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Fibronectin increases the migration induced by stromal cell-derived factor-1a (SDF-1a) in pre-B acute lymphoblastic leukemia cells

Abstract

The chemokine, stromal cell-derived factor-1a (SDF-1a) and its receptor CXCR-4 (fusin, LESTR) are thought to be involved in the trafficking of hematopoietic progenitors and stem cells, as suggested by the chemotactic effect of SDF-1a on these cells. Gene inactivation studies have shown that both SDF-1a and CXCR-4 are essential for B lymphopoiesis. Migration of leukemic cells may also be

dependent on SDF-1a and CXCR-4. Fibronectin (FN) is a component of the extracellular matrix (ECM), and one of the natural supports for cell movement in their bone hematopoietic environment. In the present study, we examined the influence of FN on the chemotactic effect of SDF-1a and on the CXCR-4 expression and function on human precursor-B acute lymphoblastic leukemia (pre-B ALL) cells at sequential stages of development. Fourteen children with pre-B ALL were studied. Their immunophenotypes belonged to the first three stages of B cell differentiation. Despite relatively high levels of CXCR-4 expression at all stages, the responsiveness to SDF-1a, measured as the percentage of migrating cells in the transwell culture system, varied with patients and seems to be less significant for pre-B3 (and pre-B1) than for pre-B2. There was no correlation (r = 0.2) between the SDF-1a induced migration (range: 2.5-39%) and the cell surface density of CXCR-4 (range: 46.5-97.5%). The extracellular matrix protein FN, either coated on the filter (for more than 18 hours) or in soluble form, enhanced the SDF-1a induced migration of pre-B ALL respectively (2 fold and 1.6 fold) without influencing CXCR-4 expression in short term cultures. Therefore, we analyzed the expression of the FN receptors, VLA-4 (CD49d) and VLA-5 (CD49e), by direct immunofluorescence, on these leukemic cells. VLA-4 was strongly expressed in all stages of pre-B ALL (range: 77-97%) while VLA-5 expression was more variable (range: 14-94%), but no correlation with the FN-dependent increased SDF-1a chemotactic effect was noted. In conclusion, the migratory behavior of pre-B leukemic cells in response to SDF-1a partly depends upon the stage of differentiation, and partly upon unexplained patient variability. Our results suggest that several molecules from the extracellular matrix, such as FN, may be implicated in this phenomenon.

Full text

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is usually a result of malignant transformation of B-lymphoid precursors in bone marrow [1, 2]. Leukemic cells usually egress from the bone marrow microenvironment and circulate in the peripheral blood. Mohle *et al.* demonstrated that this phenomenon depends in part on the chemotactic response to a chemokine, the stromal cell-derived factor (SDF-1alpha) [3]. SDF-1alpha is a member of the CXC subfamily of chemokines, initially identified as a growth factor for B cell progenitors and a chemotactic factor for T cells and monocytes [4]. The chemotactic effect of

SDF-1alpha is mediated by the chemokine receptor CXCR-4 (fusin, LESTR). However, the presence of CXCR-4 on the cell does not necessarily imply a chemotaxis induced by SDF-1alpha. For example, astrocytes express CXCR-4, but do not migrate in response to SDF-1alpha [5]. Moreover, a recent study demonstrated that SDF-1alpha chemotactic responsiveness does not correlate with CXCR-4 expression levels during the different stages of human bone marrow B cells, and the diminished responsiveness of more mature B cells to SDF-1alpha was shown to be unrelated to the level of CXCR-4 expression [6].

Progenitor cell mobilization is a multifactorial process, involving not only paracrine cytokines and chemokines, such as SDF-1alpha, but also adhesion molecules and the various components of the extracellular matrix (ECM) in the bone marrow. Effectively, the migration of human ALL cells and pre-B cell lines into stromal layers *in vitro* has been shown to be dependent on the combined action of VLA-4 and VLA-5, integrins known to be cell receptors of fibronectin (FN) [7, 8].

The purpose of this study was to compare spontaneous and SDF-1alpha-induced migration of pre-B ALL cells at different stages of maturation and to analyze the influence of FN on the chemotactic effect that SDF-1alpha exerts on these cells. Moreover, we evaluated the involvement of CXCR-4, VLA-4 and VLA-5 expression level on the SDF-1alpha migratory and chemotactic effects.

MATERIALS AND METHODS

B-lineage ALL samples

Fourteen children (under 15 years old) treated for different subtypes of pre-B ALL were studied. Blood and bone marrow samples were collected during the diagnostic procedures with the informed consent of the children's parents in agreement with the ethical laws of France. The diagnosis was established on conventional May Grünwald Giemsa and cytochemical stains according to accepted FAB criteria, and confirmed by immunophenotyping using a panel of well-characterized monoclonal antibodies (MoAbs). The immunological subtypes pre-B ($CD19^+$, $CD10^+$, cytoplasmic immunoglobulin cIg^-, surface immunoglobulin sIg^-) were determined according to the GEIL criteria as previously described [9], representing sequential stages of development; pre-

B1(n = 2), pre-B2 (n = 6), pre-B3 (n = 6). In all cases more than 85% of the leukemic cells were positive for CD19.

Leukemic samples were diluted with sterile RPMI-1640 medium and centrifuged on Ficoll-Paque (specific gravity: 1,077g/ml) (Eurobio, France). Washed mononuclear cells were then cryopreserved in 10% dimethylsulfoxide, 20% fetal calf serum (FCS) (Biowhittaker, France) and RPMI medium, prior to use. At least 60% of the cells on both peripheral blood and bone marrow samples were blast cells, and less than 30% were lymphocytes.

After thawing with Iscove's modified Dulbecco's medium (IMDM) (Gibco BRL Life Technologies, France), supplemented with 20% FCS, ALL cells were incubated in IMDM, supplemented with 0.5% bovine serum albumin (BSA) (Sigma, France) for chemotactic assay. The viability of the cells was assessed by trypan blue dye exclusion and was greater than 90% in all experiments before migration.

Reh cell line

The pre-B ALL cell line, Reh [10], was cultivated in RPMI 1640 medium supplemented with 20% FCS. After thawing with IMDM, supplemented with 20% FCS, Reh cells were incubated in IMDM, supplemented with 0.5% BSA and assessed by trypan blue dye exclusion for viability before chemotactic assay.

Cell surface staining

Indirect immunofluorescence was used to examine the expression of the chemokine receptor CXCR-4 on the surface of the different pre-B ALL cells. Pre-B ALL cells were washed with cold phosphate-buffered saline (PBS) and incubated with unlabeled anti-CXCR-4 MoAb (clone 44717.111; R&D Systems, UK) for 15 min at 4° C then washed in PBS, and incubated for 15 min with a rabbit antibody against mouse Ig, conjugated to fluoro-isothiocyanate (FITC). The surface expressions of VLA-4 and VLA-5 were analyzed by direct immunofluorescence. Cells were incubated for 15 min at 4° C with either 10 mul of phycoerythrin-conjugated CD49d (VLA-4) antibody (clone 9F10, Pharmingen, France) or with 20 mul of FITC-conjugated CD49e (VLA-5) antibody (clone SAM1, Immunotech, France). After two washes in PBS (Eurobio, France), all samples were analyzed by flow cytometry (EPICS XL-MCL, Coulter, France). Data were expressed as the percentage of positive cells.

Chemotactic assay

The *in vitro* chemotactic system used in this study was derived from the Boyden's chamber, using transwell inserts (6.5 mm diameter, 3 mum pore size, polycarbonate membrane, Dutscher, France) to define two chambers, the upper and the lower chamber, separated by the insert. The chemokine SDF-1alpha, was added to the lower chamber in 600 mul IMDM with 0.5% BSA (final concentration of 100 ng/ml) [11]. Then, 5 x 10^5 leukemic or Reh cells in 100 mul IMDM with 0.5% BSA were placed in the upper chamber. After 5 hours at 37° C in a fully humidified atmosphere flushed with a combination of 5% CO_2 in air, the migrated cells were recovered from the lower chamber. Cells were counted using a hemocytometer (Coulter Z1, Coultronics, France). Results were expressed as the percentage of migrating cells (number of migrated cells/total number of input cells) x 100. All assays were performed in duplicate.

To test the influence of FN (Sigma, France) on the chemotactic effect of SDF-1alpha, the upper and/or the lower chamber were loaded with FN diluted in IMDM, at a concentration of 150 mug/ml. Cells were added as described above.

For some experiments the membrane of the transwell system was coated on both sides with human FN (150 mug/ml in IMDM). The solution was distributed in the two chambers. After more than 18 hours incubation at 4° C, non-specific binding sites were blocked with IMDM supplemented with 1% BSA, at 37° C for 1 hour. Transwell filters were then washed twice and transferred to new wells for the chemotactic assay.

In another series of experiments, 1 x 10^6 pre-B ALL cells were incubated in IMDM containing FN (150 mug/ml) for 3 hours. Cells were then washed twice and placed in the upper chamber of the uncoated transwell (5 x 10^5 cells/well), whether or not they contained SDF-1alpha in the lower chamber.

All these experiments were performed in the absence of FCS.

Statistical tests

Wilcoxon signed-rank test was used for simple comparisons. All probability values were 2-sided and differences were considered significant at p ¾ 0.05.

Results were expressed as mean ± standard error mean (SEM). Correlation was tested using the Fisher & Yates Table.

RESULTS

*SDF-1*alpha *induced chemotaxis of pre-B ALL and Reh cells*

The capacity of SDF-1alpha alone to induce chemotaxis in leukemic cells was examined in a transwell culture system, using a 3 mum pore transwell filter (Figure 1). In the absence of SDF-1alpha, due to the small size of the pores, very few cells were recovered in the lower chamber and there was no significant spontaneous migration (3 ± 0.6%; 3 ± 0.5% of Reh and pre-B ALL input cells respectively). However, addition of SDF-1alpha, at a concentration of 100 ng/ml to the lower chamber resulted in significant migration of B-lineage ALL and Reh cells. The 10 ± 3% (mean, range 2.5-39%) of pre-B ALL cells and 14 ± 1.7% (mean, range 11-21%) of the Reh cell line added to the upper chamber were recovered in the lower chamber within 5 hours (Figure 1A, B, C and Table 1).

On Figure 1B and C, the chemotactic effects of SDF-1alpha are reported, according to the different immunological stages of differentiation of pre-B-ALL cells. SDF-1alpha seems to be more potent on pre-B2 (mean 16 ± 6%) than on pre-B3 cell migration (mean 6.5 ± 2%) ($p = 0.09$). According to the percentage of migrating cells, patients were arbitrarily classified as weak responders or strong responders (threshold value: median).

Early pre-B ALL cells (Pre-B1 ALL cells), collected from two different patients failed to respond to SDF-1alpha with a significant migration (3 and 5% respectively).

*Influence of SDF-1*alpha *on the chemotaxis of pre-B ALL cells in the presence of FN*

The role of coated or soluble forms of FN on the chemoattractive effect of a gradient of SDF-1alpha was studied. FN was tested in the different compartments of the transwell assay. FN alone in the lower chamber did not induce a significant migration by itself (data not shown). As seen in Figure 2, the soluble form of FN in the upper chamber significantly enhanced the chemoattractant effect of SDF-1alpha on pre-B ALL cells (16 ± 4.5%). In contrast,

this effect was less marked and not statistically significant when FN was added to the lower chamber with SDF-1alpha (12 ± 3%).

An increased migration was also observed when FN was added to the two compartments of the transwell assay (13.5 ± 4%, data not shown).

FN is a component of the ECM, and is one of the natural supports for cell movement in their bone hematopoietic environment. The transwell membrane was coated with FN to test its possible role on the complex relations between cell attachment and SDF-1alpha-induced migration. An important enhancement of the SDF-1alpha chemoattractive effect on pre-B-ALL cells was observed with a FN-coated insert (19 ± 6%) (Figure 2). In contrast, the enhancement was not significantly different from that obtained in experiments with soluble FN in the upper chamber.

As observed for the weakly responsive leukemic cells, FN did not enhance the Reh cells migration in the presence of a gradient of SDF-1alpha (respectively 22 ± 3%, 15 ± 2% with or without FN) (Table 1).

*Influence of pre-incubation with FN on SDF-1*alpha*-induced chemotaxis of ALL cells*

Cells were incubated for 3 hours with FN, then transferred for chemotactic assay. Figure 3 shows that in the absence of SDF-1alpha, there was no significant migration of pre-B ALL cells after pre-incubation with FN. In the presence of SDF-1alpha, there was a significantly greater migration (63 ± 17%) after pre-incubation with FN. These results suggest the induction of an intracellular signal by FN, leading to migration.

Expression of CXCR-4 on subsets of pre-B ALL cells

The magnitude of the response to SDF-1alpha could be related to the CXCR-4 expression level. CXCR-4 expression was evaluated on different subsets of pre-B ALL cells from peripheral blood and bone marrow (Table 2). On flow cytometry, pre-B ALL cells from all the patients showed a significant expression of CXCR-4 (range 46.5-97.5%). Our results did not demonstrate any correlation between the expression of CXCR-4 and the chemoattractive potential of SDF-1alpha, measured as the percentage of migrating cells (r = 0.2). In addition, the

expression of CXCR-4 on pre-B-ALL cells, after incubation in the presence of FN for three hours, was not modified (Table 3).

Absence of correlation between the chemotactic effects and the number of circulating white cells

In order to look for a relationship between the SDF-1alpha-induced migration and the number of ALL cells, the white blood cell count (WBC) *(Gen's System II Coulter)* was plotted against the percentage of migrating cells in the transwell system. There was no correlation between WBC and the chemotactic effect of the positive gradient of SDF-1alpha on pre-B ALL cells (r = 0.33) (data not shown).

Expression of VLA-4 and VLA-5 on different subtypes of pre-B ALL cells

The mean expressions of VLA-4 and VLA-5 antigens on pre-B ALL cells were respectively 93 ± 2% and 66 ± 6% (Table 4). The VLA-4 molecule was strongly expressed by all patient cells (range 77-97%). In contrast, the expression of the VLA-5 molecule varied over a large range (14-94%), but there was no correlation (r = 0.4) between VLA-5 expression and the FN-dependent increase of the chemotactic effect of SDF-1alpha.

DISCUSSION

The importance of SDF-1alpha and its receptor CXCR-4 in B lymphopoiesis has been demonstrated in murine studies involving gene inactivation [12-14]. Consider-ing leukemia, only a few studies have been published regarding the chemotactic effect of SDF-1alpha and the expression of CXCR-4 [15]. Recently, Bradstock *et al.* showed a positive effect of SDF-1alpha on the transmigration of B-ALL cells across a bone marrow stromal layer in the presence of FCS [16]. Our studies have focused specifically on the ability of SDF-1alpha to act as a chemoattractant for different subtypes of B-lineage leukemic cells under stringent conditions *i.e.* in the absence of any stromal component or serum, except BSA and FN in some experiments. The formal demonstration of this chemoattractive effect was obtained using recombinant SDF-1alpha in the lower chamber of the transwell system, with pre-B-ALL cells in the upper chamber. Within 5 hours, 2.5-39% of the pre-B ALL cells had migrated across the bare membrane but, due to the small size of the pore (3 mum), no migration was

observed during the same period in the absence of the chemokine. Our experiments suggest that SDF-1alpha may act solely, without any ECM components.

In the current analysis, the migration of pre-B2 ALL cells seems to be more important than the migration of pre-B3 ALL cells. For pre-B1 ALL, no conclusion can be drawn due to the small number of patients (n = 2). For AML cells, Möhle *et al.* found a positive correlation between the expression of CXCR-4 and the ability of these malignant cells to migrate through a monolayer of endothelial cells [3]. In contrast, for pre-B ALL cells we observed that cells at any stage of differentiation strongly expressed CXCR-4 (46.5-97.5%), but no correlation existed between CXCR-4 expression and migration. Our results on pre-B ALL cells are in agreement with three studies demonstrating that SDF-1alpha responsiveness did not correlate with levels of CXCR-4 expression on developing normal human B lymphocytes [6, 17, 18]. The chemokine receptor might not be functionally active for the cell migration. However, we can not exclude another function for CXCR-4. In two recent studies, a positive effect of SDF-1alpha on the survival of the primitive hematopoietic stem cell was observed [19, 20]. Nishii *et al.* showed that SDF-1alpha-supported survival of pre-B ALL may occur by its participation in the up-regulation of Bcl2 [21].

Additional studies are required to explain the lack of correlation between CXCR-4 expression and SDF-1alpha chemotactic responsiveness at the different stages of pre-B ALL differentiation: as for normal hematopoietic cells, the expression of CXCR-4 may be necessary for B leukemic cell migration, but not sufficient on its own.

The response of ALL cells to the chemokine may be influenced by the phase of their cell cycle. In a recent study, SDF-1alpha-induced migration was preferentially observed in $CD34^+$ cells in the S + G_2/M phase [22]. Differences in migration rates between patients observed in our study, may be related to the number of cells in this phase. Conversely, the survival and the proliferation of leukemic cells may be enhanced by SDF-1alpha.

SDF-1alpha has been shown to attract ALL cells through a bone marrow endothelial or fibroblastic cell line layer [3, 16]. In the present paper, we have provided additional information by demonstrating the effect of SDF-1alpha-mediated chemotaxis through a bare membrane, coated or not with FN.

Interestingly, migration of ALL cells in response to SDF-1alpha increased significantly when FN was added in the upper chamber but not in the lower chamber, and was more active under FN-coated conditions. This is in good agreement with a recent report that showed that SDF-1alpha increased migration of $CD34^+$ cells across FN-coated or uncoated filters [22]. These results led to the hypothesis that FN in the upper chamber might stimulate the motility of cells to respond to the chemoattractive effect of SDF-1alpha. The enhancing effect of the FN-coating on filters was observed only for cells which had a significant migratory response to SDF-1alpha. Consequently, this suggests that FN increases the chemotactic effect of SDF-1alpha, and that soluble FN may induce an intracellular signal leading ALL cells to migrate. Recently, Pelletier *et al.* showed that SDF-1alpha may be presented by FN [23]. Some of the properties of FN in SDF-1alpha-induced migration seems to be unrelated to the potential affinity between the two molecules.

Some studies have demonstrated that human leukemic lymphoblast migration is dependent on the combined presence of the beta-1 integrins, VLA-4 and VLA-5, which mediate binding to FN [8, 24]. Our results indicate that there is no correlation between the enhancement of migration by FN and the level of expression of VLA-4 and VLA-5. VLA-4 was expressed at a high level in all pre-B ALL cells, whereas there was a great variation of expression of VLA-5, with no significant correlation ($r = 0.4$) with the increased effect of FN on the chemoattractive capacity of SDF-1alpha. Clearly, other factors influencing motility were operative in our assay conditions.

Each leukemic clone is characterized by its own proliferation rate. This strong prognostic criterion correlates well to the WBC at diagnosis. We analyzed the WBC count at diagnosis, according to their migratory capacity and observed no correlation. There may be no strong relationship between proliferation and the ability to migrate in pre-B ALL.

The motility and migration of precursor-B ALL cells may have significance both for the regulation of their growth, and for the dissemination of the disease. We hypothesize that ALL cells migrate along concentration gradients of cytokines or chemokines, such as SDF-1alpha secreted by specialized stromal cells. Migration of tumoral cells is a major prognostic factor involved in the invasiveness and the metastatic expansion of a solid tumor [25]. A recent study by Scheidweiler *et al.* on cell lines indicated that the complex, SDF-1alpha/CXCR-4, is not essential for

initial adhesion of B cells to bone marrow stroma, but is required for their migration [26]. However, it is also possible that this migratory capacity may allow movement of B cell precursors to specialized microenvironmental "niches" within the marrow which are more favorable for their survival and growth. This process may be important in determining the fate of all cells surviving cytotoxic chemotherapy and may also regulate the egression of leukemic cells from initial sites of proliferation. These results warrant further investigation.

CONCLUSION

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Figures

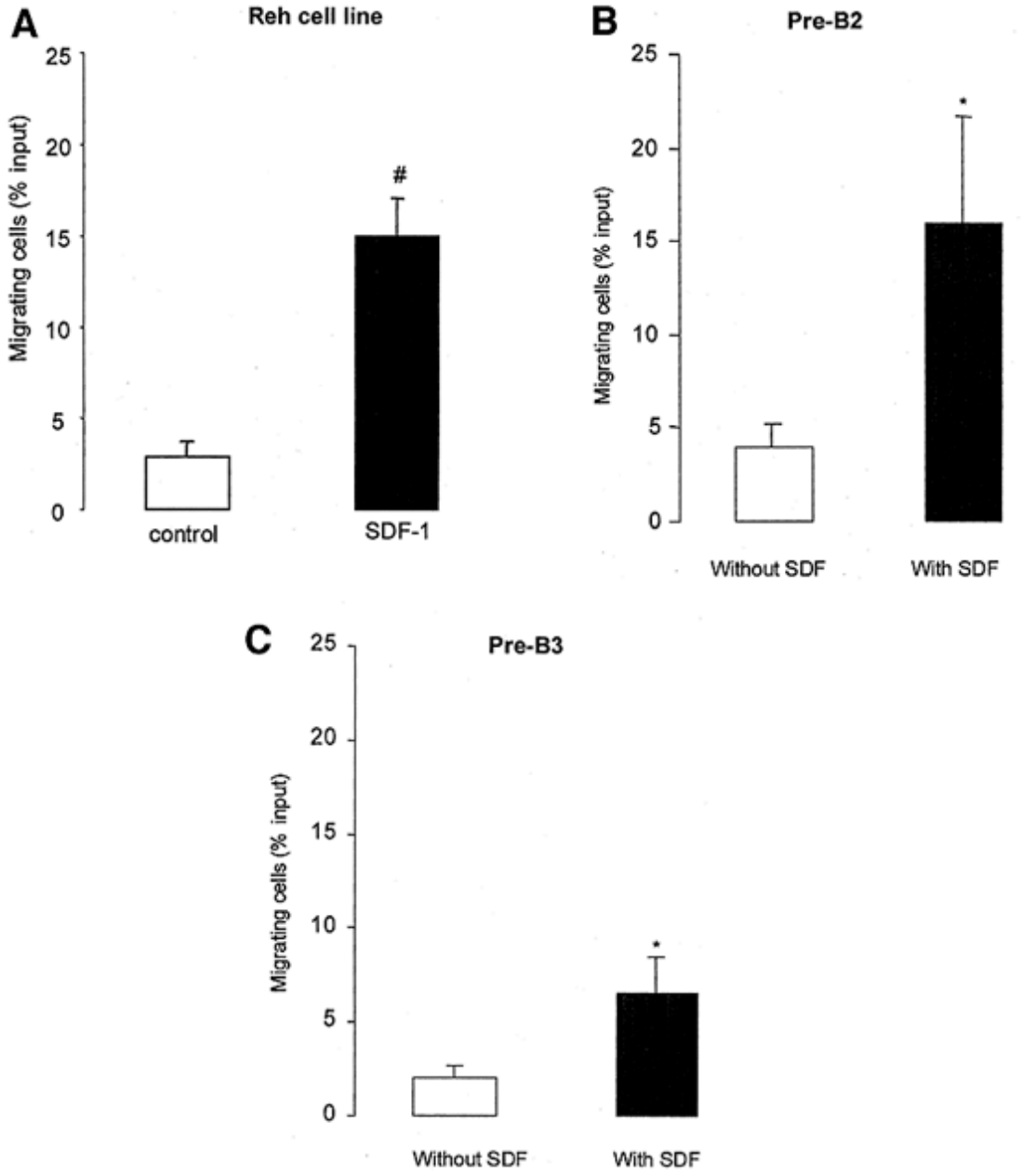


Figure 1

Chemotaxis effect of SDF-1alpha on leukemic cells and the Reh cell line. Reh cells (A) pre-B2 (B) and pre-B3 (C).

Cells (5×10^5) were added to the upper chambers of 24-well transwell plates, and the upper chambers were placed in 24-well plates containing IMDM supplemented with 0.5% BSA, with or without SDF-1alpha (100 ng/ml). After incubation of plates at 37° C for 5 hours, the upper chambers were removed, and the number of cells which had migrated to the lower chambers were counted and

expressed as a percentage of the total input cells. Columns represent mean values ± SEM of 6 independent experiments performed in duplicate with Reh, pre-B2 and pre-B3 cells (# p = 0.06, * p = 0.03).

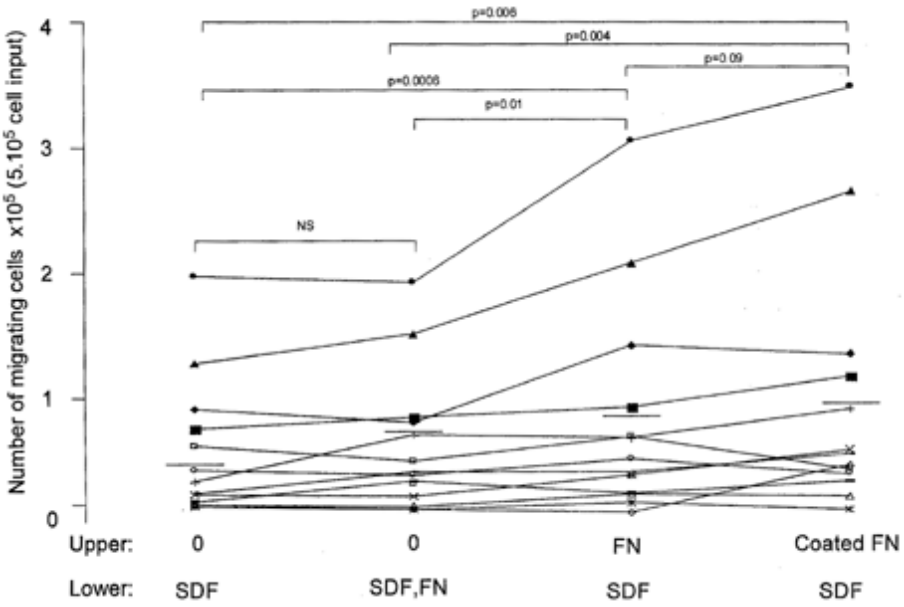


Figure 2

Role of soluble and FN-coating in the chemotaxis of ALL cells in response to SDF-1alpha.

The upper and lower sides of transwell membranes were coated with FN (150 mug/ml) in IMDM, at 4° C and left for more than 18 hours prior to use. After this incubation, membranes were washed and placed in new wells containing IMDM, with or without SDF-1alpha (100 ng/ml). For uncoated wells, FN (150 mug/ml) was loaded in the upper and/or the lower chamber. Pre-B-ALL cells (5×10^5) in IMDM were added to the upper chamber. Results represent the number of cells which had migrated to the lower chamber after 5 hours, when counted using the hemocytometer (Coulter) in 14 independent experiments

relating to 14 patients, and performed in duplicate. p ¾ 0.05 was considered as significant.

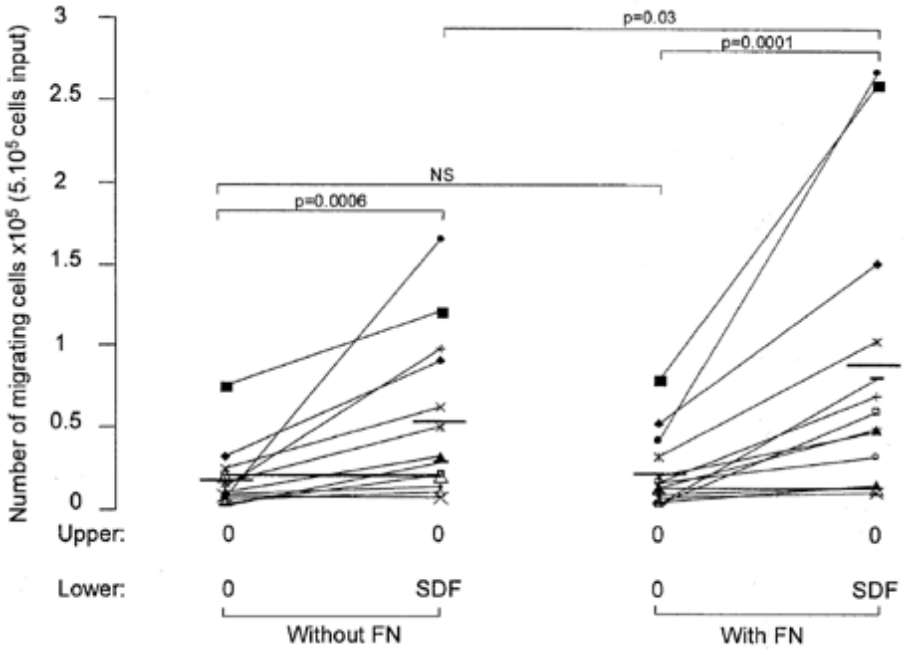


Figure 3

Effects of FN pre-incubation on the chemotaxis of leukemic cells in the presence or absence of SDF-1alpha.

Pre-B-ALL cells were incubated for 3 hours with or without FN (150 mug/ml), then washed and added to the upper chambers of the transwells. The lower chambers contained or not SDF-1alpha (100 ng/ml). The chemotactic effect was tested after 5 hours. Results represent the number of migrated cells in the lower chambers, in 14 separate experiments performed in duplicate. p ² 0.05 was considered as significant.

Table 1
Effects of FN on chemotaxis of the Reh cell line in the presence of SDF-1alpha

Reh cells (5×10^5) were added to the upper chamber with or without FN. The lower chamber contained SDF-1 with or without FN. After 5 hours, the cells migrating to the lower chamber were counted. Results are expressed as the percentage of migrating cells (number of migrating cells/total number of input cells) × 100. Results are reported as mean ± SEM of n (2-5) independent experiments. All assays were performed in duplicate.

Upper chamber	0	0	0	FN	FN	0
Filter						FN-coated
Lower chamber	0	SDF-1alpha	FN, SDF-1alpha	FN, SDF-1alpha	SDF-1alpha	SDF-1alpha
n	5	5	5	4	3	2
Mean ± SEM (%)	3 ± 0.7	15 ± 2[NS]	22 ± 3[NS]	25.5 ± 5[NS]	19 ± 4[NS]	10 ± 2[NS]

[NS]: Not significant ($p > 0.05$) compared to the control.

Table 2
Effect of CXCR-4 expression on the migration of pre-B ALL cells

Pre-B ALL subtypes cells were stained with anti-CXCR-4 antibody and analyzed for the percentage of positive cells by flow cytometry. Results are reported as mean ± SEM and compared to the migration capacity of pre-B ALL in the presence of SDF-1alpha in the lower chamber of the transwell.

Pre-B ALL	n	Expression of CXCR-4 mean ± SEM (%)	Range (%)	Migration (%)
Pre-B1	2	92 ± 4	(88-96)	4 ± 1
Pre-B2	5	91 ± 4	(76.5-97.5)	15 ± 8
Pre-B3	5	75 ± 10	(46.5-92)	6 ± 2
All points	12	85 ± 5	(46.5-97.5)	9 ± 3

Table 3
Effect of FN on the expression of CXCR-4

Pre-B ALL subtypes cells were incubated with or without FN (150 mug/ml) for 3 hours, stained with anti-CXCR-4 antibody and then analyzed for the percentage of positive cells by flow cytometry. Results are reported as mean ± SEM.

Cell type		Without FN	With FN
	n	CXCR-4 expression mean ± SEM (%)	
Pre-B2	2	70 ± 27	57.5 ± 35
Pre-B3	2	72 ± 20	84 ± 9
All points	4	71 ± 14	71 ± 17

Table 4
Integrin expression and migratory capacity of leukemic cells

Pre-B ALL subtypes cells were stained with a PE-conjugated monoclonal antibody against VLA-4 and a FITC-conjugated monoclonal antibody against VLA-5, and then analyzed for the percentage of positive cells by flow cytometry. Results are reported as mean ± SEM and compared to the migration capacity of pre-B ALL in the presence of FN-coated membrane and SDF-1alpha in the lower chamber of the transwell.

Cell type	VLA-4			VLA-5		Coated FN/SDF-1alpha
	n	Expression (%)	Range (%)	Expression (%)	Range (%)	Migration (%)
Pre-B1	2	96 ± 1	(95-97)	47 ± 11	(36-58)	10 ± 1
Pre-B2	5	94 ± 1.5	(90-97)	67 ± 13	(14-88)	23 ± 12
Pre-B3	4	92 ± 5	(77-97)	74.5 ± 7	(63-94)	16 ± 7
All points	11	94 ± 2	(77-97)	66 ± 7	(14- 94)	16 ± 6

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E. Sbaa-Ketata, M. Vasse, B. Lenormand, P. Schneider, C. Soria, J.P. Vannier
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THP-1 cells, macrophages, estrogen receptors, estrogens, monocytes.

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