Migration of human hematopoietic progenitor cells

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Citation for published version (APA):

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Chapter 4

SDF-1-induced actin polymerization and migration in human hematopoietic progenitor cells.

Submitted for publication
SDF-1-induced actin polymerization and migration in human hematopoietic progenitor cells.

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ABSTRACT

The capacity of hematopoietic progenitor cells (HPCs) to respond to chemotactic stimulation is essential for their homing efficiency, e.g. during peripheral stem cell transplantation. In previous studies, it has been established that SDF-1 and its receptor CXCR-4 play an important role in the homing of HPCs. However, the mechanisms that control HPC migration in response to SDF-1 have not been studied in detail. In the present study, the extent and kinetics of SDF-1-induced actin polymerization in, and migration of HL-60 cells and primary human CD34⁻ cells obtained from cord blood (CB) and peripheral blood (PB) were analyzed.

Addition of SDF-1 (100 ng/mL) induced a rapid and transient increase in actin polymerization in HL-60 cells as well as in CD34⁻ cells. Analysis of GFP-actin expressing HL-60 cells migrating towards a gradient of SDF-1, revealed highly localized, repetitive bursts of actin polymerization at the leading edge. To further establish the role for SDF-1-induced actin polymerization in stem cell migration, fast migrating CB CD34⁺ cells were compared with relatively slow migrating PB CD34⁺ cells. SDF-1 induced significantly more actin polymerization in CB CD34⁺ cells compared to PB CD34⁺ cells (p<0.05). Moreover, migrated CB CD34⁺ cells showed an elevated and prolonged rise in F-actin levels compared to non-migrated cells, although both cell-types expressed similar levels of CXCR-4.

In conclusion, we here show that the magnitude of the SDF-1-induced actin polymerization response correlates positively with the migration capacity of primary human HPC and is independent from CXCR-4 expression.
INTRODUCTION

Prior to migration in response to a chemotactic stimulus, leukocytes display a complex repertoire of motility-associated processes including shape changes, polarization of signaling complexes, actin polymerization and polarized adhesion. Subsequent acto-myosin based contractility moves the cell forward, in the direction of the chemotactic gradient. Chemokines bind to G-protein-coupled receptors that, through a pertussis toxin sensitive trimeric G-protein, trigger signaling cascades that lead to directed motility. This signaling is still poorly understood but likely involves Rho-like GTPases that regulate temporally and spatially coordinated actin polymerization in concert with integrin-mediated adhesion.\(^1,2\) The induction of actin polymerization by chemokines is most pronounced within the first 1-2 minutes after stimulation of the receptor. Thereafter, the amount of F-actin declines to control levels and it is therefore not obvious whether and to what extent this rapid and transient response is related to chemokine-induced spreading and migration.

The capacity of hematopoietic progenitor cells (HPC) to respond to chemotactic stimulation is an important aspect in various (patho)physiological processes. This is in particular true for peripheral stem cell transplantation, commonly used to reconstitute hematopoiesis in patients treated with myeloblastic chemo- or radiotherapy. The most effective chemoattractant for HPC is SDF-1, which is produced by stromal cells, including those from the bone-marrow.\(^3-6\) Similar to other chemoattractants, SDF-1 has been described to induce actin polymerization in human Peripheral Blood Lymphocytes (PBL) and CD34\(^+\) cells.\(^4,7,8\)

In mice lacking SDF-1 or its receptor CXCR-4, hematopoietic precursors do not shift to the bone-marrow during fetal development, suggesting that SDF-1 and/or CXCR-4 play an important role in the migration of HPC to the BM.\(^9-15\) Recently, Peled et al. found SDF-1 and CXCR-4 to be critical for murine bone marrow engraftment by SCID-repopulating stem cells.\(^16\) They also demonstrated that migration of CD34\(^+\)CD38\(^{-}\)cells to SDF-1 in-vitro correlated with in-vivo engraftment and stem cell function in NOD/SCID mice. Subsequently, we showed that significantly faster hematological recovery was observed in patients transplanted with CD34\(^+\) cells that showed high spontaneous and SDF-1-induced migration in vitro \((p<0.05)\).\(^17\)

Earlier studies in our laboratory have revealed that Cord Blood (CB)-derived CD34\(^+\) cells demonstrate higher spontaneous and SDF-1-induced migration compared to Peripheral Blood (PB)-CD34\(^+\)cells.\(^6\) Similarly, our group has recently
shown that CD34\(^+\) cells from healthy G-CSF mobilized donors exhibited a significantly higher spontaneous and SDF-1-induced migration than CD34\(^+\) cells from patients mobilized with chemotherapy and G-CSF. The lower migratory capacity of patient-derived CD34\(^+\) cells \textit{in vitro} was not due to reduced expression of CXCR-4, but probably reflected a decrease in the motile capacity of the cells.\(^{17}\)

In the present study, we analyzed the extent and kinetics of SDF-1-induced actin polymerization in HL-60 cells and primary human CD34\(^+\) cells obtained from CB or PB as well as the correlation with the efficiency of SDF-1-induced migration. Furthermore, we studied whether migration towards SDF-1 selects for cells that have an increased capacity to polymerize actin. Together, the present data show that SDF-1-induced migration of primary human HPC is positively correlated with the magnitude of the initial actin polymerization response and is independent from CXCR-4 expression or receptor polarization.

**MATERIAL AND METHODS**

**Cells**

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. The HL-60 cell lines were obtained from the ATCC (Rockville, MO) and maintained in Iscove’s Modified Dulbeco Medium (IMDM, BioWittaker, Brussel, Belgium) containing L-glutamine, Pencillin 100 U/ml, Streptomycin 100 μg/ml, β-mercaptoethanol (0.1% v/v) and 10% Fetal Calf Serum (Gibco, Life Technologies, Paisley, Scotland).

**CD34\(^+\) cell purification**

Freshly obtained PB or 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% (w/v) BSA and 5 mM EDTA. CD34\(^+\) cells were isolated with a hapten-labeled CD34-antibody (QBEND10) with the VarioMacs system according to the manufacturer’s instructions (Miltenyi Biotec GmbH, Gladbach, Germany). At least 95% of the cells isolated from PB and 90% of the cells from CB expressed CD34 as determined by FACS analysis (Becton and Dickinson (B&D) Immunocytometry Systems, San Jose, CA).
Flowcytrometric analysis

CXCR-4 expression was determined by PE-labeled anti-human Fusin (12G5, Pharmingen, Hamburg, Germany). CXCR-4 expression was determined as mean fluorescence intensity (MFI) of all CD34⁺ cells and is given after correction for the PE-labeled IgG2a isotype control. In all cases a similar homogeneous distribution of CXCR-4 was seen without any indication of sub-populations of extra bright or dull cells.

Migration assay

Migration assays were performed in Transwell plates (Costar, Cambridge, MA) of 6.5 mm diameter with 5 µm pore filters, as previously described. The upper and lower compartment of the Transwells were separated by a filter coated overnight at 4°C with (bovine) fibronectin (FN) (obtained from Sigma, St. Louis, MO) at a concentration of 20 µg/ml in PBS. Before adding cells to the upper compartment, the coated Transwells were washed three times with assay medium (IMDM with 0.25% BSA [BSA, fraction V, Sigma]). Dependent on the amount of cells available, 20000-100000 freshly isolated CD34⁺ cells, in 0.1 ml of assay medium, were added to the upper compartment and 0.6 ml of assay medium in the presence or absence of SDF-1 was added to the lower compartment. SDF-1α was purchased from Strathmann Biotech GmbH (Hannover, Germany). An 0.1-ml sample of cells in assay medium was diluted with 0.5 ml of 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. Preliminary experiments showed that after 4 hours a substantial fraction of the CD34⁺ cells had migrated. 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) had been added labeled with Calcein AM, according to the manufacturer’s instructions (Molecular Probes, Leiden, The Netherlands). The HL-60 cells were added to the FACS tubes just before analysis. FACScan analysis was used to determine the ratio between labeled cells and unlabeled cells, with characteristic light scatter parameters, in the migrated fraction as previously described. 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. All migration assays were performed with freshly obtained primary human CD34⁺ cells.
Secondary migration assay
To analyze secondary migration, cells from the migrated and non-migrated fractions were collected after 4 hours. Two samples of migrated cells were kept separate to determine the initial migration efficiency. The collected migrated and non-migrated fractions were washed three times with assay medium, were resuspended in assay medium and were adjusted to similar concentrations. 20,000-100,000 CD34+ cells in 0.1 mL of assay medium were added to the upper compartment of fresh FN-coated Transwells, and 0.6 ml of assay medium in the presence of SDF-1 (100 ng/mL) was added to the lower compartment. After 4 hours the migration efficiency of the two fractions was measured.

Actin polymerization assay
Cells were washed three times in assay medium and resuspended in assay medium (IMDM with 0.25% BSA [BSA, fraction V, Sigma]) in a concentration of 1x10^6 cells/mL. Prior to the experiment, the cells and SDF-1 were incubated separately at 37°C for 10 minutes. SDF-1 (100 ng/mL) was added to the cell suspension, and at indicated time points 100 μL of cell solution was transferred to 100 μL of fixation solution (Intraprep Permeabilization Reagent, Coulter Immunotech). Cells were incubated in the fixation solution for at least 15 minutes. Thereafter, the cells were centrifuged and resuspended in 100 μL of permeabilization reagent (Intraprep Permeabilization Reagent, Coulter Immunotech). After 5 minutes incubation in this solution, 1U/mL Alexa 488 phalloidin (Molecular Probes) was added to visualize the F-actin. After 20 minutes the cells were centrifuged and resuspended in PBS with 0.5% BSA. Mean fluorescence intensity (MFI) was measured by FACSscan (B&D). The distribution of F-actin was analyzed on cytopsins of 50000 cells. Images were analysed with a Zeiss confocal laser scanning microscope using identical instrument settings when appropriate. Z-stacks were deconvolved using the Huygens software (Bitplane AG, Zurich, Switzerland) with reconstructed point spread functions derived from 170 μm fluorescent latex beads (Molecular Probes).

Analysis of the actin cytoskeleton in migrating HL-60 cells
GFP-actin was subcloned as a NheI-BamHI fragment from the pEGFP-actin vector (Clontech), and inserted into the Xbal-BamHI sites of pBluescript. The insert was then cloned as a blunt NotI-EcoRI fragment into the Swal-EcoRI sites of the modified vector LZRS-IRES-zeocin. The resulting construct, LZRS-GFP-Actin-IRES-zeocin, was transfected into amphotropic Phoenix packaging cells using the calcium phosphate transfection system (Life Technologies) to produce retroviruses.
HL-60 cells were transduced with virus supernatant in the presence of 10 μg/mL DOTAP (Boehringer) and were centrifuged twice at 2000 rpm for 30 min. After 6 hours, supernatant was replaced with fresh medium, and the cells were allowed to recover overnight. This procedure was repeated twice on two consecutive days. Transduced cells were sorted for GFP expression by FACStar (B&D).

To monitor the actin during migration, GFP-actin expressing HL-60 cells were placed on fibronectin-coated glass slides in a closed incubation chamber (volume 400 μL) inserted in a heatstage (37°C) on the confocal microscope. SDF-1 was then added in a volume of 40 μL (final concentration 100 ng/mL) through the inlet of the chamber so that a SDF-1-gradient would form by diffusion. The establishment of this gradient was monitored and confirmed by inclusion of Texas-Red dextran 10000 with the SDF-1. Green and red fluorescence were imaged simultaneously by time-lapse confocal laser scanning microscopy with a 63x oil-immersion objective.

Statistical Analysis
For normal distributed values the arithmetic mean and standard deviation were used. Differences were tested by using the Student t-test. A p value lower than 0.05 was considered significant.

RESULTS

SDF-1 induced actin polymerization and polarization in HL-60 cells.
In an initial series of experiments, the human leukemic cell line HL-60 was used. This line strongly expresses CXCR-4 and efficiently responds to SDF-1 in (trans)endothelial migration assays (data not shown). Dose-response analysis showed that 1 ng/mL SDF-1 was already sufficient to induce transient actin polymerization after 15-30 sec (data not shown). In subsequent experiments, the maximum effective concentration of 100 ng/mL was used. This concentration is in accordance with related studies in which the MO7e cell line or megakaryocytes were used.

Figure 1A shows the detailed kinetics of SDF-1-induced F-actin formation in HL-60 cells. The response peaked already at 15 seconds, followed by a rapid decline to control levels at 60-120 sec. In parallel experiments, we analyzed the distribution of the F-actin in SDF-1 treated HL-60 cells by confocal microscopy. These data (Figure 1B) show that the rapid increase and decline in F-actin levels
SDF-1-induced actin polymerization and migration

Figure 1. SDF-1 induced actin polymerization in HL-60 cells
A The kinetics of SDF-1 (100 ng/mL) induced F-actin formation in HL-60 cells measured by FACS analysis. The line represents the mean ± sem of 4 experiments.
B Maximal-intensity projections of F-actin staining in HL-60 cells exposed to 100 ng/mL SDF-1 for a. 0 s; b. 15 s; c. 30 s; d. 60 s; e. 120 s and f. 300 s. Scale bar represents 5 μm.

Can be clearly visualized during the time course and corresponds well with the FACSscan analysis. Intriguingly, whereas the initial burst of F-actin formation occurred throughout the cells, the second phase of the response was associated with a redistribution of F-actin into large polarized clusters that contained most of the...
cellular F-actin. Again, this polarization was transient, and after 2-5 minutes F-actin was more randomly distributed, albeit that increased levels of filamentous structures remained visible that were absent in the control situation.

To visualize actin distribution directly during migration, we expressed a fusion protein of green fluorescent protein (GFP) and actin in HL-60 cells. These HL-60 GFP-actin transfectants polarize and migrate in response to a gradient of SDF-1. As recorded by confocal microscopy, GFP-actin became initially clustered at the side of cell facing the SDF-1 gradient. During the extension of cellular protrusions and the subsequent actual migration, actin was repeatedly and transiently concentrated in small spikes at the leading edge of the cell (Arrowheads in Figure 1C). At the rear, transient formation of membrane ‘blebs’ were observed that were clearly distinct from the cellular extensions at the front of the cell.

![Figure 1C Time-lapse imaging of migrating HL-60 GFP-actin cells. Images were taken of the same cell at 158 s, 329 s, 447 s, 486 s and 579 s after addition of the SDF-1 (100ng/mL) through the inlet of the incubation chamber (arrowhead below the figure). Arrow indicate lamellipodial extension, arrowheads indicate transient actin spikes at the leading edge of the cell. At the rear transient formation of membrane ‘blebs’ was observed. Scale bar represents 10 μm.](image)

These results show that addition of high concentrations of SDF-1 induce a transient and massive burst of actin polymerization, followed by a transient polarization, finally leaving the F-actin in a more random, yet altered distribution.
Gradient-exposed cells showed a more moderate response, characterized by polarization of the cell and followed by highly localized, transient and apparently cycling rises of F-actin at the leading edge.

**SDF-1 induced actin polymerization in CB and PB-derived CD34^+ cells**

From previous work it is known that spontaneous and SDF-1-induced migration of CB CD34^+ cells is significantly higher than those of PB CD34^+ cells (4 times and 3 times higher, respectively). Therefore CB- and PB CD34^+ cells were chosen to compare actin polymerization induced by SDF-1. In contrast to the HL-60 cells, 1 ng/mL SDF-1 was not sufficient to induce optimal actin polymerization in primary CD34^+ cells, but 10 to 300 ng/mL SDF-1 was required to induce a transient rise in F-actin assembly. 100 ng/mL SDF-1 induced similar effects in PB-CD34^+ cells as observed in HL-60 cells. When analyzed by confocal microscopy pronounced F-actin formation was initially observed throughout the cell, followed by transient polarization of the F-actin in large clusters (see Figure 2A). Interestingly.

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**Figure 2A.** Confocal analysis of SDF-1-induced F-actin formation in primary CD34^+ cells. Phalloidin staining of PB CD34^+ cells exposed to 100 ng/mL SDF-1 for a, 0 s, b, 15 s, c, 30 s, and d, 120 s. Arrowheads indicate polarized F-actin clusters. Scale bar represents 20 μm.
complementary FACS analysis showed that SDF-1 induced significantly more actin polymerization in CB CD34⁺ cells compared to PB CD34⁺ cells (Figure 2B). After 15, 30, 60 and 300 seconds the levels of F-actin were significantly higher in CB CD34⁺ cells as compared to PB CD34⁺ cells (p<0.05 for all the indicated time points), although the kinetics of the responses were almost similar in both cell types. For CB CD34⁺ cells, the response peaked at 15-30 sec, while PB CD34⁺ cells showed maximal F-actin levels just at 15 sec. The rapid increase in F-actin was followed by a fast decline to control levels after 60-120 sec for PB CB34⁺ cells or 120-600 sec for CB CD34⁺ cells.

![Figure 2B](image.png)

**Figure 2B.** Time course of SDF-1-induced F-actin formation in PB CD34⁺ cells (▲) and CB CD34⁺ cells (■) analyzed by FACS analysis. After 15, 30, 60 and 300 sec the levels of F-actin were significantly higher in CB CD34⁺ cells compared to PB CD34⁺ cells (p<0.05 for each indicated time point). Each line represents the mean ± SEM of 6 experiments with CB CD34⁺ and 9 experiments with PB CD34⁺ cells.

Previously it has been shown that CXCR-4 expression on CB CD34⁺ cells is significantly higher than on PB CD34⁺ cells. However, in the present experiments the mean fluorescence intensity (MFI) of CXCR4 expression on the CB CD34⁺ cells was 38±21 (n=4) and showed no significant difference compared to the CXCR-4 levels on PB CD34⁺ cells 26±17 (n=5), indicating that receptor expression is not a major factor determining the extent of actin polymerization. In addition, also the basal levels of F-actin in the two cell types were not different.

Complementary confocal analysis showed that CXCR-4 expression was not clearly polarized either in PB or in CB-derived CD34⁺ cells. This was not altered by stimulation of the cells with SDF-1 (data not shown). Absence of receptor
polarization was further confirmed with KG1a cells expressing CXCR-4-GFP. Exposure of these cells to a concentration gradient of SDF-1 did not alter the even distribution of the receptor over the cell surface, demonstrating that polarization of CXCR-4 does not occur in response to SDF-1 (data not shown).

**SDF-1-induced actin polymerization in migrated and non-migrated CD34^+ cells**

Peled et al recently demonstrated that mice, transplanted with nonmigrating (i.e. non SDF-1 responsive) human CD34^+ cells were poorly engrafted as compared to mice transplanted with migrating (SDF-1 responsive) human CD34^+ cells, while these populations did not differ in the incidence of progenitor cells. These findings were supported by our own results that significantly faster hematological recovery was observed in patients transplanted with CD34^+ cells that showed high spontaneous and SDF-1-induced migration in vitro (p<0.05).

To investigate migratory capacity and actin polymerization of CD34^+ cells from PB or CB, we performed migration assays, followed by the analysis of actin polymerization in the migrated as well as the non-migrated cells. For the PB CD34^+ cells no obvious differences were observed between the cells from the migrated and non-migrated fractions in terms of transient SDF-1-induced F-actin formation (see Figure 3A). The kinetics of SDF-1-induced actin polymerization in these two fractions were similar and comparable to the kinetics of the total population (Figure 2B). Furthermore, parallel confocal analysis showed that in the non-migrated fraction, no polarization of F-actin was observed, while the cells of the migrated fraction showed a polarized actin distribution even at t=0 (see Figure 3B).

In contrast, for CB-CD34^+ cells there was a strong tendency for the migrated cells to demonstrate a higher and prolonged rise in F-actin levels compared to the non-migrated cells (Figure 4). The response of the migrated fraction peaked between 15 and 60 sec, followed by a slow decline to control levels at 300-600 sec. In contrast, the non-migrated fraction peaked at 15 sec, followed by a rapid decline thereafter. FACS analysis showed that CXCR-4 expression on the migrated cells did not differ from the expression on the non-migrated cells (data not shown). This is in line with the CXCR-4 data of the PB and CB CD34^+ cells as described in the previous section, once more indicating that CXCR-4 expression levels are not directly proportional to the extent of SDF-1-induced F-actin formation and migration.
Commonly used isolates of primary human CD34+ cells, either from CB or PB, are heterogeneous with respect to the proportions of more primitive progenitors. It was therefore of relevance to test whether the migratory capacity was related to these subsets. In a previous study we did not observe differences between phenotypically defined immature subsets (CD34+/CD38- and CD34+/HLA-DRlow) and the total CD34+ population from cord blood (n=5) in migration towards SDF-1 across a FN-coated filter. Also in the present study no significant differences in migration between the immature CD34+ cells and the total CD34+ cell population
of PB-derived CD34+ were observed (see table 1). It is unlikely that the higher percentage of immature subsets in CB CB34+ cells is the cause of the pronounced difference in actin polymerization compared to PB CD34+ cells. Thus, our results demonstrate that migrated and non-migrated fractions of CB CD34+ cells differ intrinsically in the ability to polymerize actin in response to SDF-1, independent of CXCR-4 levels or the presence of more primitive progenitors.

Table 1. Phenotypical analysis of input and migrated CB- and PB-derived CD34+ cells*

<table>
<thead>
<tr>
<th>SOURCE/SUBSET</th>
<th>% INPUT</th>
<th>% MIGRATED</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB/CD34+/HLADR</td>
<td>5.6 ± 2.9</td>
<td>6.4 ± 2.7 NS</td>
</tr>
<tr>
<td>CB/CD34+/CD38</td>
<td>8.1 ± 6.0</td>
<td>8.0 ± 5.0 NS</td>
</tr>
<tr>
<td>PB/CD34+/HLADR</td>
<td>0.6 ± 0.3</td>
<td>1.0 ± 0.7 NS</td>
</tr>
<tr>
<td>PB/CD34+/CD38</td>
<td>1.0 ± 0.9</td>
<td>1.1 ± 0.3 NS</td>
</tr>
</tbody>
</table>

*Percentage CD34+ cells: mean ± SD of at least three experiments; NS = difference not significant

Secondary migration of migrated and non-migrated fractions of CD34+ cells
To further investigate whether migrating CD34+ cells retain their migratory response to SDF-1 and comprise a higher intrinsic migratory capacity than non-migrating CD34+ cells, we performed secondary migration assays. After 4 hours migration towards 100 ng/ml SDF-1, migrated and non-migrated CD34+ cells were washed and resuspended in assay medium. Subsequently, both fractions were used as input in a second migration assay, again using (100 ng/mL) SDF-1. As indicated in Figure 5A, the migrated fractions of CB CD34+ cells showed a 2.7 times higher secondary migration efficiency than cells derived from the originally non-migrated
fraction (p<0.05). Moreover, two similar experiments with PB-CD34" cells gave comparable results (Figure 5A). On average we observed a 3.4 times higher secondary migration efficiency of migrated CD34" cells as compared to non-migrated CD34" cells (p<0.01). As in the above discussed experiments, no significant differences in CXCR-4 expression were found between the migrated and non-migrated fractions of CD34" cells, prior to the secondary migration assay (mean MFI 65±41 and 57±40 respectively, n=5) (figure 5B). These results indicate that CB CD34" cells indeed possess an intrinsic migratory capacity that is independent of the levels of CXCR-4 expression, but correlates positively with the cells' ability to induce a rapid and transient formation of F-actin.

![Image](https://via.placeholder.com/150)

Figure 5. Secondary migration of migrated CD34" cells is significantly higher than of non-migrated CD34" cells, irrespective of CXCR-4 expression levels. A SDF-1-induced migration (100 ng/mL) of 3 independent CB CD34" cell donors and 2 independent PB CD34" cell donors. Initial migration efficiency after 4 hours is indicated in open bars. The secondary migration of the migrated and non-migrated fractions is indicated in hatched bars and filled bars, respectively. The migrated fractions of both cell types showed a 3.4 times higher secondary migration compared to the non-migrated fractions (p<0.01). B CXCR-4 expression of all CD34" cells of the fractions corresponding to those in figure 5A, is given after correction for the PE-labeled IgG2a isotype control. The MFI of the CXCR-4 expression on the CD34" cells were not significantly different during the first and second round of migration.
DISCUSSION

During the process of homing of HPC to the bone marrow, the cells must cross the bone-marrow endothelium to engraft. This process of transendothelial migration is a complex interplay of transient interactions and events, initiated by chemoattractants, mediated by various adhesion molecules and dependent on the motogenic behavior of the HPC. The mechanisms underlying cell movement in response to chemoattractants have not been clearly established.

Several studies have shown that expression of CXCR-4 is essential for SDF-1-induced migration and homing, but there is no clear correlation between receptor levels and the efficiency of stem cell migration. Surprisingly little is known about the regulation of migration of stem cells. Francis et al have described long pseudopods formed by KG1a cell line cells and primary human CD34+ cells.\(^23\) The beta-1-and beta-2 integrins, CD34 and L-selectin were found to be present on these podia.\(^24\) However, these experiments were performed in the absence of a chemokine. SDF-1 induces actin polymerization in CD34+ cells\(^4,25\), but its reorganization has never been studied in HSCs.

As a first step to define cellular factors that control HPC motility, we analyzed the SDF-1-induced F-actin formation in CD34+ cells from various sources and subfractions thereof. Initial experiments showed that, in the CXCR-4-positive leukemic HL-60 cell line, SDF-1 induced a transient burst of actin polymerization, followed by an altered cellular distribution of F-actin. These observations were in accordance with data on the control of actin polymerization during neutrophil chemotaxis, showing that chemotactic stimuli cause neutrophils to organize actin polymerization at discrete sites, the distribution of which is governed by external chemotactic gradients.\(^26\) This asymmetrical actin polymerization possibly drives directional migration of neutrophils in response to a chemoattractant.\(^26\) Our analysis of HL-60 cells (expressing GFP-actin) moving towards a SDF-1 gradient, are in line with this notion and support the idea that subtle yet highly localized bursts of actin polymerization are instrumental in cell motility.

From previous work it is known that spontaneous and SDF1-induced migration of CB CD34+ cells is significantly higher than of PB CD34+ cells.\(^6\) Consequently it was relevant to investigate whether the observed differences in migratory ability of PB CD34+ cells and CB CD34+ cells could be explained by differences in actin polymerization. In the present study, we show that SDF-1-induced F-actin formation is significantly less efficient in PB-CD34+ cells than in CB-CD34+ cells; however, maximal responses were seen at the same time points in both cell types.
In addition, no consistent differences were found when the absolute F-actin levels of cells from these sources were compared.

The enhanced F-actin formation in CB CD34⁺ cells was not caused by differences in CXCR-4 expression, because the mean CXCR-4 expression on the CD34⁻ cells of the CB CD34⁺ cells was not significantly different from the expression on mobilized CD34⁻ cells of patients. These conclusions are further corroborated by the secondary migration experiments, which showed a lack of correlation between CXCR-4 expression and remigration efficiency. It remains to be established whether the ability to reorganize the cytoskeleton is reduced in mobilized CD34⁻ cells obtained from patients due to pre-treatment with various drugs.²⁷,²⁸

Thus, the current data indicate that, for CD34⁺ cells obtained from PB of patients or from CB, the SDF-1-induced migration and actin polymerization is not dependent on the CXCR-4 expression level alone. We have previously described that CB-derived CD34⁺ cells show enhanced migration in comparison to BM-derived CD34⁺ cells, although their CXCR-4 expression was similar.⁶ Also for other cell types it is known that receptor expression is often not the sole determinant of responsiveness to chemokines. For example, only a minority of CXCR-4-expressing T cells responds to SDF-1 with a rise in calcium influx.²⁹ Also in developing human bone-marrow B cells, SDF-1 responsiveness does not correlate with CXCR-4 expression levels.³⁰ This lack of correlation between receptor expression and function is probably due to differences in CXCR-4 signaling among the different cells.¹⁷

Former studies have produced contradictory observations with respect to receptor localization in moving cells. It has been described for lymphocytes that polarization of chemokine receptors to the leading edge occurs during lymphocyte chemotaxis.¹,³¹ In contrast, our confocal analysis of permeabilized primary cells as well as of living KG1a cell-line cells expressing CXCR-4-GFP, demonstrates that CXCR-4 is not polarized to the leading edge during migration towards (or in the presence of) SDF-1. These observations are in accordance with recent data obtained in migrating PLB-985 cells that showed no preferential accumulation of chemokine receptors at the leading edge of these cells.³²

In conclusion, we here show for the first time that the magnitude of the SDF-1-induced actin polymerization response correlates positively with migration capacity of primary human HPC and is independent from CXCR-4 expression or receptor polarization. The phenomenon of intrinsic migratory capacity thus seems to be
defined by heterogeneous, cell-specific differences in the signaling between the chemokine receptor and the actin polymerization machinery. Many cellular components involved in cell motility have recently been identified. Careful molecular analysis of various types of CD34+ cells will be required to allow the identification of proteins and signaling complexes that ultimately determine the velocity and efficiency of stem cell migration.

ACKNOWLEDGMENTS

We thank Dr. D. Roos for critically reading the manuscript. We also thank Irma de Jong and Romano Baardman for their help with the isolation of CD34+ cells. Furthermore, we acknowledge all technicians of the Stem Cell laboratory at the CLB for providing leukapheresis material.

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