Identification of Hematopoietic Stem Cells by the SE-9000™ Automated Hematology Analyzer in Peripheral Blood Stem Cell Harvest Samples

K.	Katsuhiko	Takekawa
T.	Takahisa	Yaane
K.	Kenichi	Suzuki
M.	Masayuki	Hino
N.	Noriyuki	Tatsumi

Department of Hematology, Osaka City University Medical School, Osaka, Japan

Takahisa Yamane, Department of Hematology, Osaka City University Medical School, 1-5-7 Asahi-machi, Abeno-ku, Osaka 545 (Japan)

Peripheral blood stem cell (PBSC) transplantation is now used extensively to provide rapid and durable hematopoietic reconstitution following supralethal myeloablative therapies [1].

Although the quantification of the cells responsible for reconstitution is a major clinical issue, there has been no convenient method to identify the presence of stem cells in PBSC harvest (PBSC-H) samples, and until now, flow-cytometric identification or stem cell culturing has been used for detection. However, these methods require time, technical expertise and expensive reagents. We have already reported a simple and easy method of identifying the stem cells in fresh PBSC-H samples using a conventional blood cell counter (SE-9000™; TOA Medical Electronics, Kobe, Japan) with a white blood cell (WBC) differential function [2]. The SE-9000 measures WBC immaturity using a separate Immature Information (IMI) channel [3]. This report describes our investigation of the correlation between the ratio of CD34+ cells and the ratio of the cells detected by the IMI channel (IMI+ cells) for WBC counts in fresh and frozen PBSC-H samples.

Between February 1995 and March 1996, 49 PBSC-H samples were collected from 48 patients with hematological malignancies at our hospital using a CS-3000 plus blood cell separator (Baxter Health Care Corporation Fenwal Division, Deerfield, Ill., USA). The total blood volume processed in each apheresis was 7 liters. In 16 patients from whom mobilized PBSCs were to be collected, granulocyte-colony-stimulating factor (G-CSF) (Chugai, Tokyo, Japan) was administered at a dose of 5 µg/kg/day, given intravenously 2 days before the start of apheresis. In 32 patients treated by conventional chemotherapy, PBSCs were collected on the day the WBC counts exceeded $5 \times 10^9/l$ after the administration of G-CSF (5 µg/kg/day, given intravenously). The product volume was 50 ml. For cryopreservation, hydroxyethyl starch and dimethylsulfoxide were added at concentrations of 6 and 5 %, respectively. The cells were stored at -85°C in a mechanical freezer (Hitachi, Tokyo, Japan). Cryopreserved samples were thawed rapidly in a water bath at 37°C and adjusted to a leukocyte count of $10 \times 10^9/l$ using phosphate-buffered saline. The staining antibodies included

FITC-conjugated HPCA-2 (Becton Dickinson, Mountain View, Calif, USA) to detect CD34 antigen. Single-color flow cytometry on a Cytoron (Ortho, Westwood, Mass., USA) with mononuclear cell gates was used for analysis. Both fresh and frozen samples were analyzed on the SE-9000, and the percentage of IMI+ cells was calculated by dividing the total IMI+ cell count by the total WBC count. The statistical correlation was determined by regression and Pearson's p value.

The values given are means $\pm$ SD unless stated otherwise. A significant correlation ($r = 0.86$; $p < 0.01$) was found between the percentage of CD34+ cells in the fresh PBSCH samples ($0.27 \pm 0.42\%$; range 0.018-2.47%) and that in the frozen PBSCH samples ($0.20 \pm 0.24\%$; range 0.080-1.15%). An excellent correlation ($r = 0.82$; $p < 0.01$) was found between the percentage of CD34+ cells ($0.48 \pm 0.68\%$; range 0.026-2.86%) and IMI+ cells ($1.61 \pm 2.35\%$; range 0.026-8.20%) in the fresh PBSCH samples (fig. 1a). Evaluating the relationship between the percentages of CD34+ cells ($0.24 \pm 0.25\%$; range 0.080-1.15%) and of IMI+ cells ($0.62 \pm 0.84\%$; range 0.10-3.60%) in the measurement of cryopreserved PBSCH

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samples, a significant correlation ($r = 0.62$; $p < 0.01$) was observed, but it was lower than the correlation in the fresh samples (fig. 1b).

Siena et al. [4] reported that determination of the total number of CD34+ cells could be used to estimate the number of hematopoietic progenitors in PBSCH samples. We report that a significant positive correlation exists between the percentages of CD34+ cells and of IMI+ cells in fresh PBSCH samples. It is appropriate, therefore, to use the total number of IMI+ cells detected by the SE-9000 to estimate the number of hematopoietic progenitor cells that can be harvested clinically. Generally, the cytoplasm and cell membrane of blood cells, including stem cells, are damaged by freezing, and a deterioration in cell function and morphological destruction can be recognized [5]. Since flow cytometry detects cells using antigen-ic reaction on cell membranes, CD34 can react with the deteriorated or destroyed cells. The SE-9000 selectively 'protects' immature WBCs with cell-specific reagents, and measures and analyzes them by radio frequency and direct current detection, using the differences in chemical and electrical characteristics between immature and normal WBCs, so it has limited ability to detect cells deteriorated or destroyed by freezing [6]. The major cause of the lower correlation ratio between the percentages of CD34+ cells and of IMI+ cells in cryopreserved samples than in fresh samples is that the SE-9000 cannot measure cells deteriorated or destroyed by the stress of freezing. Therefore, it is possible that hematopoietic stem cells with differentiation activity in cryopreserved samples were included in the IMI+ cell count, and the total infused numbers of IMI+ cells provide a more useful indication of the recovery of the hematopoietic function in PBSC transplantation than the numbers of CD34+ cells.

1 2
CD34 positive cells (%)
0,4 0,6 0,1
CD34 positive cells (%)

Fig. 1. Correlation between the percentages of CD34+ cells and of IMI+ cells in the fresh (a) and cryopreserved (b) PBSCH samples.

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