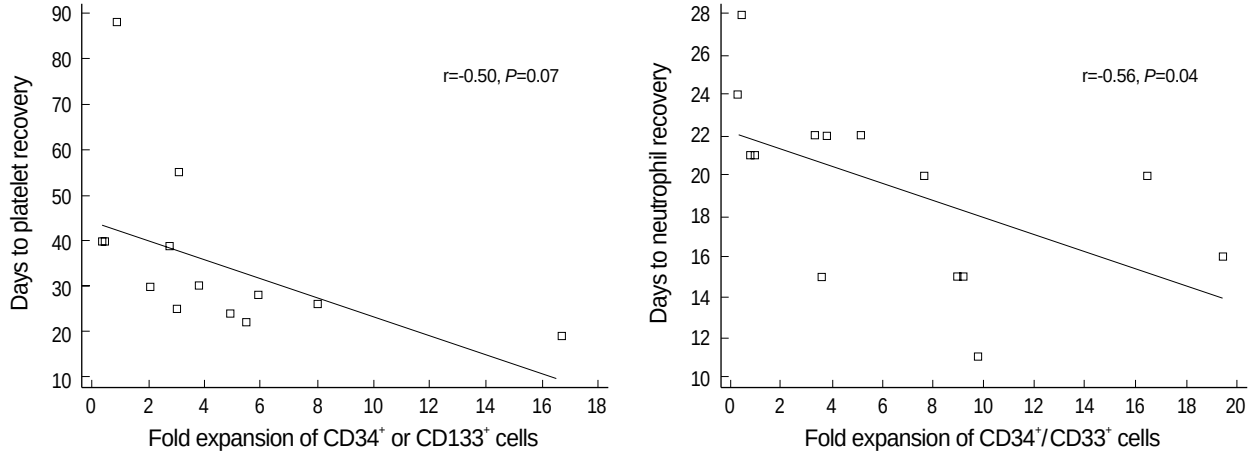

Fig. 2. Correlation of expansion potential of progenitor cells with neutrophil or platelet recovery (days) after bone marrow transplantation (n=14).

Table 3. Correlation of infused number ($\times 10^6$/kg) or expansion potential of lymphoid progenitor cells with the occurrence of acute GVHD

Parameters	without aGVHD or with grade 1 aGVHD (N=10)	with grade 2-4 aGVHD (N=4)	*P*-value
$CD34^+/CD19^+$			
Number	0.88 (0.28-1.39)	0.66 (0.42-0.88)	0.89
Fold expansion	1.3 (0.7-5.1)	3.4 (0.7-6.1)	1.0
$CD34^+/CD7^+$			
Number	0.22 (0.14-0.63)	0.43 (0.17-0.56)	0.78
Fold expansion	4.3 (1.6-5.3)	2.2 (1.9-2.2)	0.32

The data are expressed as median (interquartile range).
Abbreviation: aGVHD, acute graft-versus-host disease.

Table 4. Correlation of infused number ($\times 10^6$/kg) or expansion potential of lymphoid progenitor cells with the occurrence of acute GVHD

Parameters	without cGVHD (N=5)	with cGVHD (N=6)	*P*-value
$CD34^+/CD19^+$			
Number	1.01 (0.62-1.49)	0.47 (0.20-1.07)	0.20
Fold expansion	0.3 (0.2-1.6)	5.0 (1.0-7.2)	0.09
$CD34^+/CD7^+$			
Number	0.23 (0.20-1.10)	0.18 (0.11-1.20)	0.47
Fold expansion	1.6 (0.5-7.6)	4.3 (2.2-7.0)	0.11

The data are expressed as median (interquartile range).
Abbreviation: aGVHD, acute graft-versus-host disease.

without chronic GVHD, the group with chronic GVHD showed a higher fold expansion of lymphoid progenitor cells, but was not statistically significant ($P=0.09$, $P=0.11$) (Table 4).

DISCUSSION

In this study, we chose unselected MNCs instead of selected $CD34^+$ cells for an ex vivo expansion of HSCs. The reason behind this was the advantage posed by the MNCs.

First, the colony density and proportion of $CD34^+$ cells were increased from day 0 to day 7 in MNCs culture. These, however, showed a continuous decline in the culture of selected $CD34^+$ cells. Second, the $CD34^-$ cells in MNCs were seen to transform into $CD34^+$ cells and induce $CD34^+$ cells to mature colony forming cells[14].

Ex vivo expansion potential was highly variable between individuals even in the same hematopoietic source. Donor to donor variability of $CD34^+$ cells in ex vivo expansion capacity has been observed in cord blood (CB) and BM[15, 16]. It is likely that this variability results from genetic factors or other factors difficult to establish now.

Several studies using unexpanded cells have demonstrated a correlation between the time to engraftment and the number of $CD34^+$ cells per kilogram infused[4, 17]. The substitution of PB progenitor cells for BM progenitor cells has shown a reduction in the duration of neutrophil or platelet engraftment[18].

In our study, patients receiving the autologous PBSCT had a significantly earlier hematologic recovery than patients receiving allogeneic BMT. Also, patients with a large amount of infused progenitor cells had a shorter hematologic recovery time than those with a small infused amount of progenitor cells. The effect of the number of infused progenitor cells on hematologic recovery was not statistically significant.

Zubair et al.[7] suggested the CFU-GM infused was significantly associated with neutrophil engraftment. Dercksen et al.[19] and Feng et al.[20] suggested $CD34^+$-$CD41^+$ cells were related to platelet recovery, but Drayer et al.[21] reported the number of CFU-MK and an ex vivo expansion potential in the megakaryocytic lineage showed no correlation with platelet recovery. The present study shows the number of infused committed progenitors has no significant correlation with engraftment.

Patients with a high expansion potential of progenitor cells and committed progenitor cells had a shorter time to hematologic recovery than the patients with a low expansion potential. In fact, the expansion potential of $CD34^+/CD33^+$ was statistically related to the neutrophil recovery in BMT. But, most expansion potentials of progenitor cells and committed subsets showed no significant correlation with hematologic recovery in stem cell transplantation. We could not prove that the expansion potential of progenitor cells and committed progenitor cells was a factor in predicting the early hematologic recovery.

Some studies proposed adhesion molecules such as L-selectin, CD44 or a few integrins play an important role in hematologic reconstitution[22, 23]. This study did not analyze these adhesion molecules. There is a possibility that the correlation between expansion potential of committed progenitor cells and neutrophil or platelet engraftment was masked by the effect of adhesion molecules. A small number of patients can be a major cause for the lack of statistical significance. Therefore, further studies

with more patients are required to establish the relationship between the expansion potential of committed progenitor subsets and neutrophil or platelet recovery.

The infused number of erythroid progenitor cells and expansion potential showed no difference in erythroid recovery in BMT. So, we assume that the erythroid recovery is a part of a continuous process of general hematopoietic reconstitution and the effect of erythroid progenitor cells on erythroid recovery is minimal.

Previous studies suggested that the depletion of T lymphoid cells from the donor bone marrow reduce acute GVHD. But recent studies indicated that T cell depletion is less effective and that HLA disparity and ABO incompatibility increase acute GVHD risk[24]. In our study, the infused number of B- and T-lymphoid progenitor cells was not related to the development of a moderate to severe acute GVHD, and the expansion potential of lymphoid progenitor cells also showed no effect on the development of acute GVHD.

The pathophysiology of chronic GVHD is still poorly understood. It has not been determined whether chronic GVHD is a distinct entity or a continuation of acute GVHD. One explanation is that expansion and effector functions of auto reactive T-cells will promote auto reactive B-cell activation and that production of auto antibodies will damage target organs[25].

Although fold expansion of B- or T-lymphoid progenitors was higher in patients with chronic GVHD, the expansion potential of lymphoid progenitors was not related to the development of chronic GVHD. Also, the infused number of lymphoid progenitors displayed no effect on the development of chronic GVHD. These findings support the fact that infused doses or expansion potential of lymphoid progenitors might not be directly related to the development of acute or chronic GVHD.

In the present study, the total infused number of progenitor cells ($CD34^+$ or $CD133^+$ cells) or ex vivo expansion potential of progenitor cells and committed progenitor cells except $CD34^+/CD33^+$ cells is not related to hematologic recovery in stem cell transplantation although there was a noticeable inverse tendency. However, ex vivo expansion potential of $CD34^+/CD33^+$ progenitor cells in BMT is significantly correlated with neutrophil engraftment. B- or T-lymphoid progenitor cells are not associated with the development of acute and chronic GVHD. So, we suggest that further studies on the value of expansion potential in large populations are required.

요 약

서론 : 이식시 투여되는 $CD34^+$ 세포 및 계열별 조혈모세포의 수는 혈액학적 생착에 있어 중요한 인자이다. 그러나, 조혈모세포의 증폭 정도가 혈액학적 생착의 속도와 관련되는지 여부는 분명하지 않다. 저자들은 조혈모세포의 증폭 정도가 이식 후 혈액학적 생착의 속도를 예측할 수 있는 인자로 적용 가능한지를 평가하였다.

재료 및 방법 : 가동화한 말초혈액과 골수로부터 분리한 단핵세포를 성장인자들을 가하여 7일간 배양하였다. 조혈모세포 및 계열별 조혈모세포는 조혈모세포 표지자(CD34와 CD133)와 계열별 표지자를 이용하여 유세포분석법으로 분석하였다. 수혈없이 500/μL 이상의 호중구 및 20,000/μL 이상의 혈소판 수가 유지되는 상태를 혈액학적 생착으로 간주하였다. 급성 및 만성 이식대숙주병의 발생은 진단 기준에 의거하여 조사하였다.

결과 : 조혈모세포 및 계열별 조혈모세포(과립구계 및 거핵구계)의 이식한 세포수와 체외 증폭력은 생착소요시간과 역의 관련성을 보였으며 특히, $CD34^+/CD33^+$ 세포의 체외 증폭력은 골수이식시 호중구의 회복과 의미있는 상관관계를 나타냈다($r=-0.56$, $P=0.04$). 림프구계 조혈모세포의 이식한 세포수와 체외 증폭력은 급성 및 만성 이식대숙주병의 발생과 관련되지 않았다.

결론 : $CD34^+/CD33^+$ 세포의 체외 증폭력이 호중구의 회복과 상관관계는 나타냈으나 조혈모세포 및 다른 계열별 조혈모세포의 체외 증폭력이 혈액학적 생착과 관련되는지는 증명할 수 없었다. 그러나, 체외 증폭력이 클수록 호중구와 혈소판의 생착이 빨라지는 경향을 보였으므로 조혈모세포의 체외 증폭력의 유용성에 대한 추가적인 연구가 필요하다.

참고문헌

1. Krause DS, Fackler MJ, Civin CI, May WS. CD34: structure, biology, and clinical utility. Blood 1996;87:1-13.
2. Yin AH, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, Leary AG, et al. AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood 1997;90:5002-12.
3. Siena S, Bregni M, Brando B, Belli N, Ravagnani F, Gandola L, et al. Flow cytometry for clinical estimation of circulating hematopoietic progenitors for autologous transplantation in cancer patients. Blood 1991;77:400-9.
4. Faucher C, Le Corroller AG, Chabannon C, Viens P, Stoppa AM, Bouabdallah R, et al. Autologous transplantation of blood stem cells mobilized with filgrastim alone in 93 patients with malignancies: the

number of CD34+ cells reinfused is the only factor predicting both granulocyte and platelet recovery. J Hematother 1996;5:663-70.
5. McAdams TA, Winter JN, Miller WM, Papoutsakis ET. Hematopoietic cell culture therapies (Part II): Clinical aspects and applications. Trends Biotechnol 1996;14:388-96.
6. Weaver CH, Hazelton B, Birch R, Palmer P, Allen C, Schwartzberg L, et al. An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collection in 692 patients after the administration of myeloablative chemotherapy. Blood 1995; 10:3961-9.
7. Zubair A, Zahrieh D, Daley H, Schott D, Gribben JG, Freedman A, et al. Early neutrophil engraftment following autologous BMT provides a functional predictor of long-term hematopoietic reconstitution. Transfusion 2003;43:614-21.
8. Hogge DE, Lambie K, Sutherland HJ, Benny WB, Dalal B, Currie C, et al. Quantitation of primitive and lineage-committed progenitors in mobilized peripheral blood for prediction of platelet recovery post autologous transplant. Bone Marrow Transplant 2000;25:589-98.
9. Astori G, Malangone W, Adami V, Risso A, Dorotea L, Falasca E, et al. A novel protocol that allows short-term stem cell expansion of both committed and pluripotent hematopoietic progenitor cells suitable for clinical use. Blood Cells Mol Dis 2001;27:715-24.
10. Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. J Hematother 1996;5:213-26.
11. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 1995;15:825-8.
12. Shulman HM, Sullivan KM, Weiden PL, McDonald GB, Striker GE, Sale GE, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med 1980; 69:204-17.
13. Przepiorka D, Anderlini P, Saliba R, Cleary K, Mehra R, Khouri I, et al. Chronic graft-versus-host disease after allogeneic blood stem cell transplantation. Blood 2001;98:1695-700.
14. Wang B, Kang ZZ, Chi ZY, Xu L, Tan WS. Study on the ex vivo expansion characteristics of umbilical cord blood CD34+ cells and mononuclear cells. Zhonghua Xue Ye Xue Za Zhi 2003;24:74-7.
15. De Bruyn C, Delforge A, Bron D, Bernier M, Massy M, De Hemptinne D, et al. Ex vivo expansion of CD34+CD38- cord blood cells. J Hematother 1997;6:93-102.
16. Koller MR, Manchel I, Brott DA, Palsson BO. Donor-to-donor variability in the expansion potential of human bone marrow cells is reduced by accessory cells but not only soluble growth factors. Exp Hematol 1996;24:1484-93.
17. Ketterer N, Salles G, Raba M, Espinouse D, Sonet A, Tremisi P, et al. High CD34(+) cell counts decrease hematologic toxicity of autologous peripheral blood progenitor cell transplantation. Blood 1998;91: 3148-55.
18. To LB, Roberts MM, Haylock DN, Dyson PG, Branford AL, Thorp D, et al. Comparison of haematological recovery times and supportive care requirements of autologous recovery phase peripheral blood stem cell transplants, autologous bone marrow transplants and allogeneic bone marrow transplants. Bone Marrow Transplant 1992;9: 277-84.
19. Dercksen MW, Rodenhuis S, Dirkson MK, Schaasberg WP, Baars JW, van der Wall E, et al. Subsets of CD34+ cells and rapid hematopoietic recovery after peripheral-blood stem-cell transplantation. J Clin Oncol 1995;13:1922-32.
20. Feng R, Shimazaki C, Inaba T, Takahashi R, Hirai H, Kikuta T, et al. CD34+/CD41a+ cells best predict platelet recovery after autologous peripheral blood stem cell transplantation. Bone Marrow Transplant 1998;21:1217-22.
21. Drayer AL, Smit Sibinga CT, Esselink MT, de Wolf JT, Vellenga E. In vitro megakaryocyte expansion in patients with delayed platelet engraftment after autologous stem cell transplantation. Ann Hematol 2002;81:192-7.
22. Watanabe T, Dave B, Heimann DG, Jackson JD, Kessinger A, Talmadge JE. Cell adhesion molecule expression on CD34+ cells in grafts and time to myeloid and platelet recovery after autologous stem cell transplantation. Exp Hematol 1998;26:10-8.
23. Peled A, Kollet O, Ponomaryov T, Petit I, Franitza S, Grabovsky V, et al. The chemokine SDF-1 activates the integrins LFA-1, VLA-4, and VLA-5 on immature human CD34(+) cells: role in transendothelial/stromal migration and engraftment of NOD/SCID mice. Blood 2000;95:3289-96.
24. Keever-Taylor CA, Bredeson C, Loberiza FR, Casper JT, Lawton C, Rizzo D, et al. Analysis of risk factor for the development of GVHD after T cell-depleted allogeneic BMT: effect of HLA disparity, ABO incompatibility, and method of T-cell depletion. Biol Blood Marrow Transplant 2001;7:620-30.
25. Kansu E. The pathophysiology of chronic graft-versus-host disease. Int J Hematol 2004;79:209-15.