Migration of human hematopoietic progenitor cells

Voermans, C.

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Chapter 2

Increased migration of cord blood-derived CD34\(^+\) cells, as compared to bone marrow and mobilized peripheral blood CD34\(^+\) cells across uncoated or fibronectin-coated filters.

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Increased migration of cord-blood-derived CD34^+ cells, as compared to bone marrow and mobilized peripheral blood CD34^+ cells across uncoated or fibronectin-coated filters

C. Voermans^1,2, W.R. Gerritsen^3, A.E.G. Kr. von dem Borne^4 and C.E. van der Schoot^2,4

^1 Division of Medical Oncology, Netherlands Cancer Institute, Amsterdam, the Netherlands
^2 CLB, Sanquin Blood Supply Foundation, and Laboratory for Experimental and Clinical Immunology, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands
^3 Gene Therapy Program, Department of Medical Oncology, Academic Hospital of the Free University, Amsterdam, the Netherlands
^4 Department of Hematology, Academic Medical Centre, Amsterdam, the Netherlands

ABSTRACT

Hematopoietic progenitor cells (CD34^+ cells) migrate to the bone marrow after reinfusion into peripheral veins. Stromal cell-derived factor-1 (SDF-1) is a chemokine produced by bone marrow stromal cells that induces migration of CD34^+ cells. In this study we compared spontaneous and SDF-1-induced migration of CD34^+ cells from bone marrow (BM), peripheral blood (PB) and cord blood (CB) across Transwell filters. Under all circumstances, CB-CD34^+ cells showed significantly more migration than did BM- or PB-CD34^+ cells. SDF-1 induced migration of BM-CD34^+ cells was higher than that of PB-CD34^+ cells, possibly due to differences in sensitivity towards SDF-1. Indeed, PB-CD34^+ cells showed a significantly lower expression of the receptor for SDF-1 (CXCR-4) than did BM- and CB-CD34^+ cells. The sensitivity to SDF-1, as measured by migration towards different concentrations of SDF-1, was identical for BM- and CB-derived CD34^+ cells and correlated with their equal CXCR-4 receptor expression. Coating of the filters with the extracellular matrix protein fibronectin (FN) strongly enhanced the SDF-1-induced migration of PB-CD34^+ cells (2.5 times) and of BM-CD34^+ cells (1.5 times). SDF-1 induced migration of PB-CD34^+ cells over FN-coated filters was blocked by antibodies against β_1-integrins.

Subsequently, analysis was performed to determine whether SDF-1 preferentially promoted migration of subsets of CD34^+ cells. Actively cycling CD34^+ cells, which were present in BM (14%) but hardly in PB (2.2%) or CB
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(1.2%), were found to migrate preferentially towards SDF-1. In the input, 14 ± 2.5% of the BM-CD34+ cells were in G2/M and S phase, whereas in the migrated fraction 20 ± 5.7% of the cells were actively cycling (p<0.05). We did not observe preferential migration of phenotypically recognizable primitive CD34+ subsets, despite the fact that CB-CD34+ cells are thought to contain a higher percentage of immature subsets. In conclusion, the relatively lower migration of PB-CD34+ cells seems to be due to a lower sensitivity towards SDF-1, and the higher migrational capacity of CB-CD34+ cells, in comparison to BM- and PB-CD34+ cells, seems to have an as yet unknown intrinsic cause. The increased migration of CB-CD34+ cells may favor homing of these cells to the bone marrow, which might reduce the number of cells required for hematological reconstitution after transplantation.

INTRODUCTION

In healthy adults, hematopoiesis takes place in the bone marrow (BM), where the majority of hematopoietic progenitor cells (HPC) are located. In patients undergoing chemo- and/or radio-therapy, hematopoiesis is seriously disturbed. Bone-marrow function in these patients can be reconstituted by bone-marrow transplantation and by the more recently developed method of peripheral stem-cell transplantation. In this latter technique, the combination of chemotherapy and G-CSF is applied to mobilize CD34+ cells into peripheral blood (PB), and the cells are harvested for reinfusion after high-dose chemo- and radiotherapy [1,2]. After reinfusion, CD34+ cells derived from peripheral blood, bone marrow or cord blood (CB) home to the bone marrow, and progenitor cells from all three sources are able to reconstitute BM function [3,4]. Different factors determine the outcome as measured by hematopietic recovery of stem cell transplantations. The number of transplanted CD34+ cells seems to be the major factor [5]. Possibly, the efficiency of the homing process may also play a role. For example, we have previously shown that the number of L-Selectin positive CD34+ cells is a better predictor of rapid recovery than the total number of CD34+ cells [6].

Homing of HPC can be considered as a multistep process, in which various adhesion molecules present on both HPC and endothelial cells are involved, similar to what has been found for transendothelial migration of leukocytes at places of inflammation [7]. The first step in migration consists of tethering and rolling of HPC along the endothelium [8-10]. Chemokines (chemotactic cytokines) or growth factors located on the endothelial surface or bound to proteoglycans in the endothelial extracellular matrix, activate β1- and β2-integrins on the rolling HPCs,
leading to firm adhesion to Ig-like receptors on the endothelial cells [11-13]. This process is followed by transendothelial migration of HPC and finally leads to anchoring of the HPC in the bone-marrow microenvironment [14-16]. This anchoring of HPC depends mainly on adhesion via integrins to stromal cells and to extracellular matrix proteins [17-19]. This multistep process, involving the different adhesion molecules that are activated by a range of modifiers, may explain selective migration at specific places. Chemokines or other growth factors can activate integrins by binding to their specific receptors, which are either seven-transmembrane spanning G-protein coupled or cell surface growth factor receptors [20]. Recently, the first powerful chemoattractant for CD34+ cells was described, i.e. stromal cell-derived factor-1 (SDF-1), produced by several stromal cells including, bone-marrow stromal cells [14,21]. It has also been shown that SDF-1 induces transendothelial migration of HPC in vitro [14,22]. SDF-1 is classified as a CXC-chemokine and is also a chemoattractant for monocytes and lymphocytes [23]. The receptor for SDF-1 is a G-protein coupled receptor, called Fusin, LESTR or CXCR-4 [24-27]. In SDF-1 or CXCR-4 knockout mice, hematopoietic precursors do not shift to the bone-marrow during fetal development, suggesting that SDF-1 plays an important role in the migration of HPC to the BM [28-30].

In this report, we compared the spontaneous and SDF-1 induced migration of CD34+ cells derived from BM, PB or CB and examined the effects of FN on this process, in an in vitro Transwell system. Subsequently, we analyzed whether SDF-1 preferentially promoted migration of subsets of CD34+ cells.

**MATERIAL AND METHODS**

**Cells**

Normal bone marrow (BM) was aspirated from patients undergoing cardiac surgery (in the Academic Medical Centre, Amsterdam, The Netherlands) after informed consent. Peripheral blood (PB) progenitor cells were obtained from patients treated with chemotherapy and G-CSF to induce stem cell mobilization. Cord blood (CB) was collected after delivery, according to the guidelines of Eurocord Nederland. BM, PB and CB mononuclear cells were enriched by density gradient centrifugation over Ficoll-paque (1.077 g/ml) (Pharmacia Biotech, Uppsala, Sweden). Mononuclear cells were resuspended in PBE buffer, containing PBS, 0.5% BSA and 5mM EDTA.
The HL-60 cell lines were obtained from the ATCC (Rockville, MO) and maintained in Iscove’s Modified Dulbecco Medium (IMDM, BioWittaker, Brussel, Belgium) containing L-glutamine, Pencillin 100 U/ml, /Streptomycin 100ug/ml, β-mercaptoethanol (0.1%) and 10% Fetal Calf Serum (Gibco, Life Technologies, Paisley, Scotland).

**CD34**

CD34** cells were isolated with the VarioMacs system (Miltenyi Biotec GmbH, Gladbach, Germany); which was a gift from Amgen. Firstly, the mononuclear cells in PBE were incubated for 15 min at 4°C with a hapten-labelled antibody directed against CD34 (QBEND10) in the presence of human IgG as a blocking reagent. The cells were washed in PBE and then incubated with anti-hapten microbeads for another 15 min at 4°C. The labelled cells were washed, resuspended in PBE and applied to a VS or RS separation column that was placed in the magnetic field of the VarioMacs. The column was washed four times to remove CD34** cells. Thereafter, the column was removed from the VarioMacs and the CD34** cells were eluted with 1 ml PBE. The cells were further purified by means of a new RS column, after which at least 95% of the cells isolated from PB or BM and 90% of the cells from CB expressed CD34 as determined by FACS analysis (Becton and Dickinson (B&D) Immunocytometry Systems, San Jose, CA).

**Chemokines and monoclonal antibodies (MoAbs)**

SDF-1 was purchased from Santertech (Heerhugowaard, The Netherlands). Monoclonal antibody HP2/1 (CD49d, anti-VLA-4) was purchased from Immunotech SA (Marseille, France). SAM-1 (CD49e, anti-VLA-5) is a MoAb produced in our laboratory (CLB, Amsterdam, The Netherlands). MoAb IB4 (CD18) was a kind gift from Dr. L Koenderman, Dept. of Pulmonology, Academic Hospital Utrecht, The Netherlands. For blocking studies, MoAbs were used at saturating conditions.

For phenotypical analysis the following fluorescein isothiocyanate (FITC)-conjugated MoAbs were used, IgG1 isotype control (CLB-203; CLB), CD34 (581, Immunotech), CD38 (AT13/5, Dakopatts, Glostrup, Denmark). Phycoerythrin (PE)-conjugated MoAbs that were used are: IgG1 isotype control (X40; B&D), CD34 (581;Immunotech). Biotin-conjugated MoAbs that were used are: IgG1 isotype control (DAK-G01, Dakopatts), HLA-DR (L243; B&D) and were stained with Streptavidin-Cy5 (Dakopatts). CXCR-4 expression was determined by PE-labelled anti-human Fusi in (12G5, Pharmingen, Hamburg, Germany)
Cell cycle analysis
Cell cycle analysis was performed by flow cytometry (FACScan, B&D). Input and migrated cells were incubated for 1 hour at 4°C in PBS containing propidium iodide (50 μg/ml) to stain the DNA, RNase (100 μg/ml) and 0.15%(v/v) Tween 20 (Merck, Darmstadt, Germany). The data were analyzed with software Modfit for Mac (B&D).

Migration assay
Migration assays were performed in Transwell plates (Costar, Cambridge, MA) of 6.5 mm diameter, with 5 μm pore filters. For some experiments filters were used with 3 μm pores. The upper and lower compartment of the Transwells were separated by an uncoated filter or by a filter coated overnight at 4°C with various extracellular matrix proteins, such as (bovine) fibronectin (FN), laminin (LN) (both obtained from Sigma) or ICAM-1 (a kind gift from Dr. R Soede, Netherlands Cancer Institute, Amsterdam), at a concentration of 20 μg/ml in PBS. Before adding cells to the upper compartment, the coated and uncoated Transwells were washed three times with assay medium (IMDM with 0.25% BSA [BSA, fraction V, Sigma]). 20000-100000 freshly isolated CD34+ cells, in 0.1 ml of assay medium, were seeded in the upper compartment and 0.6 ml of assay medium in the presence or absence of SDF-1 (in indicated concentrations) was added to the lower compartment. In blocking experiments, the cells were preincubated for 10 minutes at 37°C with monoclonal antibodies. A 0.1 ml sample containing cells in assay medium was diluted with 0.5 ml assay medium and was kept as an input control for the quantitation of the number of migrated cells (see below). The Transwell plates were incubated at 37°C, 5% CO2 for 4 hours. Cells that had migrated to the lower compartment were collected in a FACS tube to which a fixed number of control cell-line cells (HL-60) labeled with Calcein AM had been added, according to the manufacturer’s instructions (Molecular Probes, Leiden, The Netherlands). The HI-60 cells were added to the FACS tubes just before FACS analysis. FACScan analysis was used to determine the ratio between labeled cells and unlabeled cells, with characteristic light scatter parameters, in the migrated fraction. By comparing this ratio to that in the input control, the number of migrated cells was quantitated. Using this method, we were able to reliably determine a minimum number of 200 migrated cells.
Statistical analysis
All results were expressed as the mean ± standard deviation (SD). Significance levels were determined using a two-sided Student’s t-test.

RESULTS

Migration of CD34+ cells over uncoated and coated filters
We compared the spontaneous and SDF-1 induced migration of CD34+ cells, derived from various sources, in a Transwell system with uncoated and FN-coated filters. As presented in figure 1, hardly any spontaneous migration of PB-CD34+ cells or BM-CD34+ cells was observed over uncoated filters. CB-CD34+ cells showed 7.4 ± 3.9% spontaneous migration. However, over FN-coated filters increased spontaneous migration of CD34+ cells from all three sources was noted. Again, CB-CD34+ cells exhibited a spontaneous migration (22.4 ± 14.7%) that was significantly higher than the migration of BM- and PB-derived CD34+ cells (2.3 ± 0.8%, p<0.01; and 5.4 ± 3.7%, p<0.01; respectively).

As has been described previously, we found that the presence of SDF-1 in the
lower compartment of the Transwell increased migration of CD34+ cells. The presence of SDF-1 in the upper compartment completely abolished this migration, which is in agreement with the absence of chemokinetic activity for HPC as described by Kim and Broxmeyer [21]. However, as shown in figure 2, clear differences seem to exist between the migration of CD34+ cells derived from various sources, towards a concentration of 200 ng/ml SDF-1. When the values found for spontaneous migration (as shown in figure 1) are subtracted from the values observed for SDF-1 induced migration, CB-CD34+ cells amounted 58.4 ± 10.8% migration; BM-CD34+ cells 36.5 ± 10.2% and PB-CD34+ cells 20.2 ± 8.1%. Thus even after correction, the SDF-1 induced migration over FN-coated filters of CB-CD34+ cells was significantly higher than BM-CD34+ cells (p<0.01) and PB-CD34+ cells (P<0.001). Migration of PB-CD34+ cells was significantly lower than the migration of BM-CD34+ cells (P<0.01). Considerably higher numbers of CB-CD34+ cells migrated across FN-coated and uncoated filters than PB- and BM-CD34+ cells. Across uncoated filters, PB-CD34+ cells showed the lowest percentage of migrating cells (10.5 ± 6.6%), which is significantly lower than the migration of BM-CD34+ cells (26.2 ± 10.7%, p<0.01) and CB-CD34+ cells (68 ± 10%, p<0.001). Again, migration was enhanced by coating the filters with fibronectin. This was most evident for PB-CD34+ cells, where migration was 2.5 times higher across FN-coated filters than across uncoated filters (p<0.0001). This effect was less pronounced for BM-CD34+ cells (1.5 times, p<0.01) and was not seen for CB-CD34+ cells. BM-CD34+ cells migrated significantly better across FN-coated filters than PB-CD34+ cells (p<0.05). However, when circumstances for migration were made less optimal by decreasing the pore size of the filters to 3 μm or by using a lower concentration of SDF-1 (100 ng/ml), FN also enhanced the SDF-1 induced migration of CB-CD34+ cells (data not shown).

We also investigated the effect on migration by coating the filters with laminin (LN) and ICAM-1. For these experiments PB-CD34+ cells were used, because migration of these cells appeared to be more dependent on the coating of the filters than migration of the CD34+ cells from both other sources. No migration-enhancing effect was seen for LN-coating, whereas ICAM-1 enhanced the SDF-1-induced migration of PB-CD34+ cells two-fold in comparison to uncoated control filters (p<0.05) (data not shown).
Figure 2. SDF-1 induced (200ng/ml) migration after 4 hours of BM-CD34⁺ cells, PB-CD34⁺ cells and CB-CD34⁺ cells across uncoated and FN-coated filters (5 μm). FN enhanced migration of BM (**=p<0.01) and PB (**=p<0.01). SDF-1-induced migration of CB-CD34⁺ cells (n=6) across uncoated filters was significantly different (p<0.001) from PB (n=7) and BM (n=6). SDF-1 induced migration of CB-CD34⁺ cells over FN-coated filters was also significantly different (p<0.01) from PB and BM. Migration of BM was significantly different from PB across uncoated (p<0.01) and FN-coated (p<0.05) filters.

**CXCR-4 expression**

To determine whether the differences in migrational capacity between CB-, BM- and PB-CD34⁺ cells are due to the sensitivity to SDF-1, the CXCR-4 expression of the different CD34⁺ cells was measured (table 1). PB-CD34⁺ cells showed a significantly lower expression of the CXCR-4 receptor than the BM- and CB-CD34⁺ cells (p=0.008 and p=0.01, respectively). Expression of the CXCR-4 receptor on CB- and BM-CD34⁺ cells was not significantly different.

**SDF-1 dose-response curves**

The observed differences in migratory capacity of the CD34⁺ cells from different sources might be due to differences in their responsiveness to SDF-1. Therefore, we compared the relative percentage migration of the CD34⁺ cells from different sources at various SDF-1 concentrations. As shown in figure 3 the shape of the dose response curves of CB- and BM-derived CD34⁺ cells is identical. For both types of CD34⁺ cells maximal migration was observed at 600 ng/ml, whereas considerable migration was already seen at a lower concentration such as 100
Table 1. CXCR-4 expression of CB-, PB- and BM-CD34\(^+\) cells

<table>
<thead>
<tr>
<th>Source</th>
<th>CXCR-4 EXPRESSION (MFI*)</th>
<th>P</th>
<th>N.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB CD34(^+) cells n=5</td>
<td>112.7 ± 36.8</td>
<td>P=0.01</td>
<td></td>
</tr>
<tr>
<td>PB CD34(^+) cells n=20</td>
<td>35.6 ± 18.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BM CD34(^+) cells n=9</td>
<td>109.4 ± 61.4</td>
<td>P=0.008</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

* MFI of all CD34\(^+\) cells is given after correction for the PE-labelled IgG2a isotype control. In all cases a similar homogeneous distribution of CXCR-4 was seen without an indication of subpopulations of extra bright or dull cells. 'N.S. = Not Significant

ng/ml. In contrast, the dose-response curve of the PB-derived CD34\(^+\) cells seemed to have shifted to the right. These cells showed maximal migration at a higher concentration (1000 ng/ml) and less response was seen at lower concentrations. At 100 ng/ml only 38 ± 2.9% of PB-CD34\(^+\) cells migrated compared to 70.3 ± 20.8% of BM-CD34\(^+\) cells (p=0.05). These results suggest that PB-derived CD34\(^+\) cells are less sensitive to SDF-1 than BM- and CB-derived cells.

**Figure 3. Migration of BM-, PB- and CB-CD34\(^+\) cells across FN-coated filters (5 \(\mu m\)) at different SDF-1 concentrations.** % of maximum migration is the result of absolute % migration divided by the maximal % migration per experiment x 100. Each curve represents the mean of at least three experiments.

### Integrins involved in the migration of CD34\(^+\) cells over filters

Blocking experiments were performed to determine the integrins involved in SDF-1-induced migration. No effects of MoAbs directed against the \(\alpha_4\) or \(\alpha_5\) subunits of
Figure 4. Inhibition of SDF-1 induced migration of PB-CD34⁺ cells across FN-coated filters (5 μm) by blocking antibodies against β₁ or β₂-integrins. Migration of PB-CD34⁺ cells (n=6) was significantly inhibited by antibodies against VLA-4 (*=p<0.05), VLA-5 (*=p<0.05) or by the combination of VLA-4 and VLA-5 (**=p<0.01). No inhibition of migration was seen by adding a MoAb against the β₂-integrins (CD18).

the β₁-integrins or of MoAbs against the β₂-integrins were seen on the migration of PB-, BM- or CB-CD34⁺ cells across uncoated filters (data not shown). As shown in figure 4, the migration of PB-CD34⁺ cells over FN-coated filters (5.0 μm) was partly inhibited by antibodies against the α₄ (VLA-4) or α₅ subunits (VLA-5) of the β₁-integrins (24% and 33% inhibition, respectively, p<0.01 and p<0.05). The combination of both antibodies resulted in 66% inhibition of migration (p<0.01). In contrast, the migration of BM-CD34⁺ cells over FN-coated filters was only inhibited by a combination of antibodies against the α₄ and the α₅ subunit (37% inhibition, p<0.05) but not by the separate antibodies against the α-subunits (data not shown). Similar results were obtained for CB-CD34⁺ cells over FN-coated 3 μm filters. An inhibition of 80% was found only when a combination of α₄ and α₅ antibodies was used (data not shown). In contrast, migration of CB-CD34⁺ cells over FN-coated 5.0 μm filters could not be inhibited.

**Cell-cycle analysis of migrated PB, BM and CB derived CD34⁺ cells**

PB-CD34⁺ cells which are normally all in G₀/G₁ phase can be partially brought into S+G₂/M phase by stimulation with growth factors. It has recently been described that this growth factor stimulation has no effect on the adhesion of these cells, but results in enhanced migration across HUVECs and BMECs [15]. Therefore we
performed cell-cycle analysis to determine whether the differences in cell-cycle status were responsible for the different responses towards a SDF-1 gradient. We determined the proportion of cells in G0/G1 and G2/M or S-phase of the cells used for migration experiments. The input cells and the cells that had migrated in response to SDF-1 were analyzed. CB- and PB-CD34+ cells hardly contained any cells in G2/M or S-phase (see table 2) In contrast, BM-derived CD34+ cells, showed a higher percentage of cells in S phase and in G2/M phase compared to PB- or CB-derived cells (p<0.001 and p<0.0001, respectively). We also found a significantly higher percentage of migrated BM-CD34+ cells in the S- or G2/M phase compared to input BM-CD34+ cells (p<0.05).

Table 2. Cell-cycle analysis of CD34+ cells

<table>
<thead>
<tr>
<th>Source</th>
<th>% cells in G0/G1 phase</th>
<th>% cells in S+G2/M phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB CD34+ cells</td>
<td>98.8 ± 0.05</td>
<td>1.2 ± 0.12</td>
</tr>
<tr>
<td>PB CD34+ cells</td>
<td>97.8 ± 1.7</td>
<td>2.2 ± 1.7</td>
</tr>
<tr>
<td>BM CD34+ cells input</td>
<td>86.0 ± 2.5</td>
<td>14.0 ± 2.5</td>
</tr>
<tr>
<td>BM CD34+ cells migrated</td>
<td>80.3 ± 5.7 *</td>
<td>19.7 ± 5.7 *</td>
</tr>
</tbody>
</table>

a Percentage cells: mean ± SD of at least three experiments. Results of BM-CD34+ cells: mean of 6 experiments. * Input and migrated fraction of BM-CD34+ cells were significantly different, p<0.05.

Phenotypical analysis of migrated BM- and CB-derived CD34+ cells

The higher migratory ability of CB-CD34+ cells might be due to a phenotypically recognizable subset of CD34+ cells that has a higher migratory capacity. Therefore, the phenotype of the migrated and the input cells was analyzed. BM- and CB-CD34+ derived cells were analyzed for the following subsets: CD34+/CD38- and CD34+/HLA-DR- (see table 3). PB-derived CD34+ cells were not analyzed because previous studies have shown that the immature subsets CD34+/CD38- and CD34+/HLA-DR+, are usually very small in this source [5]. No significant differences were found in the percentages of primitive CD34+/CD38- or CD34+/HLA-DR+ subsets in the input and migrated fraction of either CB or BM-CD34+ cells.

DISCUSSION

It has been reported that CD34+ cells express CXCR-4, the receptor for SDF-1, and that SDF-1 is chemotactic for CD34+ cells [14,21,22,27]. In this study we show that the migratory behavior of CD34+ cells depends on the source from which the
cells are obtained. Far more CB-derived CD34\(^+\) cells migrated in response to SDF-1 compared to PB- and BM-CD34\(^+\) cells. Moreover, PB-CD34\(^+\) cells were less responsive to SDF-1 than BM-CD34\(^+\) cells. Gene-inactivation studies and experiments on NOD/SCID mice, have shown that SDF-1 and CXCR-4 are critical for stem cell homing [28-31]. Our results may indicate that cord-blood derived CD34\(^+\) cells home more rapidly than CD34\(^+\) cells from other sources. This is in line with clinical data, which show that cord blood stem cell grafts are capable of inducing stable engraftment, despite the very low numbers of CD34\(^+\) cells present [32].

There are several explanations possible for the increased migratory response of CB-CD34\(^+\) cells, such as differences in sensitivity towards SDF-1. CB- and BM-CD34\(^+\) cells were found to express CXCR-4 at the same level. However, comparable receptor expression does not directly implicate a similar activity of the receptor, e.g. SDF-1 is not chemotactic for astrocytes, although these cells do express CXCR-4 [33]. But, our observation that CB- and BM-derived CD34\(^+\) cells show a similar dose-response relationship indicates that they have no differential sensitivity to SDF-1.

Another explanation could be that cord blood contains a subset of highly SDF-1 responsive CD34\(^+\) cells, that is absent in BM. Therefore, we investigated whether specific characteristics of migrating cells could be identified by immunophenotype or cell cycle analysis. As cord blood cells are thought to contain relatively more primitive progenitors, we focussed on CD38\(^-\) and HLA-DR dull subsets [34]. As shown in Table 3, no differences in migration between primitive and more mature precursors were detected. Also Möhle et al. found that the expression of CXCR-4 on CD34\(^+\) cells is not related to differentiation [22], and Aiuti et al. showed no preferential SDF-1-induced migration of primitive precursors [14].

Next, we analyzed the cell cycle phase of the migrating cells. Yong et al. [15] have recently shown that growth factor stimulation of CD34\(^+\) cells enhances their spontaneous transendothelial migration. We here present evidence that SDF-1-induced migration is preferentially exhibited by CD34\(^+\) cells in S+G2/M.

### Table 3. Phenotypical analysis of input and migrated CB- and BM-derived CD34\(^+\) cells

<table>
<thead>
<tr>
<th>Source/subset</th>
<th>% input</th>
<th>% migrated</th>
<th>N.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB / CD34(^+) / HLADR(^-)</td>
<td>5.6 ± 2.9</td>
<td>6.4 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>CB / CD34(^+) / CD38(^-)</td>
<td>8.1 ± 6.0</td>
<td>8.0 ± 5.0</td>
<td></td>
</tr>
<tr>
<td>BM / CD34(^+) / HLADR(^-)</td>
<td>8.8 ± 12.2</td>
<td>12.9 ± 17.8</td>
<td></td>
</tr>
<tr>
<td>BM / CD34(^+) / CD38(^-)</td>
<td>2.1 ± 1.4</td>
<td>1.4 ± 1.6</td>
<td></td>
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</table>

*Percentage CD34\(^+\) cells: mean ± SD of at least three experiments. N.S.=Not Significant
However, similar to other publications, our BM-CD34+ cells contained a larger proportion of CD34+ cells in (S+G2/M) phase than did CB- and PB-CD34+ cells [35-37]. Thus the cell cycle status of the CD34+ cells also does not explain the enhanced migration of CB cells. The preferential migration of proliferating cells might be a mechanism underlying the specificity of the homing process. Only in the bone marrow will CD34+ cells be exposed to growth factors at concentrations that induce cell activation, thereby directly influencing the transmigratory ability of the cells. In addition it might be related to the physiological function of circulating cells. It has been suggested that circulating cells are capable of redistributing hematopoiesis, especially when increased blood cell production is needed. Under these circumstances homing of actively cycling cells would be preferable.

Strikingly, we observed that not only the SDF-1 induced migration but also the spontaneous migration of CB-CD34+ cells is greatly increased in comparison to CD34+ cells from other sources. Therefore, we assume that the enhanced ability of CB-CD34+ cells to migrate across filters is mainly due to a higher chemokinetic activity of these cells. Control of actin assembly and disassembly is important for cell migration [38]. In fact, our preliminary results indicate that SDF-1 induced actin polymerization, is higher in CB-CD34+ cells than in BM.

In contrast, the diminished migration of PB-CD34+ cells compared to BM- and CB-CD34+ cells appeared to be due to differences in chemotactic activity towards SDF-1 rather than intrinsic differences in chemokinetic activity. PB-CD34+ cells showed a significantly lower expression of the CXCR-4 receptor. Furthermore, the SDF-1 concentration at which half of the maximal response was observed, was higher for PB-CD34+ cells. The reduced expression of CXCR-4 on PB-CD34+ cells may be due to downregulation, which might be necessary for egress from the bone marrow during mobilization. A similar mechanism has been suggested for VLA-4 expression [6]. G-CSF did not decrease CXCR-4 expression in vitro, but we did find that CXCR-4 was downregulated on migrated cells (data not shown). The increased SDF-1 sensitivity of cycling cells might be an explanation for the low number of actively cycling CD34+ cells in peripheral blood, both in steady state and after mobilization.

In the present study we have demonstrated a direct link between integrin function and migration [39]. For migration, the cell has to deform by extending cytoplasmic projections and generating contractile forces as it moves. Proper adhesion of cytoplasmic extensions allows cells to generate contractility, necessary for motility. Recently, Francis et al. have shown that human CD34+ cells form
pseudopods that mediate adhesion to substrates coated with extracellular matrix proteins [40]. Our results support this model. Coating of the filters with a ligand for \( \beta_1 \)- or \( \beta_2 \)-integrins that are active on CD34\(^+\) cells, such as FN or ICAM-1, but not LN, can clearly enhance the migration of these cells across the filters. Presumably, a pseudopod of the CD34\(^+\) cell drifts freely until its adhesion receptors encounter suitable ligands on the substrate. Recently, Yamaguchi et al. have shown that cycling CD34\(^+\) cells express more VLA-4 and adhere more efficiently to stromal cells than CD34\(^+\) cells in G\(_0\)/G\(_1\) [37]. Both the enhanced function of VLA-4 and a higher sensitivity towards SDF-1 might favor the migration of cycling CD34\(^+\) cells to the bone marrow, as discussed above.

The enhancing effect of FN coating on the filters was most clearly seen when the migration was suboptimal. \( \alpha_4 \beta_1 \) integrin (VLA-4) and \( \alpha_5 \beta_1 \) integrin (VLA-5) were found to mediate binding to FN-coated filters. However, for each source of CD34\(^+\) cells, different blocking effects were observed. In the present study, migration of PB-CD34\(^+\) cells could be inhibited by adding either MoAbs against VLA-4 or VLA-5. However, migration of BM-CD34\(^+\) cells could only be inhibited by adding a combination of both VLA-4 and VLA-5 MoAbs. This might indicate that there is lower functional activity of the \( \beta_1 \)-integrins on PB-CD34\(^+\) cells than on BM-CD34\(^+\) cells. By blocking one of the receptors, the other receptor may be unable to mediate FN-binding by itself. Indeed, we and others, have previously found a significantly lower expression of VLA-4 on PB-CD34\(^+\) cells than on BM-CD34\(^+\) cells [6,37]. Another explanation may be that PB-CD34\(^-\) cells require a stronger interaction with the filter than the BM-CD34\(^+\) cells and are therefore more sensitive to inhibition. Evidently, for optimal migration, reversible binding of the cells to the filter is required. In case of irreversible adhesion, as seen when cells are activated by PMA or the \( \beta_1 \)-MoAb 8A2 [41], migration is blocked (data not shown).

To summarize, we have shown that migration of CD34\(^+\) cells is facilitated by reversible integrin-mediated interactions with FN-coated filters. PB-derived CD34\(^+\) cells have a lower sensitivity towards SDF-1 than BM- and CB-CD34\(^+\) cells, which could be due to its lower CXCR-4 expression. Preferential SDF-1 induced migration was observed for cells in active phases of the cell cycle. Finally, CB-CD34\(^+\) cells showed increased migration, possibly because these cells have a higher chemokineti c activity. This increased migration of CB-CD34\(^+\) cells may favor homing of these cells, thus reducing the number of cells required for hematological reconstitution after transplantation.
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REFERENCES

Chapter 2


