Expression of Adhesion Molecules on CD34$^+$ Cells: CD34$^+$ L-Selectin$^+$ Cells Predict a Rapid Platelet Recovery After Peripheral Blood Stem Cell Transplantation

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Adhesion molecules play a role in the migration of hematopoietic progenitor cells and regulation of hematopoiesis. To study whether the mobilization process is associated with changes in expression of adhesion molecules, the expression of CD31, CD44, L-selectin, sialyl Lewis^x, β1 integrins very late antigen 4 (VLA-4) and VLA-5, and β2 integrins lymphocyte function–associated 1 and Mac-1 was measured on either bone marrow (BM) CD34$^+$ cells or on peripheral blood CD34$^+$ cells mobilized with a combination of granulocyte colony-stimulating factor (G-CSF) and chemotherapy. β1 integrin VLA-4 was expressed at a significantly lower concentration on peripheral blood progenitor cells than on BM CD34$^+$ cells, procured either during steady-state hematopoiesis or at the time of leukocytapheresis. No differences in the level of expression were found for the other adhesion molecules. To obtain insight in which adhesion molecules may participate in the homing of peripheral blood stem cells (PBSCs), the number of CD34$^+$ cells expressing these adhesion molecules present in leukocytapheresis material was quantified and correlated with hematopoietic recovery after intensive chemotherapy in 27 patients. The number of CD34$^+$ cells in the subset defined by L-selectin expression correlated significantly better with time to platelet recovery after PBSC transplantation ($r = -.86$) than did the total number of CD34$^+$ cells ($r = -.55$). Statistical analysis of the relationship between the number of CD34$^+$L-selectin$^+$ cells and platelet recovery resulted in a threshold value for rapid platelet recovery of 2.1×10^6 CD34$^+$ L-selectin$^+$ cells/kg. A rapid platelet recovery ($\leq$14 days) was observed in 13 of 15 patients who received $\geq 2.1 \times 10^6$ CD34$^+$ L-selectin$^+$ cells/kg (median, 11 days; range, 7 to 16 days), whereas 10 of 12 patients who received less double positive cells had a relative slow platelet recovery (median, 20 days; range, 13 to 37 days). The L-selectin$^+$ subpopulation of CD34$^+$ cells also correlated better with time to neutrophil recovery ($r = -.70$) than did the total number of reinfused CD34$^+$ cells ($r = -.51$). However, this latter difference failed to reach statistical significance. This study suggests that L-selectin is involved in the homing of CD34$^+$ cells after PBSC transplantation.

TRANSPLANTATION of mobilized peripheral blood stem cells (PBSCs) is increasingly used to facilitate hematologic recovery following high-dose chemotherapy.[1,2] CD34$^+$ progenitor cells normally circulate in very low numbers, but they can be mobilized into the peripheral blood with a variety of regimens, including different combinations of cytotoxic drugs and hematopoietic growth factors.[1-5] Highly enriched CD34$^+$ progenitor cells can reconstitute hematopoiesis in vivo in primates and humans.[6-8] Direct interactions between these hematopoietic progenitor cells (HPCs) and bone marrow (BM) endothelial and stromal cells play an important role in the egress of these cells from the BM into the peripheral blood[9] as well as in the homing of the intravenously infused HPCs to the BM.[10] In addition, direct interactions may also play a role in the regulation of hematopoiesis.[11] The molecular basis of recognition, the egress of stem cells from and the homing to the BM is not yet completely defined.

A number of known cell adhesion molecules may be involved in these processes. On hematopoietic progenitor cells, L-selectin and sialyl Lewis^x, a known counter structure for vascular E-selectin and P-selectin, are expressed.[12-15] In addition, HPCs express platelet/endothelial cell adhesion molecule 1 (PECAM-1/CD31) and homing-associated cell adhesion molecule (H-CAM/CD44), both of which can bind to extracellular matrix components in the BM.[16,17] The β1 integrins, very late antigen 4 (VLA-4) and VLA-5 and the β2 integrin lymphocyte function–associated 1 (LFA-1) have been implicated in the adhesive interactions of HPC and BM stroma.[10,18-22] VLA-4 seems to play an especially important role, because it has been shown that blocking VLA-4 antibodies both inhibit the homing[19] and mobilize stem cells into the peripheral blood in animal models.[9]

To investigate which of these adhesion molecules may be involved in mobilization of PBSCs, we compared the expression of adhesion molecules on BM HPCs and mobilized progenitor cells. We hypothesized that reinfused CD34$^+$ cells that express the proper set of adhesion molecules might home more easily to the BM environment and possibly lead to a relative rapid hematopoietic recovery after high-dose chemotherapy. In this study, subsets of peripheral blood CD34$^+$ cells defined by the expression of these adhesion antigens were identified and quantified. To test our hypothesis, the numbers of CD34$^+$ cells belonging to each of the reinfused subsets were correlated with hematopoietic recovery after intensive chemotherapy.

MATERIALS AND METHODS

Monoclonal antibodies (MoAbs). The following antibodies were used in this study: IgG1 and IgG2a isotype control antibodies were obtained from CLB (Amsterdam, The Netherlands). Phycoerythrin (PE)-labeled rat MoAb against the (κ) light chain of mouse Ig, MoAb

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Leu-8 (CD62L, L-selectin) and fluorescein isothiocyanate (FITC)-labeled HPCA-2 (CD34) were purchased from Becton Dickinson (San Jose, CA). The antisialyl-Le^x MoAb CSLEX-1 (CD15s) was purchased from P. Terasaki (University of California Medical School, Los Angeles, CA). MoAb HPA 2/1 (CD49d, VLA-4) was purchased from Immunotech SA (Marseille, France).

MoAbs OKM (CD11b, Mac-1) and P2 (CD44, H-CAM) were obtained by Fourth International Workshop studies.[23] MoAbs CLB-mon/1 (CD14), CLB-T11/1 (CD2), CLB-LFA 1/2 (CD11a, LFA-1), ES12F11 (CD31, PECAM-1), and SAM-1 (CD49e, VLA-5) are all MoAbs produced in our laboratory and clustered in the International Workshops on Leukocyte Differentiation Antigens.[23]

Patient characteristics. The 27 patients studied (median age, 35 years; range, 19 to 52) included 9 patients with breast cancer, 8 patients with germ cell cancer, 4 patients with Hodgkin's disease, 3 patients with non-Hodgkin's lymphoma, 1 patient with nasopharynx carcinoma, 1 patient with medulloblastoma, and 1 patient with neuroblastoma. The patients were either in their first chemotherapy-induced (near) complete remission (patients with breast cancer)[24] or in second partial or complete remission (remaining patients). The patients with germ cell cancer underwent a tandem transplantation procedure with a 5-week interval. All patients had World Health Organization (WHO) performance status 0 or 1, adequate renal and hepatic functions (creatinine clearance $\geq$ 50 mL/min, bilirubin $\leq$ 25 μmol/L) and normal BM functions (white blood cell (WBC) count $\geq 3.5 \times 10^9$/L, platelets $\geq 100 \times 10^9$/L.

At the time of leukocytapheresis, BM was aspirated from patients with breast cancer. In addition, BM was aspirated from patients undergoing cardiac surgery (L. Eijsman, Department of Cardiac Surgery, Academic Medical Center, Amsterdam, The Netherlands) and patients with malignancies without evidence of BM localization of their disease.

All patients gave informed consent, and the separate protocols were approved by the Ethical and Scientific Review Committees of the Netherlands Cancer Institute, the Free University Hospital, and the Academic Medical Center (Amsterdam, The Netherlands).

Mobilization procedure, PBSC harvest and reinfusion. Hematopoietic progenitor cells were mobilized by chemotherapeutic treatment followed by 300 μg/d subcutaneous granulocyte colony-stimulating factor (G-CSF) daily (Filgrastim, Amgen Inc, Thousand Oaks, CA) until completion of the leukocytapheresis. In the patients with breast cancer, the mobilizing regimen consisted of 5-fluorouracil (500 mg/m^2), epirubicin (120 mg/m^2) and cyclophosphamide (500 mg/m^2) given on day 1 with G-CSF starting on day 2.[24] In the remaining patients, PBSCs were mobilized with ifosfamide (4 g/m^2, day 1) and etoposide (100 mg/m^2, day 1 to 3), followed by G-CSF onward from day 4.

From the seventh day of G-CSF administration, the percentage of CD34$^+$ cells in the peripheral blood was determined daily. As soon as the WBC count exceeded 3.0×10^9/L and an unequivocal increase in CD34$^+$ cell percentage was observed, leukocytapheresis procedures were started. At the start of each leukocytapheresis procedure, the number of platelets had to be $\geq 50 \times 10^9$/L. The leukocytaphereses were performed as an outpatient procedure with a continuous-flow blood cell separator (Fenwal CS3000, Baxter Deutschland GmbH, Germany) on 2 to 4 consecutive days, depending on the number of CD34$^+$ cells procured, which was determined at the end of each pheresis day. The cells were cryopreserved in physiologic saline solution, containing 0.1% glucose, 0.38% trisodium citrate, 10% human serum albumin and 10% dimethylsulfoxide at a cell concentration of about 50×10^6 mononuclear cells/mL. For cryopreservation, the cell suspensions were frozen at a controlled rate in a Kryo10 (Cryothec, Schagen, The Netherlands). The frozen cells were stored in the vapor phase of liquid nitrogen until reinfusion.

As a high-dose regimen, the patients with nonhematologic malignancies received 1,600 mg/m^2 carboplatin, 480 mg/m^2 thiotepa, and 6,000 mg/m^2 cyclophosphamide intravenously, divided over 4 days (CTC).[25]

Patients with malignant lymphoma received the BEAM regimen (300 mg/m^2 carmustine, 800 mg/m^2 etoposide, 800 mg/m^2 cytarabine, and 140 mg/m^2 melphalan[26]). Forty-eight hours after the last dose of chemotherapy in the CTC regimen or 24 hours after the last dose of chemotherapy in the BEAM regimen, the cryopreserved apheresis products were thawed rapidly at the bedside and were reinfused via an indwelling subclavian catheter. All patients received G-CSF, which was started on the day of PBSC transplantation and continued until the WBC count in the peripheral blood exceeded 5 $\times 10^9$/L.

Detection of CD34$^+$ cells. The percentage of cells expressing the CD34 antigen was determined in a sample of the leukocytapheresis product just before cryopreservation by a direct immunofluorescence procedure. After lysis of erythrocytes with isomolar NH_4Cl buffer for 10 minutes, 1×10^6 cells were incubated with MoAb CD34-FITC. All incubations were performed at 4°C, and after each incubation, the cells were washed with phosphate buffered saline (PBS) containing 0.2% (wt/vol) bovine serum albumin (BSA). Flowcytometric analyses were performed with a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA). A minimum of 20,000 cells were acquired in list mode. The percentage of CD34$^+$ cells present was assessed after correction for cells reactive with an isotype control. Analysis of the five-dimensional data was performed with Consort 30 software (Becton Dickinson, San Jose, CA). Absolute numbers of CD34$^+$ progenitor cells were calculated by multiplication of the total amount of nucleated blood cells in the leukocytapheresis product with the percentage of CD34$^+$ cells in the total leukocytapheresis product.

Flow cytometric analysis of CD34$^+$ cells. Subsets of CD34$^+$ cells expressing the different adhesion antigens were quantified with thawed samples of the leukocytapheresis product. The erythrocytes were lysed with isomolar NH_4Cl buffer for 10 minutes at 4°C and were subsequently washed twice with PBS containing 0.2% (wt/vol) BSA. BM mononuclear cells (MNCs) were isolated by density centrifugation over Ficoll-Paque (Pharmacia, Uppsala, Sweden; density, 1.077 g/cm^3). To facilitate further analysis of subsets of CD34$^+$ cells, we enriched for immature progenitors by one to two rounds of immunomagnetic depletion of more mature MNCs. MNCs (1×10^7/mL) were incubated for 40 minutes at 4°C with a mixture of CD2 and CD14 in the presence of DNAse (20 U/L) and 10 mmol/L $MgCl_2$. Cells were washed twice and incubated with immunomagnetic beads coated with rat-antimouse Ig (ratio beads:cells, 3:1; Dynal, Hamburg, Germany). Magnetic beads, together with bound cells, were then removed with a magnet, and the remaining cells were washed with PBS/BSA. Two rounds of immunomagnetic depletions were usually performed. This procedure did not influence the relative proportions of the different CD34$^+$ cell subsets (data not shown).

For dual-color immunofluorescence analysis, cells were incubated for 30 minutes with the primary MoAb, followed by incubation with PE-labeled rat antimouse Ig. An isotype matched mouse Ig served as control. Residual binding sites of rat-antimouse Ig were blocked with a mixture of irrelevant murine MoAbs of the IgG1 and IgG2a subclasses. Subsequently, cells were incubated with MoAb CD34-FITC. All incubations were performed at 4°C. After each incubation, cells were washed with PBS/BSA. For the determination of the phenotype of CD34$^+$ cells, a minimum of 2,500 CD34$^+$ cells were analyzed. Absolute numbers of the various subsets of CD34$^+$ cells defined by the expression of adhesion molecules were calculated by multiplication of the absolute numbers of CD34$^+$ cells with the percentage of CD34$^+$ cells that express the adhesion molecule.

Statistical analysis. For nonnormal distributed values, data were summarized by means of median and ranges; otherwise, the arithme-

tic mean and standard deviation were used. Differences were calculated by means of the Mann-Whitney U test. The correlations are Spearman Rank correlations. A P value lower than $\alpha = .05$ was considered significant.

Thresholds for rapid hematopoietic recovery were defined by the optimum of both sensitivity and specificity of a tested parameter in the receiver-operating characteristic curve.

RESULTS

Procurement of PBSCs and hematopoietic recovery after transplantation. PBSCs were mobilized with a combination of G-CSF and two different chemotherapeutic regimens (5-fluorouracil/epirubicin/cyclophosphamide or ifosfamide/etoposide) in 27 patients. In 92 leukocytapheresis procedures (median, 3 per patient; range, 1 to 8) a median of 6.7×10^6 $CD34^+$ cells/kg were harvested (range, 1.6 to 42.0×10^6/kg). The two mobilization regimens were equally effective in mobilizing $CD34^+$ cells.

After high-dose chemotherapy, PBSCs were reinfused and the patients were then followed for hematologic recovery. Primary graft failures were not observed. However, in one patient with breast cancer, secondary graft failure occured and has been reported elsewhere.[27] Reinfusion of PBSCs with a median of 6.0×10^6 $CD34^+$ cells/kg (range, 1.6 to 39.4×10^6/kg) resulted in a rapid recovery of neutrophils to values higher than 0.5×10^9/L within 14 days (median, 10 days; range, 8 to 28) in the majority of the patients (22/27) (Fig 1A). Fifteen of 27 patients achieved platelet transfusion independence (defined as the platelet count remaining $\geq 20 \times 10^9$/L without platelet transfusions) within 2 weeks after PBSC transplantation (median, 14 days; range, 7 to 37) (Fig 1B). No significant differences in the rate of neutrophil or platelet recovery were found with either high-dose chemotherapy regimen (CTC or BEAM) or between the different diagnoses (data not shown).

Expression of adhesion molecules on $CD34^+$ BM stem cells and mobilized PBSCs. To investigate which adhesion molecules may be involved in mobilization of PBSC, the expression of eight different adhesion molecules was examined in a double-color fluorescence assay with an FITC-conjugated CD34 antibody on $CD34^+$ progenitors from normal BM (n = 10) and on mobilized PBSCs (n = 27) (Table 1). Equally high expression on both types of $CD34^+$ cells was found for PECAM-1 (CD31), H-CAM (CD44), and sialyl Lewis^x, whereas a variable expression was found for L-selectin (CD62L), the $\beta 1$ integrins VLA-4 (CD49d) and VLA-5 (CD49e), and the $\beta 2$ integrins LFA-1 (CD11a) and Mac-1 (CD11b). In leukocytapheresis material, a significantly lower percentage of $CD34^+$ cells were found positive for VLA-4 ($P < .0001$), whereas a significantly higher percentage of $CD34^+$ cells were found positive for LFA-1 as compared with BM $CD34^+$ cells ($P = .03$). However, the mean fluorescence intensity (MFI) was significantly different only for VLA-4 ($P < .0001$). No significant differences in expression were found for the other adhesion molecules (Table 1). No significant differences in adhesion molecule expression were found between the two mobilizing regimens (data not shown).

To investigate whether this difference in VLA-4 expression was caused by the mobilization procedure, the expression of adhesion molecules was measured on $CD34^+$ cells from BM and peripheral blood of the same patients, both procured at the time of leukocytapheresis (n = 5). In peripheral blood, a significant lower percentage of $CD34^+$ cells expressed VLA-4 (median, 24.3%; range, 12.4 to 35.9) with a lower MFI (median, 10.1; range, 6.1 to 14.6) as compared with BM $CD34^+$ cells (percent expression, 57.7% (range, 9.8% to 67.1%); MFI, 25.0 (range, 5.6 to 27.3) (paired t-test, $P < .05$) (Fig 2). The expression pattern of other adhesion molecules did not differ between BM or peripheral blood $CD34^+$ cells (data not shown).

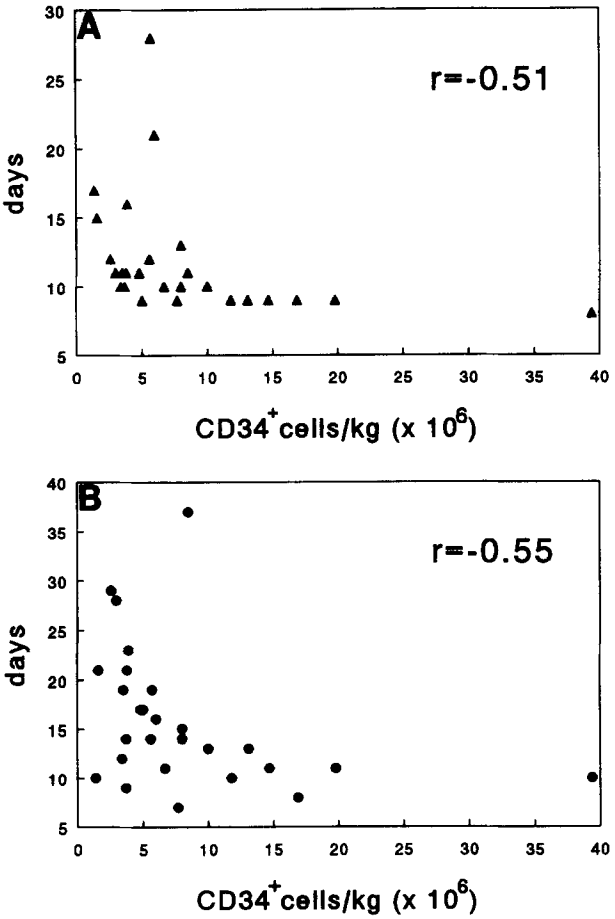

Fig 1. Correlation between reinfusion of $CD34^+$ cells and time to neutrophil recovery to values above 0.5×10^9/L (A) and time to platelet transfusion independence (B).

L-selectin improves correlation with platelet recovery after PBSC transplantation. Spearman Rank correlation assays were performed to correlate the numbers of $CD34^+$ cells belonging to each of the reinfused subsets present in the leukocytapheresis samples with time to recovery of either neutrophils or platelets after PBSC transplantation. The results show that the number of reinfused $CD34^+$ cells expressed per kilogram body weight correlated with time to neutrophil recovery to values higher than 0.5×10^9/L ($r = -.51$, 95% confidence interval (CI), -0.75 to -0.16) as well

Table 1. Distribution of Adhesion Molecules on CD34⁺ Cells

Antigen	CD	Leukocytapheresis Samples (n = 27) Median % Positive	Range	MFI (SD)	BM (n = 10) Median % Positive	Range	MFI (SD)
Irrelevant mouse IgG1		—	—	4.6 (1.4)	—	—	5.1 (2.5)
PECAM-1	CD31	93.3	83.9-96.2	92.8 (24.7)	92.3	85.3-98.2	82.6 (27.4)
H-CAM	CD44	95.1	89.4-98.6	165.8 (49.8)	94.4	83.4-97.1	136.6 (41.8)
Sialyl-Le^x	CD15s	95.2	90.4-95.0	809.7 (162.0)	93.7	40.3-97.5	697.6 (164.0)
L-selectin	CD62L	34.5	15.5-65.5	24.0 (15.9)	37.0	15.7-74.1	24.9 (11.1)
VLA-4*	CD49d	11.1†	2.0-36.8	9.7† (2.5)	40.7	10.3-95.8	22.6 (12.2)
VLA-5*	CD49e	75.2	46.8-89.8	38.3 (22.1)	79.8	23.9-98.4	34.5 (16.3)
LFA-1	CD11a	75.7‡	5.6-95.0	61.9 (22.3)	64.0	45.7-81.2	66.7 (24.0)
Mac-1	CD11b	19.3	7.1-41.5	16.5 (5.9)	20.9	7.2-32.6	19.6 (8.1)

* Expression of VLA-4 and VLA-5 was measured on BM CD34⁺ cells obtained from 25 patients.
† Significantly different from BM CD34⁺ cells ($P < .0001$).
‡ Significantly different from BM CD34⁺ cells ($P = .03$).

as time to platelet transfusion independence ($r = -.55$, 95% CI, -0.77 to -0.21).

To investigate whether subsets of CD34⁺ cells expressing adhesion molecules resulted in a better correlation with hematopoietic recovery than CD34⁺ cells alone, the numbers of CD34⁺ cells belonging to each of the reinfused subsets were correlated with time to neutrophil and platelet recovery (Table 2). When subsets of CD34⁺ cells were correlated with time to platelet transfusion independence, a significantly better correlation ($r = -.86$) was found with CD34⁺ subset defined by L-selectin expression than with the total number of CD34⁺ cells ($P < .01$). Although the number of L-selectin–expressing CD34⁺ cells tended to correlate somewhat better with neutrophil recovery ($r = -.70$) than did the total number of CD34⁺ cells, this difference was not statistically significant ($P = .14$). Subsets defined by the expression of other adhesion molecules did not improve the correlation with neutrophil or platelet recovery as compared with CD34⁺ cells alone. A better correlation with neutrophil as well as platelet recovery was found for VLA-4⁻ subsets ($r = -.54$ and $r = -.62$, respectively) than for VLA-4⁺ subsets. However, this difference did not reach statistical significance.

To assess a threshold for rapid recovery (arbitrarily set as the time to platelet transfusion independence within 14 days after PBSC transplantation), the optimum of both sensitivity and specificity of the tested parameter were defined. When L-selectin–expressing CD34⁺ cells was used as a parameter, this threshold was calculated to be 2.1×10^6 CD34⁺ L-selectin⁺ cells/kg. In 13 of 15 patients, reinfusion of over 2.1×10^6 CD34⁺ L-selectin⁺ cells/kg resulted in a rapid platelet recovery (median, 11 days; range, 7 to 16 days), whereas reinfusion of less double-positive cells resulted in a significant delayed ($P < .001$) platelet recovery (median, 20 days; range, 13 to 37). In this group, only 2 of 12 patients had recovered within 2 weeks (Fig 3).

DISCUSSION

In the current study, expression of adhesion molecules was measured on CD34⁺ cells from BM and peripheral blood of the same patients, both procured at the time of leukocytapheresis, and on steady-state BM CD34⁺ cells from other patients. Significantly lower expression on mobilized CD34⁺ cells was shown for VLA-4 as compared with BM-derived

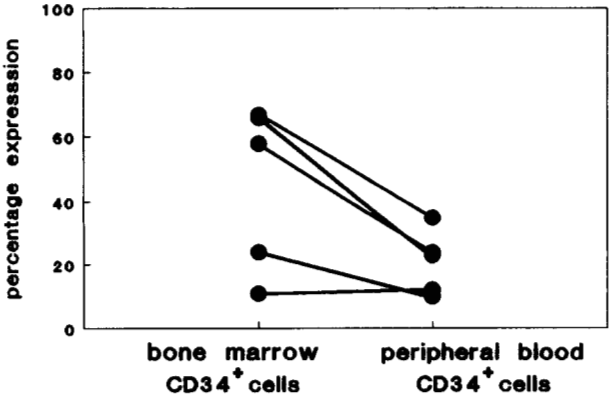

Fig 2. Percentage of CD34⁺ cells expressing VLA-4. Expression of VLA-4 was measured on CD34⁺ cells from BM and peripheral blood of the same patients, both harvested at the time of leukocytapheresis (n = 5).

Table 2. Correlation of Subsets of CD34⁺ Cells With Time to Neutrophil and Platelet Recovery

	Time to Neutrophil Recovery (>0.5 × 10⁹/L) Correlation Coefficient	95% Confidence Interval	Time to Platelet Transfusion Independence Correlation Coefficient	95% Confidence Interval
All CD34⁺ cells	−0.51	−0.75-−0.16	−0.55	−0.77-−0.21
Subset of CD34⁺ cells				
CD34⁺ CD31⁺	−0.52	−0.75-−0.17	−0.60	−0.80-−0.28
CD34⁺ CD44⁺	−0.52	−0.75-−0.17	−0.59	−0.79-−0.27
CD34⁺ Sialyl-Le^x⁺	−0.52	−0.75-−0.17	−0.58	−0.79-−0.26
CD34⁺ L-selectin⁺	−0.70	−0.85-−0.44	−0.86*	−0.93-−0.71
CD34⁺ L-selectin⁻	−0.39	−0.67-−0.01	−0.45	−0.71-−0.08
CD34⁺ VLA-4⁺	−0.37	−0.66-0.01	−0.29	−0.60-0.10
CD34⁺ VLA-4⁻	−0.54	−0.76-−0.20	−0.62	−0.81-−0.31
CD34⁺ VLA-5⁺	−0.52	−0.75-−0.17	−0.62	−0.81-−0.31
CD34⁺ LFA-1⁺	−0.40	−0.68-−0.02	−0.45	−0.71-−0.08
CD34⁺ Mac-1⁺	−0.46	−0.72-−0.10	−0.63	−0.81-−0.33

* Significantly different from the correlation between the total number of CD34⁺ cells and time to platelet transfusion independence ($P < .01$).

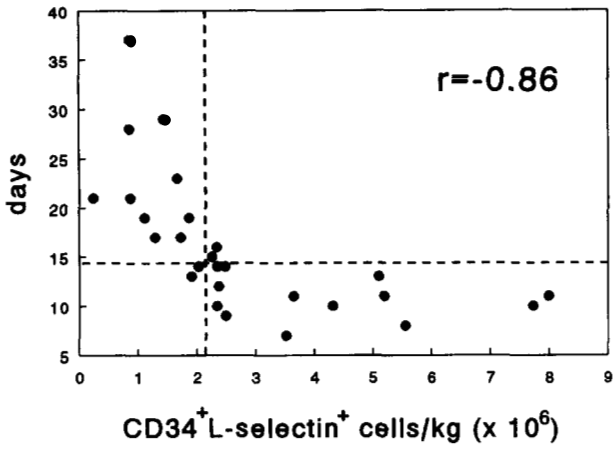

Fig 3. Correlation between reinfusion of CD34+ L-selectin+ cells and time to platelet transfusion independence. Calculated threshold of reinfused CD34+ L-selectin+ cells (2.1×10^6 CD34+ L-selectin+ cells/kg) for rapid platelet recovery and the time for rapid recovery (arbitrarily set as the time to platelet transfusion independence within 14 days after PBSC transplantation) are depicted as dashed lines.

$CD34^+$ cells from both patient groups, whereas no significant differences in expression were found for PECAM-1, H-CAM, sialyl $Lewis^x$, L-selectin, the $\beta 1$ integrin VLA-5, and the $\beta 2$ integrins LFA-1 and Mac-1. Subsets of PBSCs defined by adhesion molecule expression were quantified and the number of reinfused $CD34^+$ cells of the various subsets were correlated with hematopoietic recovery after intensive chemotherapy. It was shown that the number of L-selectin–expressing $CD34^+$ cell subsets present in the leukocytapheresis products correlated significantly better with platelet recovery after PBSC transplantation than did the total number of $CD34^+$ cells. Subsets of $CD34^+$ cells expressing L-selectin also correlated better with neutrophil recovery than did the total number of reinfused $CD34^+$ cells. However, this difference failed to reach statistical significance. This may be because of the fact that in only 5 of 27 patients of this patient group, reinfusion of PBSCs resulted in a slow neutrophil recovery (more than 14 days) and, apparently, in most of the other patients an excess of PBSC was reinfused. In addition, L-selectin was not found to be preferentially expressed on $CD34^+CD41^+$ cells (putative megakaryocyte progenitors) (data not shown). The significantly better correlation between the number of $CD34^+$L-selectin$^+$ cells with platelet recovery and the total number of $CD34^+$ cells indicates that determination of L-selectin can help to predict short-term repopulation capacity of the PBSC and can be useful in the establishment of a practical minimum of progenitor cells required for rapid engraftment. This study indicates that reinfusion of at least 2.1×10^6 $CD34^+$ L-selectin$^+$ cells/kg body weight predicts a rapid engraftment of both platelets and neutrophils.

Several adhesion molecules are involved in the homing of hematopoietic progenitor cells and in the regulation of hematopoiesis. The significantly better correlation between subsets of $CD34^+$ cells expressing L-selectin with hematopoietic recovery suggests that L-selectin is involved in the hematopoietic reconstitution after PBSC transplantation. Several mechanisms can account for these results. L-selectin might be involved in the homing of $CD34^+$ cells to the BM by presenting itself to a proper ligand present on BM endothelial cells. At least three glycoproteins present on endothelial cells have been reported to function as a ligand for leukocyte L-selectin. These glycoproteins were defined as Glycam-1, which is expressed predominantly in high endothelial venules of peripheral lymph nodes,[22,28] and CD34,[29] which is constitutively expressed on endothelial cells in a diversity of blood vessels including those in the BM stroma.[30] Another study showed the involvement of E-selectin in L-selectin–mediated binding of neutrophils.[31] Moreover, L-selectin–associated sialyl $Lewis^x$, which is also present on HPC,[13] appears to be a ligand for E-selectin expressed on vascular endothelial cells.[32] Constitutive expression of E-selectin on BM endothelial cells has been shown.[33] Furthermore, unidentified ligands for L-selectin may be expressed on BM endothelial cells. The importance of carbohydrates in the homing of progenitors is also reflected by the murine studies of Aizawa and Tavassoli.[34] They showed that the homing of stem cells could be prevented by galactosyl and mannosyl residues.

Selectins are reported to act in concert with other cell adhesion molecules to effectuate adhesive interactions of leukocytes, platelets and endothelial cells.[35] Moreover, the VLA-4/vascular cell adhesion molecule (VCAM-1), the VLA-5/fibronectin, and the LFA-1/intercellular adhesion molecule 1 pathways have been implicated in adhesive interactions with cultured BM stroma.[18,20,22,36,37] Both the $\beta 1$ integrins (VLA-4 and VLA-5) and the $\beta 2$ integrins (LFA-1, Mac-1) are expressed to varying degrees on $CD34^+$ progenitor cells, as well as on differentiating cells of several lineages.[22,38-41] In our study subsets of $CD34^+$ cells, defined by either expression or absence of expression of $\beta 1$ integrins (VLA-4 and VLA-5) or $\beta 2$ integrins (LFA-1, Mac-1), did not correlate better with hematopoietic recovery than did $CD34^+$ cells alone. Interestingly, significantly lower expression of VLA-4 was found on mobilized HPC as compared with normal BM progenitor cells. This was not a direct consequence of the mobilizing regimen or G-CSF levels because lower expression of VLA-4 was also found on peripheral blood $CD34^+$ cells as compared with BM $CD34^+$ cells procured from the same patients at the time of leukocytapheresis. Furthermore, in vitro incubation with G-CSF did not induce downregulation of VLA-4 expression on $CD34^+$ cells (data not shown). Because we have previously shown that during myeloid differentiation VLA-5 is lost at an earlier stage of differentiation than is VLA-4,[22] the decreased expression of VLA-4 cannot only be caused by the presence of more differentiated $CD34^+$ cells in the peripheral blood. Recently, Papayannopoulou and Nakamoto[9] suggested a role for VLA-4 in the egress of HPCs. Intravenous treatment of baboons or macaques with MoAbs against VLA-4 induced a significant mobilization of HPCs of all cell lineages.[9] The lower expression of VLA-4 on mobilized HPCs is in accordance with this study and supports the suggestion that $\beta 1$ integrins have a role in stabilization of the interaction of progenitor cells with the stroma.

In conclusion, we have presented data that $CD34^+$ cells from BM and peripheral blood express different amounts of VLA-4. Correlation studies between subsets of mobilized PBSCs and hematopoietic recovery after high-dose chemotherapy suggest that L-selectin present on $CD34^+$ cells is involved either in the homing of PBSCs to the BM or in regulation of hematopoiesis.

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