c-kit-Positive Cells and CD34-Positive Cells in Cryopreserved Peripheral Blood Stem Cell Samples

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Human hematopoietic stem and progenitor cells are contained within the CD34-positive (CD34+) cell populations. The number of CD34+ cells has been used as a reference in predicting neutrophil engraftment in bone marrow and mobilized blood stem cell transplantation [1]. Stem cell factor, which binds to the c-kit receptors, stimulates in synergy with other hematopoietic growth factors the growth and differentiation of early progenitor cells. A portion of human marrow cells has been found to express c-kit receptors [2]. This communication describes our investigation of the correlation between the percentages of c-kit-positive (c-kit+) cells and CD34+ cells in cryopreserved peripheral blood stem cell (PBSC) samples.

Between May 1993 and June 1994, 46 samples of PBSCs were collected from 14 patients at the Department of Hematology of the Osaka City University Hospital. The diseases included malignant lymphoma (n = 11), acute lymphocytic leukemia (n = 1) and acute myelocytic leukemia (n = 2). PBSCs were mobilized with granulocyte-colony-stimulating factor (G-CSF; Chugai, Tokyo, Japan) at 5 µg/kg/day. PBSC collection was initiated 2 days after the first G-CSF injection using a CS-3000plus (Baxter HealthCare, Deerfield, Ill., USA) blood cell separator with a small-volume collection chamber; 7 l of blood were processed during each leukapheresis session. The product volume was 50 ml. Hydroxyethyl starch (6%) and dimethylsulfoxide (5%) were used for cryopreservation. 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 $1 \times 10^4/\mu\text{l}$ using phosphate-buffered saline. Flow cytometry was then used to measure cell surface markers. The staining antibodies included FITC-conjugated HPCA-2 (Becton Dickinson, Mountain View, Calif., USA), to detect CD34 antigen, and FITC-conjugated Anti-Nu-c-kit (Nichirei, Tokyo, Japan), to detect c-kit receptors. Single-color flow cytometry on Cytoron (Ortho, Westwood, Mass., USA) with lymphocyte gates was used for analysis of both markers. Statistical correlation was determined by regression and Pearson's r value. Mean ± SD values are given unless stated otherwise.

A significant correlation ($r = 0.92$, $p < 0.01$) was found between the nucleated cell counts ($5.9 \pm 2.8 \times 10^9$, range: 1.8 - 12.6×10^9) and the percentage of CD34+ cells ($0.45 \pm 0.28\%$, range: 0.12 - 1.3%) in the fresh and cryopreserved PBSC samples (cell counts: $5.7 \pm 2.7 \times 10^9$, range: 1.5 - 10.7×10^9 ; CD34+: $0.28 \pm 0.18\%$, range: 0.08 - 0.98%). A significant correlation was also found between the percentages of CD34+ cells and c-kit cells ($0.33 \pm 0.17\%$, range: 0.08 - 0.68%) in the cryopreserved samples ($r = 0.78$, $p < 0.01$; fig. 1).

Since the 1980s, transplantation of mobilized PBSCs has been used to treat malignant lymphoma [3]. Results have been comparable to or better than those achieved using autologous bone marrow transplantation [4]. Measurement of blood stem cells in PBSC samples has been

0.8-
0.6-
0.4-
0.2-
-0.2 1.0

traditionally obtained by CFU-GM colony-forming assays [5]. Recently, a correlation was reported between total infused CD34+ cells and neutrophil recovery period [6]. An increasing number of hospitals have shifted to the use of CD34+ cells as a reference for PBSC collection and transplantation [7]. Our study identified a significant correlation between the nucleated cell counts and the percentage of CD34+ cells in the fresh and cryopreserved PBSC samples. Although we were not able to examine the percentage of c-kit+ cells among CD34+ cells by two-color flow cytometry, a significant correlation was found between the percentage of c-kit+ cells and CD34+ cells in the thawed PBSC samples. It is possible that the total infused c-kit+ cell number, like that of CD34+ cells, may provide a useful indication of recovery of hematopoietic functions following PBSC transplantation. We are now collecting data on the recovery of hematopoietic functions of these patients. The results will be used to determine whether the number of infused c-kit+ cells can be correlated with engraftment.

0.5 % CD34 positive cell!

Fig. 1. Correlation between percent of CD34+ c-kit+ cells.

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