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Decrease in Thy-1 Expression on Peripheral CD34 Positive Cells Induced by G-CSF Mobilization

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TSUCHIYA, S., KIKUTA, A., SHIMIZU, Y., TAKANO, N., ITO, E., WATANABE, A., IMAIZUMI, M., KONNO, T. and TOHOKU CHILDREN LEUKEMIA STUDY GROUP. *Decrease in Thy-1 Expression on Peripheral CD34 Positive Cells Induced by G-CSF Mobilization.* Tohoku J. Exp. Med., 1997, **182** (2), 157-162 —— In order to ascertain the cytological features of peripheral hematopoietic progenitor cells (PHPC) mobilized after administration of chemotherapeutic agents and G-CSF, lineage- and progenitor cell-specific surface markers on CD34 positive (+) cells were sequentially examined. Nineteen evaluable samples were obtained from a malignant lymphoma, an acute lymphoblastic leukemia and 5 neuroblastoma patients. CD38 and HLA-DR were respectively expressed on more than 95% and approximately 85% of CD34+PHPC cells. CD19 was also expressed on the majority and CD117 on 10 to 20% of the CD34+cells. The most striking finding was that the Thy-1(CDw90)+/CD34+ population was decreased at the peak of mobilization of CD34+ cells as compared to the early phase after G-CSF administration (approximately 20% vs. 60%). These results suggest that decrease in Thy-1 expression on CD34+ cells is related to mechanisms easing CD34+ cell mobilization to the peripheral blood. —— Thy-1; CD34; mobilization;

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Peripheral hematopoietic progenitor cells (PHPC) are easily mobilized to the peripheral blood by chemotherapeutic agents and cytokines (Siena et al. 1989; Teshima et al. 1993). Many studies have shown that hematopoietic stem cells are actually included in the mobilized PHPC (Sonoda et al. 1994; Teofili et al. 1994). Based on these findings autologous peripheral blood stem cell transplantation has been widely carried out instead of autologous bone marrow transplantation (Chopra et al. 1992; Martin 1995). However, the mechanisms underlying mobilization of PHPC by chemotherapeutic reagents and/or cytokines are still poorly understood.

Thy-1 (CDw90), a member of the immunoglobulin superfamily, is a 25–35 kDa membrane glycoprotein expressed on approximately one fourth of CD34+ human hematopoietic cells (Craig et al. 1993). Cells positive for CD34, an immunological marker for PHPC, in fact comprise extremely heterogeneous populations, with the CD34+/Thy-1+ subpopulation being sometimes considered as a candidate hematopoietic progenitor cell. This is not necessarily the case, however, because CD34+/Thy-1+ cells have themselves proved to be phenotypically and functionally heterogeneous (Humeau et al. 1996). In view of the fact that Thy-1 is an adhesion molecule in the mouse immune system (Nishimura et al. 1991), we examined sequential patterns of Thy-1 expression on mobilized peripheral (MP)-CD34+ cells in order to determine any mobilization associated change. As a result we found thy-1 expression on MP-CD34+ cells to be decreased at the time of their peak mobilization.

Materials and Methods

Patients

Seven pediatric patients with malignancies were enrolled in this study after obtaining informed consent. Morphological contamination of bone marrow by residual tumor/leukemia cells was not detected in any of the patients at the time of mobilization. All patients received G-CSF (filgrastim; Sankyo, Tokyo; 100 $\mu g/m^2$ intravenously or 50 $\mu g/m^2$ subcutaneously) for several days from 2 to 10 days after cytotoxic chemotherapy. A total of 22 samples of heparinized venous blood from the 7 patients was sequentially drawn and the surface marker profiles of cells obtained were analyzed by flow cytometry.

Immunofluorescence staining and flow cytometric analysis

For immunofluorescence analysis 20 to 100 μl aliquots of whole blood were incubated for 30 minutes at 4°C with the fluorescein (FITC) conjugated monoclonal antibody HPCA-2 (CD34; Becton-Dickinson, San Jos, CA, USA) and one of the following phycoerythrin (PE)-conjugated monoclonal antibodies: CD38 (OKT10; Ortho, Tokyo), CD19 (HIB19; Pharmingen, San Diego, CA, USA),

HLA-DR (L243; Becton-Dickinson), CD117 (c-kit, 95C3; Immunotech, Marseille, Cedex, France) and Thy-1 (CDw90, 5E10; Pharmingen). Isotype-specific antibodies served as controls. After the incubation, red blood cells (RBC) were lysed with a lysing solution (Becton-Dickinson) and then washed twice with phosphate buffered saline.

Flow cytometric analysis of at least 65,000 cells was carried out according to the method of Craig et al. (1993) using a FACScan (Becton-Dickinson). Briefly, side scatter (SSC) versus forward scatter (FWD) were used to discriminate between white blood cells and RBC or debris. After gating of the WBC region, the CD34+ cells were analyzed in a fluorescence vs. SSC plot. Only cells demonstrating positive fluorescence with a lymphoid or lymphoblastoid appearance were considered as CD34+ cells and their percentages of total WBCs were obtained. Then PE positive cells vs. 100% FITC-CD34+ cells were plotted on cytograms and the percentages were determined and compared with those gained using isotype matched PE labeled control antibodies.

Results

PHPC as assessed in terms of CD34+ cells were successfully mobilized after administration of cytotoxic reagents and G-CSF. Out of 22 samples from 7 patients, 19 were found to contain CD34+ cells as 0.1% to 15.3% of the total WBCs and therefore considered to be evaluable for further dual staining analyses. CD34+ cells were mobilized in at least one sample of each patient. The results of dual staining of surface antigens on CD34+ cells are shown in Fig. 1. More than 95% and approximately 85% of CD34+ cells were positive for CD38 and

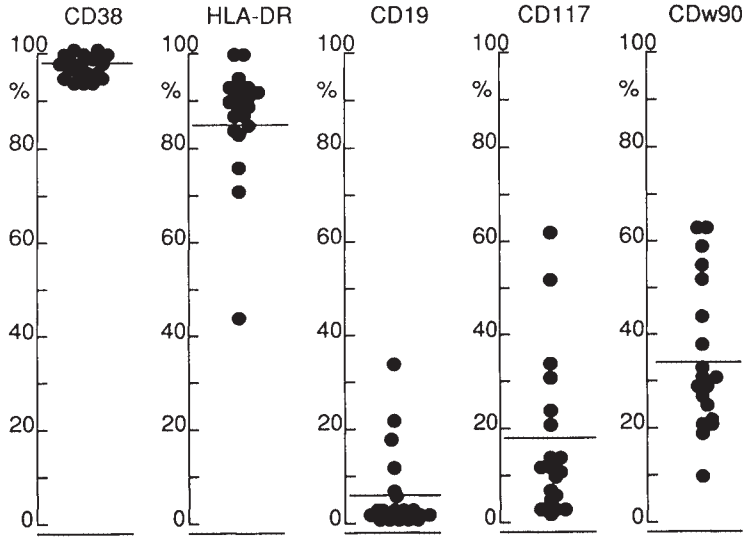

Fig. 1. Two-color immunofluorescence analysis of CD34+ cells. Nineteen peripheral blood samples from 7 patients under G-CSF mobilization after chemotherapy were plotted for expression of each of the monoclonal antibodies. The Y axis indicates the percentage of positive fluorescent cells in the total CD34+ population. The horizontal line represents the mean.

TABLE 1. *Sequential determination of CD34+/THY-1+ cells in G-CSF mobilized peripheral blood*

Patient	Days after G-CSF administration	White blood cell count /μl	Absolute number of CD34+ cells/μl	Percentage of CD34+/THY-1+ cells	Percentage of CD34+/CD117+ cells
B.I. (2)[a]	1	1,600	1.6	54.0	61.9
	3	2,500	7.5	38.2	50.4
	7	6,700	53.6	20.5	32.0
M.R.	4	1,200	10.8	62.7	8.1
	6	3,500	157.5	51.8	4.9
	7	5,600	235.2	28.1	2.3
	11	11,200	280.0	21.1	12.6
O.F.	2	1,700	1.7	61.1	10.1
	7	4,300	4.3	30.9	1.9
	9	11,500	11.5	25.2	3.7
	10	14,400	43.2	28.2	11.1
K.M.	4	2,600	397.8	57.3	23.9
	5	10,200	734.4	43.1	20.1
B.I. (1)[a]	8	2,500	20.0	31.6	13.6
	13	2,300	13.8	26.6	13.1

[a]The number in parentheis indicates the cycle of chemotherapy for neuroblastoma.

HLA-DR, respectively. CD117, c-kit tyrosine kinase, was expressed on from 10 to 20% of the CD34+ cells. The majority of CD34+ cells were negative for CD19, a B lineage marker. However, in one patient (B.I.) who received second cycle chemotherapy for neuroblastoma, blood samples drawn on days 1, 3 and 7 after G-CSF administration demonstrated exceptionally high expression of CD117 (61%, 50% and 32%), correlated to a certain extent with high expression of CD19 (34%, 11.5% and 21.5%, respectively). On the other hand, a tendency for decrease in expression of Thy-1 on CD34+ cells was uniformly found in sequentially collected samples from 4patients as shown in Table 1. The percentage of Thy-1+ cells were approximately 60% among CD34+ cells at the beginning of G-CSF administration and this fell to approximately 20% at the peak of mobilization.

Discussion

There have been several reports of sequential changes of surface antigen expression on MP-CD34+ cells after chemotherapy and cytokine administration. After administration of GM-CSF (Stewart et al. 1995), and G-CSF or GM-CSF (Murray et al. 1995) to patients with malignant diseases, findings similar to ours were obtained, though mainly with GM-CSF; decrease in percentage of Thy-1+ cells among CD34+ cells from 77% to 37% (Stewart et al. 1995), gradual decline from 40–65% to 20% (Murray et al. 1995) at the peak of mobilization. Usually, Thy-1 expression has been reported for around 23% (Haas et al. 1995) or 15–30% (Nimgaonkar et al. 1995) of MP-CD34+ cells, and for approximately 10% of bone marrow derived (BM)-CD34+ cells (Haas et al. 1995). Only sequential analysis revealed high Thy-1 expression on CD34+ cells at the initial few days of mobilization irrespective of cytokines used. However, peripheral blood CD34+/Thy-1+ cells are phenotypically and functionally heterogeneous populations and can not be utilized as a marker of pluripotent hematopoietic progenitor cells like CD34+/CD38- cells (Humeau et al. 1996). Sequential changes in degree of Thy-1 expression might reflect still unknown functions of the molecule in connection with mobilization of CD34+ cells to peripheral blood. A well characterized example of the interaction between stromal cells and PHPC during mobilization is that observed for the c-kit receptor and its membrane bound form (Heinrich et al. 1993). CD117 is expressed on more than 70% of BM-CD34+ cells, but among CD34+ cells mobilized to peripheral blood the figure is less than 20% (Table 1, Haas et al. 1995). Taking these findings into consideration, decrease in Thy-1 might be a mobilization associated change of surface antigens on CD34+ cells along with that of CD117, and therefore presumably related to the mechanisms underlying mobilization.

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