

Stromal cell-derived factor-1 (SDF-1)/CXCR4 couple plays multiple roles on haematopoietic progenitors at the border between the old cytokine and new chemokine worlds: survival, cell cycling and trafficking

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ABSTRACT. Generation of haematopoietic cells is regulated by cellular and humoral interactions in which stromal cells, adhesion molecules, cytokines and chemokines play a crucial role. Among the chemokines, SDF-1 and its CXCR4 receptor have been reported to be key players in the nesting of haematopoietic progenitors within the bone marrow. Disruption of the SDF-1/CXCR4 axis results in cell mobilization and may participate in leukaemia extramedullary infiltration. In this review we will discuss the manifold roles of the SDF-1 chemokine and of its receptor in haematopoiesis regulation. By recruiting quiescent progenitors, by participating in their survival/cycling and by sensitizing them to further cytokine synergistic action, SDF-1 likely contributes to haematopoiesis homeostasis under physiological conditions and in stress situations. The complexity of the SDF-1/CXCR4 interactions in the regulation of haematopoiesis illustrates a dynamic and sequential cross-talk between chemokine and cytokine/growth factor worlds. Because of their pleiotropic effects on haematopoietic progenitor trafficking, survival and proliferation, the SDF-1/CXCR4 couple could be considered as promising molecules for improvement of cell-based therapy protocols in haematopoietic transplantation.

Keywords: chemokines, SDF-1/CXCR4, haematopoiesis, leukaemia, CD34⁺ cells

INTRODUCTION

Haematopoiesis is a cell-renewal process that leads to the continuous production of functional differentiated blood cells from bone marrow haematopoietic stem (HSC) and progenitor (HPC) cells. Maintenance of haematopoietic homeostasis is tightly orchestrated by cellular and humoral interactions between HSC/HPC and stromal cells, adhesion molecules, cytokines and chemokines.

Cytokines act on HSC/HPC quiescence/proliferation, self-renewal/differentiation, survival/apoptosis and migration/homing processes through binding and activation of their cognate receptor [1]. Most cytokines exert their biological activity through positive regulation [2]. Some, however, such as transforming growth factor- β (TGF- β), can also elicit negative regulation of HPC [3]. Cytokine specificity appears to be related to their target cells; immature HSC/HPC are responsive to a large number of cytokines, mainly acting in synergy [4], while more mature cells preferentially respond to single cytokines [5].

For a long time regarded as members of the cytokine superfamily, the chemokines now have their own identity, as a class of small molecules (70 to 100 amino acids; 8 to 14 kDa), mainly involved in leucocyte recruitment to inflammatory sites [6]. The name “chemokine” comes from the combination of “chemotactic” and “cytokine”.

Thus, by quickly mobilizing blood cells, chemokines are involved in various biological processes such as neural development, atherosclerosis, angiogenesis, inflammation, viral pathogenesis and, in the context of this review, haematopoiesis. Because of its dual action on HSC/HPC chemotaxis and proliferation, stromal cell-derived factor-1 (SDF-1) could be considered as the flagship chemokine. SDF-1 acquired its identity among chemokines suppressing haematopoiesis such as interleukin 8 (IL-8), platelet factor 4 (PF4) and macrophage inflammatory protein 1 α (MIP-1 α), thanks to a stimulatory effect on haematopoiesis [7]. This review will focus on the role of the SDF-1/CXCR4 couple in trafficking, survival and proliferation of normal haematopoietic progenitors. In this respect, its involvement in leukaemia progression will be discussed.

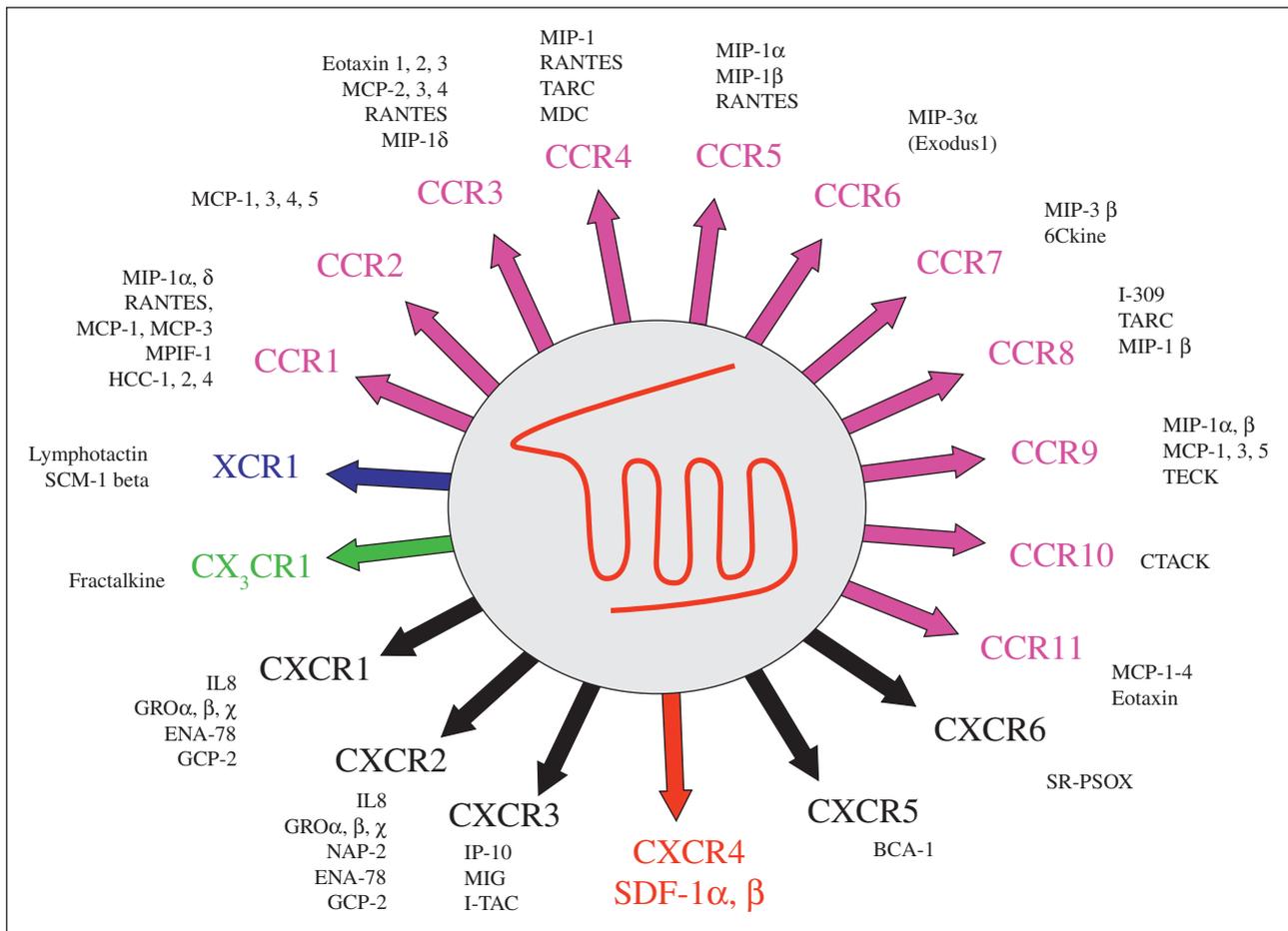


Figure 1

Schematic overview of chemokine receptors and their ligands.

We will show how a chemokine such as SDF-1 and its receptor play a major role in the complexity of humoral haematopoietic regulation and, as such, could also be considered as belonging to the cytokine world.

TO BE OR NOT TO BE A CHEMOKINE!

Are redundancy and duality chemokine hallmarks?

Of nearly 100 chemokines, only 50 have been identified to date. Beside their chemotactic function, which constitutes the central concept of these molecules, chemokines are characterized by structural similarities, redundancy and functional duality. Chemokines are produced by a great number of haematopoietic and non-haematopoietic cells such as leucocytes, platelets, endothelial cells, stromal cells (fibroblasts, osteoblasts...) and keratinocytes [7, 8]. They are classified into four different subgroups characterized by their chemokine domains defined by the presence of two cysteines in highly conserved positions. The two largest chemokine subfamilies are the "CXC" and "CC" groups, depending on the presence or not of any amino acid called "X" [9]. Two exceptions, lymphotactin and fractalkine, are characterized by a "C" and "CX3C" structure, respectively.

Except for PF4, the receptor of which is still unknown, all members of these families exert their biological activity through 7-transmembranes spanning, G-protein-coupled

receptors. Chemokine receptors are classified into two major groups, according to their sequence similarities, which correspond to the ligand subfamilies [10, 11]. The CXC receptors (CXCR) bind the CXC chemokines, and the CC receptors (CCR) bind the CC chemokines. Until now, six CXCR and 11 CCR have been identified (Figure 1). Their expression is not restricted to leucocytes, since they are also found on endothelial, neuronal, fibroblastic and epithelial cells, and is dependent on cell activation and differentiation status. Interestingly, some cytokines, mainly inflammatory ones such as interleukin 6 (IL-6), interleukin 10 (IL-10), tumour necrosis factor (TNF), interferon (INF) and stem cell factor (SCF), may modulate their expression.

In contrast to cytokines, the relationship between chemokines and their receptors is of a "polygamous" type, since chemokine receptors bind several different chemokines and chemokines bind several different receptors [12]. Such an unusual relationship between chemokines and their receptors generates similar biological responses through different pathways. This redundancy could explain why deficiency in some chemokine- or receptor-coding genes does not necessarily induce a major *in vivo* abnormality. There are a very few exceptions to this rule. Among them, CXCR4 is the archetypal example. In addition to interactions with their own receptors, chemokines may also interact with cell surface or stromal proteoglycans [13]. At physiological concentrations, biologically active chemok-

ines are found as monomers [14]. At high concentrations (100 μ M), they form dimers and oligomers. In the stroma, binding of chemokines to glycosaminoglycans promotes their dimerization and therefore participation in receptor regulation [15]. Such a dimerization/oligomerization process led to the concept that chemokines exhibit dual biological activities. Because it acts differently on haematopoiesis when in its mono- and dimerization forms, MIP-1 α is a well-known model of this functional duality [16-18].

Do chemokines have specific transduction signalling pathways?

The activation cascade of the G-protein-coupled receptors (GPCR) schematically consists of three partners: a receptor, G proteins and an effector. G proteins are composed of an α sub-unit associated with a cytoplasmic membrane-bound $\beta\gamma$ complex. When GPCR are activated by ligands, heterotrimeric G proteins stimulate their own activation, resulting in signal amplification. In its resting state, the G-binding form of $G\alpha$ is tightly associated with $G\beta\gamma$. Upon receptor binding, the GDP/GTP exchange activity of $G\alpha$ results in the GTP-bound form and free $G\beta\gamma$ subunit. Activated $G\alpha$ or free $G\beta\gamma$ subsequently triggers downstream target molecules [19].

Multiple transduction pathways are activated by chemokine receptors and are ligand- and cell type-dependent [20-22]. Among these pathways, the most important are phospholipase C/PKC, phosphatidylinositol-3-kinase (PI3K)/AKT, adenylate cyclase/cAMP and MEK/MAP kinases [23, 24]. Lastly, JAK/STAT signalling could alternatively be used by some receptors such as CCR5 and CXCR4 [25, 26]. Thus, the linear cascade (receptor, G proteins and effector) signalling, associated with a high degree of complexity resulting from the multiple downstream pathways, allows the cell to respond specifically to each stimulus. Transduction signals are negatively controlled by various mechanisms [27], of which receptor "sensitization/desensitization" plays an important role. Desensitization is a major down-regulation process that limits duration of the receptor activation signal. Three types of regulatory proteins participate in this process: (i) PKA and PKC kinases that modify the receptor conformation, (ii) GRK that are specific G protein serine/threonine kinases, and (iii) arrestines which, by binding to clathrin vesicles, lead to receptor internalization, recycling or degradation [28]. Another negative receptor regulation process involves the RGS (regulator of G protein signalling) elements which, by increasing the GTPase activity of the $G\alpha$ proteins, sequester receptor signalling [29].

Therefore, chemokine signalling intrinsically contrasts with that of cytokines. Several chemokines may bind to a sole receptor, generating, through a common G-protein mediator, different pathways associated with specific biological functions. In contrast, cytokine receptors are activated by a unique and specific ligand that generates two main downstream pathways (MAP kinase and JAK-STAT cascades), depending on their receptor family (cytokine-receptor, tyrosine kinase-receptor or Ig superfamilies).

SDF-1, AN ATTRACTIVE CHEMOKINE!

In human beings, the *SDF1* gene is located on chromosome 10q11.1 [30]. It is a large gene with four exons.

SDF-1 α cDNA results by alternative splicing from the first three exons, and SDF-1 β cDNA from the first four exons. Winkler *et al.* [31] reported a single nucleotide polymorphism (SNP) within the *SDF1* gene that consists of a G-to-A transition in a 3' untranslated region of exon 4. The frequency of the variant 3'A allele (*SDF1*-3'A) is 21 % in Caucasians but only 6 % in African Americans. This polymorphism is suggested to have a biological relevance since *SDF1*-3'A homozygosity is associated with lower plasma SDF-1 levels [32] and is reported to be related to HPC mobilization capability [33].

SDF-1 is constitutively expressed by virtually all tissues, including bone marrow (BM) [34], while most of the other chemokines are usually induced by pro-inflammatory cytokines. SDF-1 α represents the predominant isoform and SDF-1 β contains four additional C-terminus amino acids. SDF-1 is a homeostatic chemokine initially cloned from the murine ST-2 and PA6 bone marrow stromal cell lines [30] and then purified from supernatant from the murine MS-5 cell line [35]. SDF-1 has a CXC sequence, while its structure is of the CC type [36]. Both isoforms display similar functions and no particular tissue expression. They are produced by mucosal epithelial cells and by medullar, endothelial and stromal cells as well as by immature osteoblasts within the haematopoietic niches [37]. SDF-1 is also expressed by CD34⁺ HPC purified from bone marrow (BM), cord blood (CB) and unmobilized peripheral blood (PB) [38, 39]. The constitutive expression of SDF-1 in various tissues and cells, as well as its highly conserved nucleotide and amino acid sequences [40, 41], argue for its key biological role.

SDF-1/CXCR4, A MONOGAMOUS COUPLE

SDF-1 is a chemokine which binds a unique receptor. It has been identified as the ligand of the "leucocyte-derived seven transmembrane domain receptor" (LESTR). This 7-transmembrane-spanning G-protein-coupled receptor was originally known as an orphan receptor with a structure very similar to that of IL-8 receptors [35, 42]. Due to the similarity of its sequence to that of other CXC chemokine receptor genes, LESTR was also named CXCR4 [43]. It was also called fusin because it mediates HIV-1-CD4 T lymphocyte cell fusion [44]. Whereas its extracellular domain plays a significant role in ligand binding, the intracellular domain, enriched in phosphorylatable residues, is required for G protein activation and is involved in the receptor internalization process.

Cellular and tissue distribution of CXCR4 (clustered as CD184) is constitutive and broad. Its expression on various non-haematopoietic and haematopoietic cells distinguishes it from the other chemokine receptors, the expression of which is more restricted. CXCR4 is present on dendritic [45], endothelial [46], neural cells [47] and on mature blood cells (lymphocytes, monocytes, megakaryocytes/platelets) [48-50]. CXCR4 is also detected on CD34⁺ HPC with a specific profile according to their source (BM, PB or CB) [42, 43, 51, 52]. Its expression is highly versatile: it is rapidly up-modulated by cytokines such as IL-2, IL-6 and SCF [24, 53], adhesion molecule activation [54] - especially VLA-4 and CD44 [55] - and temperature (personal results). Intracellular storage of CXCR4 is partly responsible for this rapid membrane upregulation [51]. This phenotypic versatility could be

significant as a prerequisite for the bio-availability of SDF-1-responsive cells. Such a reversible modulation is also observed for other migration-mediated cytokine receptors and could be a common feature shared by molecules involved in the mobilization process (Lataillade J.-J. *et al.*, submitted manuscript, 2004).

The ligand/receptor interaction induces a fast and reversible endocytosis of the receptor after ligand withdrawal [56], a phenomenon that could also result from anti-CXCR4 antibody ligation. As previously described for chemokine receptors, the internalization/desensitization process of CXCR4 plays a significant role in the CD34⁺ HPC migration between BM and PB. The lower sensitivity of bone marrow HPC CD34⁺ to this type of regulation would explain their nesting within the SDF-1-enriched environment [57]. The lack of correlation between the CXCR4 expression on medullar CD34⁺ cells and their unresponsiveness to the proliferative and survival effect of SDF-1 argues for the hypothesis that expression and function of CXCR4 are not obligatorily related [39, 51]. These data afford a glimpse of the complexity and subtlety of SDF-1/CXCR4 interactions, which seem to be dependent on the cell type and environment.

SDF-1/CXCR4, AN EMANCIPATED COUPLE

The various biological functions of SDF-1/CXCR4 confer on this monogamous couple an emancipated nature. This univocal character, which is preserved during evolution, demonstrates its essential role in physiology. The similarity of the phenotypes of CXCR4^{-/-} and SDF-1^{-/-} knock-out mice reinforces this concept [58-60].

SDF-1/CXCR4: an established HPC homing/mobilization regulator

Homing of circulating HPC to BM is a multi-step process, including attachment to sinus endothelial cells (EC), transendothelial migration and anchoring within the haematopoietic niche (Figure 2). This process involves interactions of HPC with cell adhesion molecules (CAMs) expressed on sinus vascular cells and extra-cellular matrix (ECM) adhesive molecules, as well as the intervention of chemotactic factors. SDF-1 is considered to be the major *in vitro* chemotactic factor and the SDF-1/CXCR4 couple appears to be crucial for *in vivo* HPC engraftment [53, 61]. In contrast to EC from non-haematopoietic tissues, BM EC constitutively express CAMs, such as E-selectin or vascular cell adhesion molecule-1 (VCAM-1), and produce predominantly SDF-1 [62]. They bind SDF-1 [63] via heparan sulphate (HS) proteoglycans present on their luminal face according a tissue-specific affinity [64]. Concentration of SDF-1 on the BM EC surface thereby favours the presentation of its CXCR4-binding site to HPC [65]. Such particularities of BM EC could explain specific SDF-1-induction of HPC engraftment within BM, while this chemokine is broadly and strongly expressed in many other tissues.

The lower expression of CXCR4 on circulating HPC, as compared to their BM counterparts [66, 67], is a reversible phenomenon that restores sensitivity of circulating HPC to SDF-1 [53]. SDF-1 located on the endothelial surface and bound to HS can optimally interact with its counter-receptor CXCR4 expressed on HPC, resulting in activation

of VLA-4 and LFA-1 by inside-out signalling on the rolling HPC. VLA-4 and LFA-1 activation converts to firm adhesion their interactions with VCAM-1 and ICAM-1, inducing HPC arrest [68]. Other consequences of CXCR4 engagement are a transient increase in intracytoplasmic calcium [61] and cytoskeleton actin polymerization [69] that trigger HPC migration through the endothelium. Following transendothelial extravasation, HPC interact with the fibronectin present in the sinusal ECM by using VLA-4 and VLA-5 receptors, these interactions also being enhanced by SDF-1 [70]. Moreover, proteoglycans present in the subendothelial matrix bind the SDF-1 produced by stromal cells. Such an interaction helps to establish a local gradient of SDF-1, guiding polarized HPC into specific niches in the BM [71]. SDF-1 production by stromal cells can be increased by pre-transplant conditioning regimens including radiotherapy and chemotherapy [34] and therefore helps enhance BM homing of HPC. The final anchoring depends on interactions of HPC/VLA-4 with stromal cell/VCAM-1 and of HPC/VLA-5 with ECM fibronectin [72-75] and is maintained by a continuous production of SDF-1 by stromal cells. Recently, a major role for the CD44 adhesion molecule and its ligand, hyaluronic acid, in SDF-1-dependent transendothelial HPC migration and their anchorage within BM niches has been demonstrated in an elegant study by Lapidot's group [55].

Unlike homing, mechanisms leading to HPC mobilization are less well understood (Figure 2). It occurs when SDF-1 levels are artificially increased by *in vivo* administration of SDF-1 analogue [57], adenovirus-expressing SDF-1 [76] or sulphated polysaccharides [77]. Nevertheless, a reversion of the SDF-1 gradient between BM and PB is not observed during clinical mobilization since it has been demonstrated that SDF-1 concentrations remain lower in PB than in BM after *in vivo* administration of G-CSF [66, 78]. On the other hand, it is now well established that HPC mobilization is associated with an increased local production of proteases, such as leucocyte serine proteases (elastase, cathepsin G) and matrix metalloproteinases (MMP) within the BM, which are able to degrade the ECM. G-CSF-induced mobilization has recently been associated with SDF-1 degradation by elastase [79]. The role of MMP-9 in HPC mobilization is also suspected, since *in vivo* G-CSF treatment has been associated with an increase in MMP-9 expression in BM [80], while its plasma levels have been correlated with HPC mobilizing capacity [66]. The impairment of HPC mobilization found in MMP-9^{-/-} mice strongly argues for a role of this protease in the process [80], by acting through a possible local degradation of SDF-1. Indeed, N-terminus cleavage of SDF-1 can be induced *in vitro* by MMPs, including MMP-9, which inactivates SDF-1 and results in loss of binding to CXCR4 and of its chemoattractant activity for CD34⁺ cells [81]. In addition, proteolysis of the extracellular domain of CXCR4 by elastase, first demonstrated *in vitro* [82], has been demonstrated *in vivo* on CD34⁺ cells after mobilization by G-CSF or chemotherapy [83], in parallel to the cleavage of VCAM-1 [84]. Such CXCR4 degradation could lead to its internalization, an event that appears to be critical in the HPC mobilizing process [57]. This could explain the decrease in CXCR4 expression reported on PB CD34⁺ cells [66, 67, 83] and the correlation with HPC mobilizing capacity already described [66, 78, 85]. Nevertheless, direct blocking of the SDF-1/CXCR4 interaction

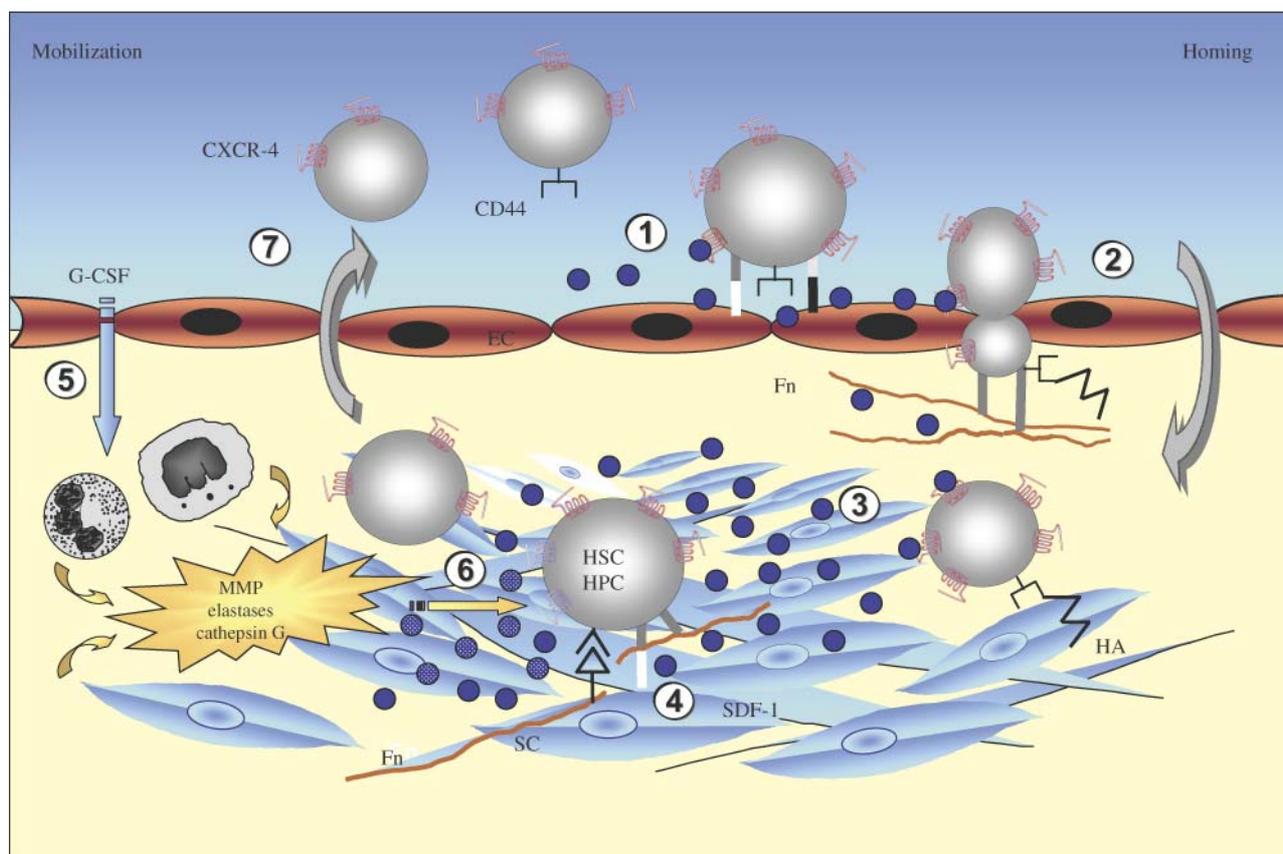


Figure 2

Role of the SDF-1/CXCR-4 couple in HSC/HPC trafficking.

- ① SDF-1 located on the surface of endothelial cells (EC) and bound to heparan sulphates can optimally interact with its counter-receptor CXCR-4 expressed on the rolling HSC/HPC. CXCR-4 engagement induces an activation of integrins VLA-4 and LFA-1 by inside-out signalling which converts their interactions with VCAM-1 and ICAM-1 constitutively expressed on EC to firm adhesion.
- ② This activation induces the arrest of HSC/HPC, and stimulates actin polymerization, which results in transendothelial migration mediated by VLA-4 and VLA-5 in the presence of fibronectin (Fn), these interactions being enhanced by SDF-1.
- ③ HSC/HPC then polarizes, migrates toward a local gradient of SDF-1 continuously produced by stromal cells (SC) and reaches the haematopoietic niche. SDF-1-induced migration is related to the presence of CD44, the receptor for hyaluronic acid (HA).
- ④ The final anchoring of HSC/HPC within the niche depends mainly on interactions with SC and extracellular matrix (Fn, HA...). Anchoring is maintained by the continuous production of SDF-1 by SC.
- ⑤ *In vivo* administration of G-CSF induces local production of proteases (MMP, elastases and cathepsin G) from leucocytes or SC. These molecules are able to degrade ⑥ the ECM and to disrupt VLA-4/VCAM-1, c-Kit/SCF and also CXCR-4/SDF-1 interactions by degrading both CXCR-4 and SDF-1. The loss of attachment to stromal cells and to the ECM, together with the loss of SDF-1 activity, favours the release of HSC/HPC into the peripheral blood ⑦.

alone can trigger HPC mobilization from BM to the bloodstream, as recently demonstrated *in vivo* with the CXCR4 antagonist AMD3100 in humans [86].

A genetic factor influencing HPC mobilization capacity has recently been reported. It was attributed to *SDF1* gene SNP [33] since patients carrying the SDF1-3'A allele appear to be better mobilizers than other patients. Multivariate analysis showed that *SDF1* gene SNP is an independent factor for good HPC mobilization. Until now, *SDF1* gene SNP has not been associated with biological changes involving stromal cell function, and the mechanism by which it results in increased mobilization is still unclear.

SDF-1/CXCR4 displays an unexpected haematopoietic-promoting activity

SDF-1, a haematopoietic-stimulating synergistic factor

Most chemokines, and especially MIP-1 α , IL-8 and PF4, suppress haematopoiesis [7, 87-89]. Knock-out mice ex-

periments have demonstrated the specific involvement of the CXCR-2 receptor in the suppressive effect of IL-8 and of MIP-2 on haematopoiesis [90]. In contrast, chemokines such as SDF-1, fractalkine and HCC-1 have been isolated from the rest of the family as they promote haematopoiesis [51, 91-93]. SDF-1 is prominent among these non-suppressive chemokines since it stimulates PB CD34⁺CD38⁻ cell expansion and increases colony formation by primitive HPC released from quiescence by anti-TGF- β antibody treatment. The proliferating effect of SDF-1 on PB CD34⁺ cells was observed in synergy with cytokines, especially with SCF and Tpo [51], and was further confirmed by Rosu-Myles *et al.* [94] on CB CD34⁺ cells. The lack of an *in vitro* stimulating effect on medullar HPC may reflect a specific role of SDF-1 on circulating HPC. Recently, it has become increasingly clear that SDF-1 also stimulates *in vivo* haematopoiesis, as shown by the increase in the absolute number of myeloid progenitors detected in haematopoietic organs from SDF-1 transgenic mice. Interestingly, HPC from these transgenic mice resist the suppressive effect of chemokines like MIP-1 α ,

IL-8 or PF4 [95]. Impairment of lympho-myelopoiesis in mice reconstituted with bone marrow HPC expressing SDF-1-intrakine, which by binding intracytoplasmic CXCR4 blocks its membrane translocation, further argues for the stimulating role of the SDF-1/CXCR4 couple in adult haematopoiesis [96]. Interestingly, the *in vivo* anti-apoptotic and proliferative synergistic effect of SDF-1 on haematopoiesis has been recently confirmed in lethally gamma-irradiated monkeys [97].

The promoting effect of SDF-1 on haematopoiesis is observed for concentrations below 0.5 ng/mL, which is 1 000 times lower [39, 51] than that which has been found to be effective in migration tests [61]. Such a stimulating effect follows a bell-shaped curve, which characterizes chemokine biological activities [51], and its sharpness is consistent with subtle and accurate haematopoiesis regulation. Beside the key role of chemokine concentration in their regulatory effect on haematopoiesis, it is interesting to note that their activity also depends on their structural conformation, as has been well documented for MIP-1 alpha [98].

SDF-1, a survival-promoting factor

SDF-1 was initially known as a stimulating factor for normal and leukaemic B lymphoid proliferation [58, 99, 100]. Results showing that bone marrow-derived fibroblastic cells from patients with B lymphoid leukaemia produce large amounts of SDF-1 that maintain the survival of their B lymphoid cells suggest that SDF-1 plays an anti-apoptotic role in lymphopoiesis [101].

The increased production of SDF-1 by irradiated stromal cells led Tania Ponomaryov to suggest a survival-promoting effect for SDF-1 in haematopoietic stress conditions [34]. This hypothesis was further demonstrated by results from our group showing that low concentrations (0.05 to 0.5 ng/mL) of exogenous SDF-1 promote the survival of CD34⁺ cells purified from human unmobilized PB [39]. SDF-1 is able to counteract apoptosis as demonstrated by its capacity to reduce DNA fragmentation, annexin-V⁺ cell number, APO2.7 detection and to modulate bcl-2 homologue protein expression. SDF-1 also promotes survival of clonogenic progenitors from sorted CD34⁺CD38⁻ and CD34⁺CD38⁺ cells, suggesting that SDF-1 protects primitive haematopoiesis from apoptosis [39]. SDF-1 is produced by cycling CD34⁺CD38⁺ circulating cells and over-released in response to cell damage to exert an anti-apoptotic effect on CD34⁺ cells through an autocrine/paracrine regulatory loop, suggesting that SDF-1 could participate in the autonomous survival of circulating HPC [39]. Such a survival-promoting activity has been further confirmed *in vivo* since, in combination with other cytokines, SDF-1 increases the long-term survival of lethally irradiated mice [97]. Broxmeyer *et al.* have further confirmed that SDF-1 directly enhances survival/antiapoptosis of myeloid progenitor cells through CXCR4 and G(alpha)i proteins and enhances engraftment of competitive, repopulating stem cells [102]. This group also demonstrated that transgenic expression of SDF-1 enhances myeloid progenitor survival in response to growth factor withdrawal [95]. Taken together, these results imply a major role for SDF-1 in "haematopoiesis survival" by protecting the stem cell compartment in response to cell damage.

SDF-1, a cell cycle-promoting and cytokine-priming factor

Involvement of SDF-1 in cell cycle regulation was recently demonstrated using dual DNA/Ki67 staining. Low concentrations of exogenous SDF-1 trigger G₀ quiescent PB CD34⁺ cells in G₁ phase and make them progress through the S + G₂/M phases of the cell cycle when combined with Tpo or SCF [39]. Such a triggering effect on the cell cycle was confirmed by the over-expression of G₀/G₁-restricted D1 and E cyclins in response to SDF-1. Pre-treatment of PB CD34⁺ cell with SDF-1 before culture increases their further proliferation under cytokine stimulation, suggesting that SDF-1 could act as a priming/sensitizing factor rendering HPC responsive to cytokines [39, 103]. In contrast, the large amounts of SDF-1 produced by stromal cells can lead to opposite effects on cycling of HPC from BM [104]. Opposing concentration-dependent effects of SDF-1 could be linked to the bell-shaped concentration-response curve of chemokine biological activity regulation [105].

As a promoter of the G₀/G₁ transition, SDF-1 is considered to be a regulator of early cell cycle phases and could act as an antagonist of TGF-β, which is active during the G₁ phase [3]. Recent data from Wright [106] showing that TGF-β down-regulates the production of SDF-1 by medullar stromal cells argue for intimate interactions between these cell cycle regulators. Therefore, through its different activities on HSC/HPC regulation (*Figure 3*), the SDF-1 chemokine shares numerous biological functions with haematopoietic cytokines.

The communication network within the SDF-1/CXCR4 couple

Involvement of the PI3-K/Akt and MAP-kinase pathways in SDF-1 signalling has been extensively studied [103, 107-109]. By using specific inhibitors, we demonstrated that either exogenous or endogenous SDF-1 displayed its anti-apoptotic effect on CD34⁺ cells through activation of the PI3K/Akt axis [39]. On the other hand, the MAP-kinase/MEK pathway is required neither for autonomous PB CD34⁺ cell survival nor for the anti-apoptotic effect of SDF-1, although SDF-1 binding is able to phosphorylate MAP kinases [39]. Conflicting data have also been reported concerning the involvement of the MAP-kinase/MEK pathway in adhesion protein activation and chemotaxis in response to SDF-1 [110]. Lapidot's group has shown that activation of PI3K, but not of MAPK, is required for motility of immature CD34⁺ cells [111]. Interestingly, the PKC zeta isoform, which is activated by SDF-1 via PI3K, is found to be essential for the migration process [112]. The role of Rac2 and Cdc42 GTPases has also been shown to be critical in SDF-1-induced HPC adhesion and migration processes (113). Taken together these studies illustrate the complexity of SDF-1-mediated signalling, depending on the functions studied, cell types and experimental conditions (*Figure 4*).

DOES DISHARMONY WITHIN THE SDF-1/CXCR4 COUPLE PARTICIPATE IN LEUKAEMIA PROGRESSION ?

Considering the significant role played by SDF-1 in mobilization, survival and cycling/proliferation of normal pro-

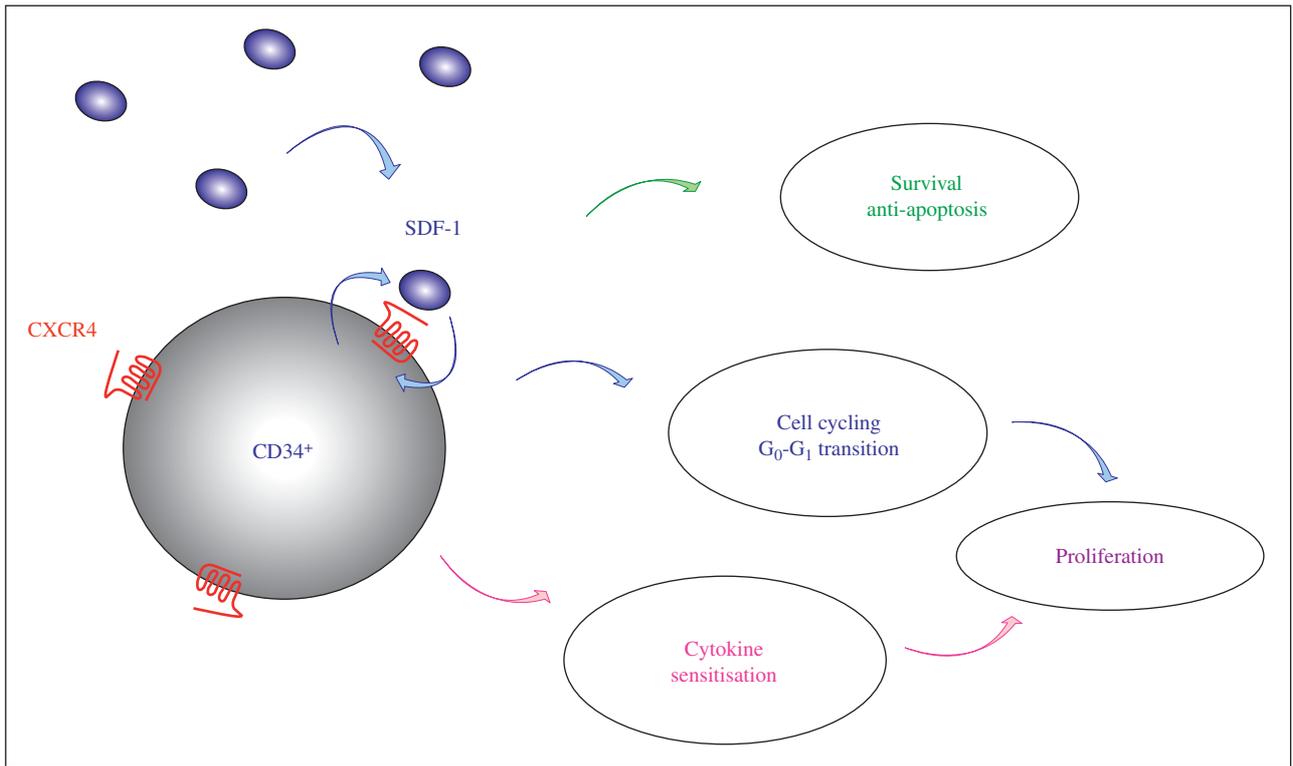


Figure 3

SDF-1/CXCR4 activities in haematopoiesis.

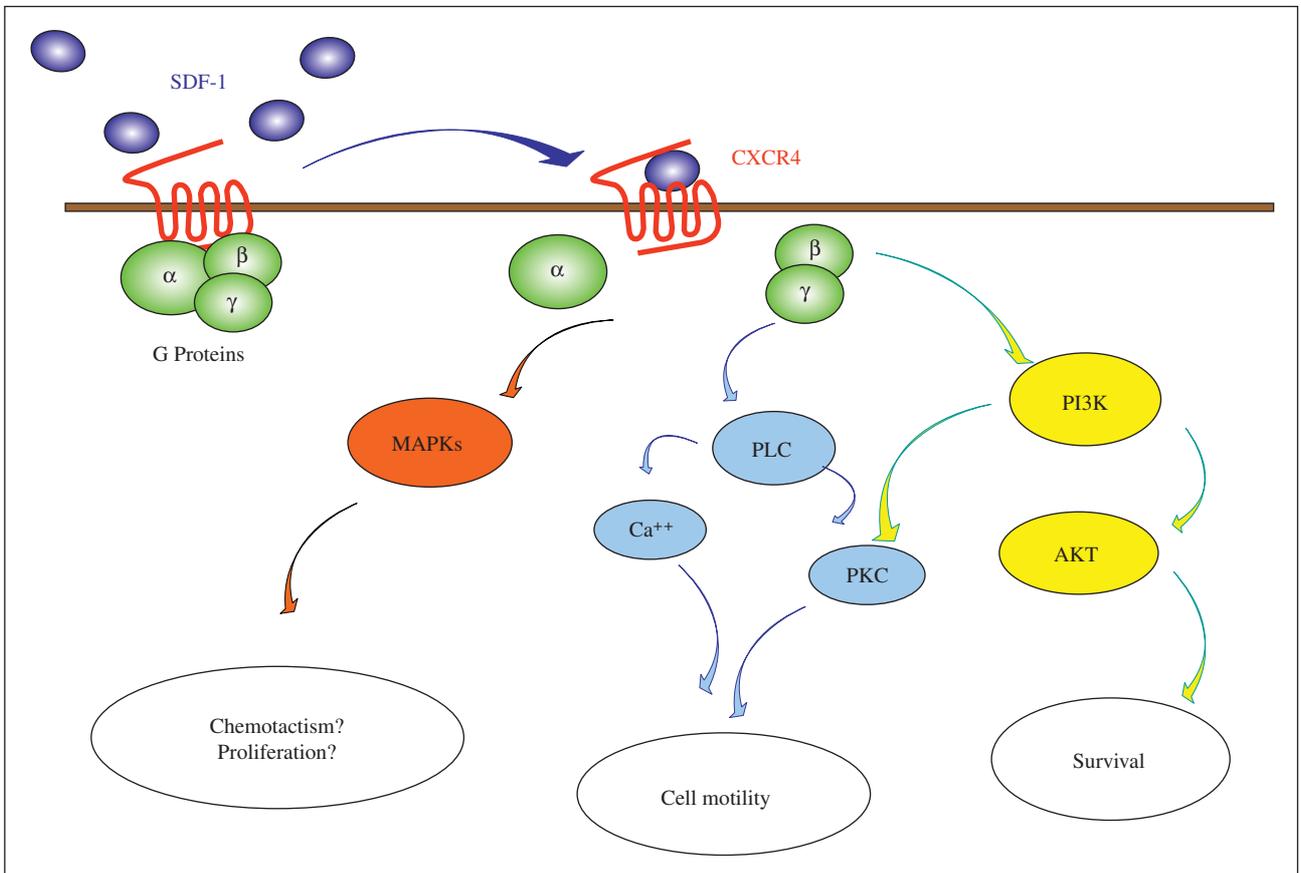


Figure 4

SDF-1/CXCR4 signaling pathways.

genitors, it is conceivable that abnormalities within the SDF-1/CXCR4 couple participate in leukaemia progression. The essential role of SDF-1/CXCR4 in B lymphopoiesis prompted several groups to study extensively its involvement in chronic (B-CLL) and acute (B-ALL) B-cell lymphocytic leukaemia development. In B-CLL, SDF-1 and its receptor are shown to govern the homing and survival of leukaemic cells, contributing to their chemotherapy-induced apoptosis resistance. This resistance is suggested to result from the protective effect of blood-derived nurse-like cells [101, 114]. CXCR4 expression on B-CLL cells is associated with survival prognosis [115] and plays a critical role in heterotypic adherence to marrow stromal cells, suggesting that such a mechanism could account for marrow infiltration by neoplastic B-cells [116, 117]. Interestingly, by decreasing the sensitivity of leukaemic cells to SDF-1 proliferative and survival effects, the use of SDF-1/CXCR4 antagonists to alter the communication within the couple has been proposed for the treatment of acute lymphocytic leukaemia [105]. Alteration of the SDF-1/CXCR4 axis signalling pathway was recently reported to be involved in leukaemic precursor-B ALL cell migration [118]. A similar dysfunction has been recently suggested to be involved in tumour progression in multiple myeloma patients [119].

More recently, the implication of the SDF-1/CXCR4 couple in myeloid leukaemia progression has been analyzed, but results are still scarce. High levels of intracellular CXCR4 and of SDF-1 are expressed by acute myeloid leukaemia (AML) cells, including cells that do not express the membrane receptor [54, 112]. CXCR4 is essential for their homing to bone marrow in NOD/SCID mice, suggesting a dynamic translocation of the receptor to the membrane [120]. As previously demonstrated in normal circulating CD34⁺ progenitors [39], autocrine SDF-1 production by AML cells likely participated in their survival and proliferation [112].

In chronic myeloproliferative disorders such as CML, it has been proposed that the impaired chemotactic response of CD34⁺ cells to SDF-1 is not due to a lack or complete uncoupling of CXCR4, but rather to an intracellular downstream signalling defect of the receptor [121]. BCR-ABL transfection of haematopoietic cell lines reduces their chemotactic response to SDF-1, suggesting that alterations in the CXCR4 signalling pathway participate in the lower migration and adhesion of CML cells in response to SDF-1 [122, 123]. In myeloid metaplasia with myelofibrosis (MMM), which is characterized by an increased number of circulating CD34⁺ cells, the mechanisms of HSC/HPC mobilization are still unclear. Whereas Xu *et al.* (124) have reported a greater proportion of CXCR4⁺ HSC/HPC in MMM patients, we found that CXCR4 expression is higher on all circulating MMM CD34⁺ cells as compared to their normal counterparts (unpublished results). Interestingly, the lower SDF-1 serum level detected in MMM patients [125] is correlated with its reduced production by spleen (unpublished results from D. Brouty-Boyé and M.C. Le Bousse-Kerdilès) or bone marrow fibroblasts [126].

CONCLUSION AND PERSPECTIVES

Considering the multiple roles played by SDF-1 in normal haematopoiesis, including HPC trafficking, survival, cell

cycling and proliferation, it is difficult to classify SDF-1 within the chemokine or the cytokine family. Such functional ambiguity is shared by other mobilization-related molecules such as IL-8, SCF, G-CSF... By recruiting quiescent HPC, by participating in their survival/cycling and by sensitizing them to further cytokine synergistic action, SDF-1 likely contributes to haematopoiesis homeostasis and illustrates a dynamic and sequential cross-talk between chemokines and cytokines/growth factors in the regulation of haematopoiesis.

A better knowledge of the SDF-1/CXCR4 involvement in HPC trafficking has allowed the development of new strategies for enhancing engraftment efficiency and for improving stem cell mobilization protocols. CXCR4 antagonists such as AMD3100 [86, 127] and SB290157, a C3aR antagonist [128], are currently used for these purposes. Furthermore, by its synergistic effect on cell survival and proliferation, SDF-1 could be proposed to improve *in vitro* HPC expansion in view of cell-based therapy protocols [51, 94].

The SDF-1/CXCR4 couple has recently found its place within haematopoiesis regulation. Its potential clinical interest means that SDF-1 and CXCR4 are promising molecules for the future. A long and happy life to the young couple!

REFERENCES

1. Ozaki K, Leonard WJ. 2002. Cytokine and cytokine receptor pleiotropy and redundancy. *J Biol Chem.* 277: 29355.
2. Zandstra Peter W, Lauffenburger Douglas A, Eaves Connie J. 2000. A ligand-receptor signaling threshold model of stem cell differentiation control: a biologically conserved mechanism applicable to hematopoiesis. *Blood.* 96: 1215.
3. Fortunel N, Hatzfeld A, Hatzfeld JA. 2000. Transforming growth factor- β 1: pleiotropic role in the regulation of hematopoiesis. *Blood.* 96: 2022.
4. Piacibello W, Fubini L, Sanavio F, Brizzi MF, Severino A, Garetto L, Stacchini A, Pegoraro L, Aglietta M. 1995. Effects of human FLT3 ligand on myeloid leukemia cell growth: heterogeneity in response and synergy with other hematopoietic growth factors. *Blood.* 86:4105.
5. Bodine DM, Crosier PS, Clark SC. 1991. Effects of hematopoietic growth factors on the survival of primitive stem cells in liquid suspension culture. *Blood.* 78: 914.
6. Rollins BJ. 1997. Chemokines. *Blood.* 90: 909.
7. Broxmeyer HE, Kim CH. 1999. Regulation of hematopoiesis in a sea of chemokine family members with a plethora of redundant activities. *Exp Hematol.* 27: 1113.
8. Laing KJ, Secombes CJ. 2004. Chemokines. *Dev Comp Immunol.* 28: 443.
9. Murphy PM, Baggiolini M, Charo IF, Hebert CA, Horuk R, Matsushima K, Miller LH, Oppenheim JJ, Power CA. 2000. International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol Rev.* 52: 145.
10. Murdoch C, Finn A. 2000. Chemokine receptors and their role in inflammation and infectious diseases. *Blood.* 95: 3032.
11. Loetscher P, Moser B, Baggiolini M. 2000. Chemokines and their receptors in lymphocyte traffic and HIV infection. *Adv Immunol.* 74: 127.
12. Devalaraja MN, Richmond A. 1999. Multiple chemotactic factors: fine control or redundancy? *Trends Pharmacol Sci.* 20: 151.

13. Luster AD, Greenberg SM, Leder P. 1995. The IP-10 chemokine binds to a specific cell surface heparan sulfate site shared with platelet factor 4 and inhibits endothelial cell proliferation. *J Exp Med.* 182: 219.
14. St Charles R, Walz DA, Edwards BF. 1989. The three-dimensional structure of bovine platelet factor 4 at 3.0-Å resolution. *J Biol Chem.* 264: 2092.
15. Clark-Lewis I, Kim KS, Rajarathnam K, Gong JH, Dewald B, Moser B, Baggiolini M, Sykes BD. 1995. Structure-activity relationships of chemokines. *J Leukoc Biol.* 57: 703.
16. Broxmeyer HE, Sherry B, Lu L, Cooper S, Oh KO, Tekamp-Olson P, Kwon BS, Cerami A. 1990. Enhancing and suppressing effects of recombinant murine macrophage inflammatory proteins on colony formation in vitro by bone marrow myeloid progenitor cells. *Blood.* 76: 1110.
17. Lord BI, Heyworth CM, Woolford LB. 1993. Macrophage inflammatory protein: its characteristics, biological properties and role in the regulation of haemopoiesis. *Int J Hematol.* 57: 197.
18. Mantel C, Kim YJ, Cooper S, Kwon B, Broxmeyer HE. 1993. Polymerization of murine macrophage inflammatory protein 1 alpha inactivates its myelosuppressive effects in vitro: the active form is a monomer. *Proc Natl Acad Sci USA.* 90: 2232.
19. Maghazachi AA. 1999. Intracellular signalling pathways induced by chemokines in natural killer cells. *Cell Signal.* 11: 385.
20. Gazitt Y. 2004. Homing and mobilization of hematopoietic stem cells and hematopoietic cancer cells are mirror image processes, utilizing similar signaling pathways and occurring concurrently: circulating cancer cells constitute an ideal target for concurrent treatment with chemotherapy and antineoplastic-specific antibodies. *Leukemia.* 18: 1.
21. Papayannopoulou T, Priestley GV, Bonig H, Nakamoto B. 2003. The role of G-protein signaling in hematopoietic stem/progenitor cell mobilization. *Blood.* 101: 4739.
22. Moore MA. 2002. Cytokine and chemokine networks influencing stem cell proliferation, differentiation, and marrow homing. *J Cell Biochem Suppl.* 38: 29.
23. Curnock AP, Logan MK, Ward SG. 2002. Chemokine signalling: pivoting around multiple phosphoinositide 3-kinases. *Immunology.* 105: 125.
24. Loetscher P, Moser B, Baggiolini M. 2000. Chemokines and their receptors in lymphocyte traffic and HIV infection. *Adv Immunol.* 74: 127.
25. Rodriguez-Frade JM, Vila-Coro AJ, de Ana AM, Albar JP, Martinez-AC, Mellado M. 1999. The chemokine monocyte chemoattractant protein-1 induces functional responses through dimerization of its receptor CCR2. *Proc Natl Acad Sci USA.* 96: 3628.
26. Vila-Coro AJ, Rodriguez-Frade JM, Martin De Ana A, Moreno-Ortiz MC, Martinez-A C, Mellado M. 1999. The chemokine SDF-1alpha triggers CXCR4 receptor dimerization and activates the JAK/STAT pathway. *FASEB J.* 13: 1699.
27. Clark RB, Knoll BJ, Barber R. 1999. Partial agonists and G protein-coupled receptor desensitization. *Trends Pharmacol Sci.* 20: 279.
28. Cheng ZJ, Zhao J, Sun Y, Hu W, Wu YL, Cen B, Wu GX, Pei G. 2000. β -arrestin differentially regulates the chemokine receptor CXCR4-mediated signaling and receptor internalization, and this implicates multiple interaction sites between β -arrestin and CXCR4. *J Biol Chem.* 275: 2479.
29. Bowman EP, Campbell JJ, Druey KM, Scheschonka A, Kehrl JH, Butcher EC. 1998. Regulation of chemotactic and proadhesive responses to chemoattractant receptors by RGS (regulator of G-protein signaling) family members. *J Biol Chem.* 273: 28040.
30. Shirozu M, Nakano T, Inazawa J, Tashiro K, Tada H, Shinohara T, Honjo T. 1995. Structure and chromosomal localization of the human stromal cell-derived factor 1 (SDF1) gene. *Genomics.* 28: 495.
31. Winkler C, Modi W, Smith MW, Nelson GW, Wu X, Carrington M, Dean M, Honjo T, Tashiro K, Yabe D, Buchbinder S, Vittinghoff E, Goedert JJ, O'Brien TR, Jacobson LP, Detels R, Donfield S, Willoughby A, Gomperts E, Vlahov D, Phair J, O'Brien SJ. 1998. Genetic restriction of AIDS pathogenesis by an SDF-1 chemokine gene variant. *Science.* 279: 389.
32. Soriano A, Martinez C, Garcia F, Plana M, Palou E, Lejeune M, Arostegui JJ, De Lazzari E, Rodriguez C, Barrasa A, Lorenzo JJ, Alcami J, del Romero J, Miro JM, Gatell JM, Gallart T. 2002. Plasma stromal cell-derived factor (SDF)-1 levels, SDF1-3'A genotype, and expression of CXCR4 on T lymphocytes: their impact on resistance to human immunodeficiency virus type 1 infection and its progression. *J Infect Dis.* 186: 922.
33. Benboubker L, Watier H, Carion A, Georget MT, Desbois I, Colombat P, Bardos P, Binet C, Domenech J. 2001. Association between the SDF1-3'A allele and high levels of CD34(+) progenitor cells mobilized into peripheral blood in humans. *Br J Haematol.* 113: 247.
34. Ponomaryov T, Peled A, Petit I, Taichman RS, Habler L, Sandbank J, Arenzana-Seisdedos F, Magerus A, Caruz A, Fujii N, Nagler A, Lahav M, Szyper-Kravitz M, Zipori D, Lapidot T. 2000. Induction of the chemokine stromal-derived factor-1 following DNA damage improves human stem cell function. *J Clin Invest.* 106: 1331.
35. Bleul CC, Farzan M, Choe H, Parolin C, Clark-Lewis I, Sodroski J, Springer TA. 1996. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature.* 382: 829.
36. Dealwis C, Fernandez EJ, Thompson DA, Simon RJ, Siani MA, Lolis E. 1998. Crystal structure of chemically synthesized [N33A] stromal cell-derived factor 1alpha, a potent ligand for the HIV-1 "fusin" coreceptor. *Proc Natl Acad Sci USA.* 95: 6941.
37. Agace WW, Amara A, Roberts AI, Pablos JL, Thelen S, Ugucioni M, Li XY, Marsal J, Arenzana-Seisdedos F, Delaunay T, Ebert EC, Moser B, Parker CM. 2000. Constitutive expression of stromal derived factor-1 by mucosal epithelia and its role in HIV transmission and propagation. *Curr Biol.* 10: 325.
38. Aiuti A, Turchetto L, Cota M, Cipponi A, Brambilla A, Arcelloni C, Paroni R, Vicenzi E, Bordignon C, Poli G. 1999. Human CD34(+) cells express CXCR4 and its ligand stromal cell-derived factor-1. Implications for infection by T-cell tropic human immunodeficiency virus. *Blood.* 94: 62.
39. Lataillade JJ, Clay D, Bourin P, Herodin F, Dupuy C, Jasmin C, Le Bousse-Kerdilès MC. 2002. Stromal cell-derived factor 1 regulates primitive hematopoiesis by suppressing apoptosis and by promoting G(0)/G(1) transition in CD34(+) cells: evidence for an autocrine/paracrine mechanism. *Blood.* 99: 1117.
40. Shirozu M, Nakano T, Inazawa J, Tashiro K, Tada H, Shinohara T, Honjo T. 1995. Structure and chromosomal localization of the human stromal cell-derived factor 1 (SDF1) gene. *Genomics.* 28: 495.
41. Jiang W, Zhou P, Kahn SM, Tomita N, Johnson MD, Weinstein IB. 1994. Molecular cloning of TPARI, a gene whose expression is repressed by the tumor promoter 12-O-tetradecanoylphorbol 13-acetate (TPA). *Exp Cell Res.* 215: 284.
42. Oberlin E, Amara A, Bachelier F, Bessia C, Virelizier JL, Arenzana-Seisdedos F, Schwartz O, Heard JM, Clark-Lewis I, Legler DF, Loetscher M, Baggiolini M, Moser B. 1996. The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. *Nature.* 382: 833.
43. Deichmann M, Kronenwett R, Haas R. 1997. Expression of the human immunodeficiency virus type-1 coreceptors CXCR-4 (fusin, LESTR) and CKR-5 in CD34+ hematopoietic progenitor cells. *Blood.* 89: 3522.
44. Feng Y, Broder CC, Kennedy PE, Berger EA. 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science.* 272: 872.
45. Sozzani S, Luini W, Borsatti A, Polentarutti N, Zhou D, Piemonti L, D'Amico G, Power CA, Wells TN, Gobbi M, Allavena P,

- Mantovani A. 1997. Receptor expression and responsiveness of human dendritic cells to a defined set of CC and CXC chemokines. *J. Immunol.* 159: 1993.
46. Gupta SK, Lysko PG, Pillarisetti K, Ohlstein E, Stadel JM. 1998. Chemokine receptors in human endothelial cells. Functional expression of CXCR4 and its transcriptional regulation by inflammatory cytokines. *J Biol Chem.* 273: 4282.
47. Hesselgesser J, Halks-Miller M, DelVecchio V, Peiper SC, Hoxie J, Kolson DL, Taub D, Horuk R. 1997. CD4-independent association between HIV-1 gp120 and CXCR4: functional chemokine receptors are expressed in human neurons. *Curr Biol.* 7: 112.
48. Loetscher M, Geiser T, O'Reilly T, Zwahlen R, Baggiolini M, Moser B. 1994. Cloning of a human seven-transmembrane domain receptor, LESTR, that is highly expressed in leukocytes. *J Biol Chem.* 269: 232.
49. Hamada T, Mohle R, Hesselgesser J, Hoxie J, Nachman RL, Moore MA, Rafii S. 1998. Transendothelial migration of megakaryocytes in response to stromal cell-derived factor 1 (SDF-1) enhances platelet formation. *J Exp Med.* 188: 539.
50. Wang JF, Liu ZY, Groopman JE. 1998. The alpha-chemokine receptor CXCR4 is expressed on the megakaryocytic lineage from progenitor to platelets and modulates migration and adhesion. *Blood.* 92: 756.
51. Lataillade JJ, Clay D, Dupuy C, Rigal S, Jasmin C, Bourin P, Le Bousse-Kerdilès MC. 2000. Chemokine SDF-1 enhances circulating CD34(+) cell proliferation in synergy with cytokines: possible role in progenitor survival. *Blood.* 95: 756.
52. Bleul CC, Wu L, Hoxie JA, Springer TA, Mackay CR. 1997. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. *Proc Natl Acad Sci USA.* 94: 1925.
53. Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T, Nagler A, Ben-Hur H, Many A, Shultz L, Lider O, Alon R, Zipori D, Lapidot T. 1999. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science.* 283: 845.
54. Mohle R, Bautz F, Rafii S, Moore MA, Brugger W, Kanz L. 1998. The chemokine receptor CXCR-4 is expressed on CD34+ hematopoietic progenitors and leukemic cells and mediates transendothelial migration induced by stromal cell-derived factor-1. *Blood.* 91: 4523.
55. Avigdor A, Goichberg P, Shvitiel S, Dar A, Peled A, Samira S, Kollet O, Hershkovitz R, Alon R, Hardan I, Ben-Hur H, Naor D, Nagler A, Lapidot T. 2004. CD44 and hyaluronic acid cooperate with SDF-1 in the trafficking of human CD34+ stem/progenitor cells to bone marrow. *Blood.* 103: 2981.
56. Amara A, Gall SL, Schwartz O, Salamero J, Montes M, Loetscher P, Baggiolini M, Virelizier JL, Arenzana-Seisdedos F. 1997. HIV coreceptor downregulation as antiviral principle: SDF-1alpha-dependent internalization of the chemokine receptor CXCR4 contributes to inhibition of HIV replication. *J Exp Med.* 186: 139.
57. Shen H, Cheng T, Olszak I, Garcia-Zepeda E, Lu Z, Herrmann S, Fallon R, Luster AD, Scadden DT. 2001. CXCR-4 desensitization is associated with tissue localization of hemopoietic progenitor cells. *J Immunol.* 166: 5027.
58. Kawabata K, Ujikawa M, Egawa T, Kawamoto H, Tachibana K, Iizasa H, Katsura Y, Kishimoto T, Nagasawa T. 1999. A cell-autonomous requirement for CXCR4 in long-term lymphoid and myeloid reconstitution. *Proc Natl Acad Sci USA.* 96: 5663.
59. Nagasawa T, Nakajima T, Tachibana K, Iizasa H, Bleul CC, Yoshie O, Matsushima K, Yoshida N, Springer TA, Kishimoto T. 1996. Molecular cloning and characterization of a murine pre-B-cell growth-stimulating factor/stromal cell-derived factor 1 receptor, a murine homolog of the human immunodeficiency virus 1 entry coreceptor fusin. *Proc Natl Acad Sci USA.* 93: 14726.
60. Nagasawa T, Tachibana K, Kishimoto T. 1998. A novel CXC chemokine PBSF/SDF-1 and its receptor CXCR4: their functions in development, hematopoiesis and HIV infection. *Semin Immunol.* 10: 179.
61. Aiuti A, Webb IJ, Bleul C, Springer T, Gutierrez-Ramos JC. 1997. The chemokine SDF-1 is a chemoattractant for human CD34+ haematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. *J Exp Med.* 185: 111.
62. Imai K, Kobayashi M, Wang J, Ohno Y, Hamada J, Cho Y, Imamura M, Musashi M, Kondo T, Hosokawa M, Asaka M. 1999. Selective transendothelial migration of haematopoietic progenitor cells: a role in homing of progenitor cells. *Blood.* 93: 149.
63. Netelenbos T, Drager AM, van het Hof B, Kessler FL, Delouis C, Huijgens PC, van den Born J, van Dijk W. 2001. Differences in suflation patterns of heparan sulfate derived from human bone marrow and umbilical vein endothelial cells. *Exp Hematol.* 29: 884.
64. Middleton J, Patterson AM, Gardner L, Schmutz C, Ashton BA. 2002. Leukocyte extravasation: chemokine transport and presentation by the endothelium. *Blood.* 100: 3853.
65. Netelenbos T, van den Born J, Kessler FL, Zweegman S, Merle PA, van Oostveen JW, Zwaginga JJ, Huijgens PC, Drager AM. 2003. Proteoglycans on bone marrow endothelial cells bind and present SDF-1 towards haematopoietic progenitor cells. *Leukemia.* 17: 175.
66. Carion A, Benboubker L, Hérault O, Roingeard F, Degenne M, Senecal D, Desbois I, Colombat P, Charbord P, Binet C, Domenech J. 2003. Stromal-derived factor 1 and matrix metalloproteinase 9 levels in bone marrow and peripheral blood of patients mobilized by granulocyte colony-stimulating factor and chemotherapy. Relationship with mobilizing capacity of haematopoietic progenitor cells. *Br J Haematol.* 122: 918.
67. Voermans C, Gerritsen WR, von dem Borne AE, van der Schoot CE. 1999. 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. *Exp Hematol.* 27: 1806.
68. Peled A, Grabovsky V, Habler L, Sandbank J, Arenzana-Seisdedos F, Petit I, Ben-Hur H, Lapidot T, Alon R. 1999. The chemokine SDF-1 stimulates integrin-mediated arrest of CD34(+) cells on vascular endothelium under shear flow. *J Clin Invest.* 104: 1199.
69. Voermans C, Anthony EC, Mul E, van der Schoot E, Hordijk P. 2001. SDF-1-induced actin polymerization and migration in human haematopoietic progenitor cells. *Exp Hematol.* 29: 1456.
70. Peled A, Kollet O, Ponomaryov T, Petit I, Franitza S, Grabovsky V, Slav MM, Nagler A, Lider O, Alon R, Zipori D, Lapidot T. 2000. The chemokine SDF-1 activates the integrins LFA-1, VLA-4, and VLA-5 on immature human CD34(+) cells: role in transendothelial/stromal migration and engraftment of NOD/SCID mice. *Blood.* 95: 3289.
71. Netelenbos T, Zuijderduijn S, Van Den Born J, Kessler FL, Zweegman S, Huijgens PC, Drager AM. 2002. Proteoglycans guide SDF-1-induced migration of haematopoietic progenitor cells. *J Leukoc Biol.* 72: 353.
72. Miyake K, Weissman IL, Greenberger JS, Kincade PW. 1991. Evidence for a role of the integrin VLA-4 in lympho-hemopoiesis. *J Exp Med.* 173: 599.
73. Oostendorp RA, Reisbach G, Spitzer E, Thalmeier K, Dienemann H, Mergenthaler HG, Dormer P. 1995. VLA-4 and VCAM-1 are the principal adhesion molecules involved in the interaction between blast colony-forming cells and bone marrow stromal cells. *Br J Haematol.* 91: 275.
74. Papayannopoulou T, Nakamoto B. 1993. Peripheralization of hemopoietic progenitors in primates treated with anti-VLA4 integrin. *Proc Natl Acad Sci USA.* 90: 9374.
75. Teixeira J, Hemler ME, Greenberger JS, Anklesaria P. 1992. Role of $\beta 1$ and $\beta 2$ integrins in the adhesion of human CD34hi stem cells to bone marrow stroma. *J Clin Invest.* 90: 358.
76. Hattori K, Heissig B, Tashiro K, Honjo T, Tateno M, Shieh JH, Hackett NR, Quidoriano MS, Crystal RG, Rafii S, Moore MA.

2001. Plasma elevation of stromal cell-derived factor-1 induces mobilization of mature and immature haematopoietic progenitor and stem cells. *Blood*. 97: 3354.
77. Sweeney EA, Lortat-Jacob H, Priestley GV, Nakamoto B, Papayannopoulou T. 2002. Sulfated polysaccharides increase plasma levels of SDF-1 in monkeys and mice: involvement in mobilization of stem/progenitor cells. *Blood*. 99: 44.
 78. Gazitt Y, Liu Q. 2001. Plasma levels of SDF-1 and expression of SDF-1 receptor on CD34 + cells in mobilized peripheral blood of non-Hodgkin's lymphoma patients. *Stem Cells*. 19: 37.
 79. Petit I, Szyper-Kravitz M, Nagler A, Lahav M, Peled A, Habler L, Ponomaryov T, Taichman RS, Arenzana-Seisdedos F, Fujii N, Sandbank J, Zipori D, Lapidot T. 2002. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat Immunol*. 3: 687.
 80. Heissig B, Hattori K, Dias S, Friedrich M, Ferris B, Hackett NR, Crystal RG, Besmer P, Lyden D, Moore MA, Werb Z, Rafii S. 2002. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. *Cell*. 109: 625.
 81. McQuibban GA, Butler GS, Gong JH, Bendall L, Power C, Clark-Lewis I, Overall CM. 2001. Matrix metalloproteinase activity inactivates the CXC chemokine stromal cell-derived factor-1. *J Biol Chem*. 276: 43503.
 82. Valenzuela-Fernandez A, Planchenault T, Baleux F, Staropoli I, Le-Barillec K, Leduc D, Delaunay T, Lazarini F, Virelizier JL, Chignard M, Pidarv D, Arenzana-Seisdedos F. 2002. Leukocyte elastase negatively regulates Stromal cell-derived factor-1 (SDF-1)/CXCR4 binding and functions by amino-terminal processing of SDF-1 and CXCR4. *J Biol Chem*. 277: 15677.
 83. Levesque JP, Hendy J, Takamatsu Y, Simmons PJ, Bendall LJ. 2003. Disruption of the CXCR4/CXCL12 chemotactic interaction during haematopoietic stem cell mobilization induced by G-CSF or cyclophosphamide. *J Clin Invest*. 111: 187.
 84. Levesque JP, Takamatsu Y, Nilsson SK, Haylock DN, Simmons PJ. 2001. Vascular cell adhesion molecule-1 (CD106) is cleaved by neutrophil proteases in the bone marrow following haematopoietic progenitor cell mobilization by granulocyte colony-stimulating factor. *Blood*. 98: 1289.
 85. Voermans C, Kooi ML, Rodenhuis S, van der Lelie H, van der Schoot CE, Gerritsen WR. 2001. In vitro migratory capacity of CD34 + cells is related to haematopoietic recovery after autologous stem cell transplantation. *Blood*. 97: 799.
 86. Liles WC, Broxmeyer HE, Rodger E, Wood B, Hubel K, Cooper S, Hangoc G, Bridger GJ, Henson GW, Calandra G, Dale DC. 2003. Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist. *Blood*. 102: 2728.
 87. Hermouet S, Corre I, Lippert E. 2000. Interleukin-8 and other agonists of Gi2 proteins: autocrine paracrine growth factors for human hematopoietic progenitors acting in synergy with colony stimulating factors. *Leuk Lymphoma*. 38: 39.
 88. Lecomte-Raclet L, Alemany M, Sequira-Le Grand A, Amiral J, Quentin G, Vissac AM, Caen JP, Han ZC. 1998. New insights into the negative regulation of hematopoiesis by chemokine platelet factor 4 and related peptides. *Blood*. 91: 2772.
 89. Dudek AZ, Nesmelova I, Mayo K, Verfaillie CM, Pitchford S, Slungaard A. 2003. Platelet factor 4 promotes adhesion of hematopoietic progenitor cells and binds IL-8: novel mechanisms for modulation of hematopoiesis. *Blood*. 101: 4687.
 90. Broxmeyer HE, Cooper S, Cacalano G, Hague NL, Bailish E, Moore MW. 1996. Involvement of Interleukin (IL) 8 receptor in negative regulation of myeloid progenitor cells in vivo: evidence from mice lacking the murine IL-8 receptor homologue. *J Exp Med*. 184: 1825.
 91. Broxmeyer HE, Kim CH, Cooper SH, Hangoc G, Hromas R, Pelus LM. 1999. Effects of CC, CXC, C, and CX3C chemokines on proliferation of myeloid progenitor cells, and insights into SDF-1-induced chemotaxis of progenitors. *Ann N Y Acad Sci*. 872: 142.
 92. Broxmeyer HE, Cooper S, Hague N, Benninger L, Sarris A, Cornetta K, Vadhan-Raj S, Hendrie P, Mantel C. 1995. Human chemokines: enhancement of specific activity and effects in vitro on normal and leukemic progenitors and a factor-dependent cell line and in vivo in mice. *Ann Hematol*. 71: 235.
 93. Durig J, Testa NG, Heyworth CM. 1999. Distinct biological effects of macrophage inflammatory protein-1alpha and stroma-derived factor-1alpha on CD34+ hemopoietic cells. *Stem Cells*. 17: 62.
 94. Rosu-Myles M, Bhatia M. 2003. SDF-1 enhances the expansion and maintenance of highly purified human hematopoietic progenitors. *Hematol J*. 4: 137.
 95. Broxmeyer HE, Cooper S, Kohli L, Hangoc G, Lee Y, Mantel C, Clapp DW, Kim CH. 2003. Transgenic expression of stromal cell-derived factor-1/CXC chemokine ligand 12 enhances myeloid progenitor cell survival/antiapoptosis in vitro in response to growth factor withdrawal and enhances myelopoiesis in vivo. *J Immunol*. 170: 421.
 96. Onai N, Zhang Y, Yoneyama H, Kitamura T, Ishikawa S, Matsushima K. 2000. Impairment of lymphopoiesis and myelopoiesis in mice reconstituted with bone marrow-hematopoietic progenitor cells expressing SDF-1-intrakinase. *Blood*. 96: 2074.
 97. Herodin F, Bourin P, Mayol JF, Lataillade JJ, Drouet M. 2003. Short-term injection of antiapoptotic cytokine combinations soon after lethal gamma-irradiation promotes survival. *Blood*. 101: 2609.
 98. Broxmeyer HE, Orazi A, Hague NL, Sledge GW Jr, Rasmussen H, Gordon MS. 1998. Myeloid progenitor cell proliferation and mobilization effects of BB10010, a genetically engineered variant of human macrophage inflammatory protein-1alpha, in a phase I clinical trial in patients with relapsed/refractory breast cancer. *Blood Cells Mol Dis*. 24: 14.
 99. Nagasawa T, Kikutani H, Kishimoto T. 1994. Molecular cloning and structure of a pre-B-cell growth-stimulating factor. *Proc Natl Acad Sci USA*. 91: 2305.
 100. Nishii K, Katayama N, Miwa H, Shikami M, Masuya M, Shiku H, Kita K. 1999. Survival of human leukaemic B-cell precursors is supported by stromal cells and cytokines: association with the expression of bcl-2 protein. *Br J Haematol*. 105: 701.
 101. Burger JA, Tsukada N, Burger M, Zvaifler NJ, Dell'Aquila M, Kipps TJ. 2000. Blood-derived nurse-like cells protect chronic lymphocytic leukemia B cells from spontaneous apoptosis through stromal cell-derived factor-1. *Blood*. 96: 2655.
 102. Broxmeyer HE, Kohli L, Kim CH, Lee Y, Mantel C, Cooper S, Hangoc G, Shaheen M, Li X, Clapp DW. 2003. Stromal cell-derived factor-1/CXCL12 directly enhances survival/antiapoptosis of myeloid progenitor cells through CXCR4 and G(alpha)i proteins and enhances engraftment of competitive, repopulating stem cells. *J Leukoc Biol*. 73: 630.
 103. Lee Y, Gotoh A, Kwon HJ, You M, Kohli L, Mantel C, Cooper S, Hangoc G, Miyazawa K, Ohyashiki K, Broxmeyer HE. 2002. Enhancement of intracellular signaling associated with hematopoietic progenitor cell survival in response to SDF-1/CXCL12 in synergy with other cytokines. *Blood*. 99: 4307.
 104. Cashman J, Clark-Lewis I, Eaves A, Eaves C. 2002. Stromal-derived factor 1 inhibits the cycling of very primitive human hematopoietic cells in vitro and in NOD/SCID mice. *Blood*. 99: 792.
 105. Juarez J, Bendall L. 2004. SDF-1 and CXCR4 in normal and malignant hematopoiesis. *Histol Histopathol*. 19: 299.
 106. Wright N, de Lera TL, Garcia-Morujia C, Lillo R, Garcia-Sanchez F, Caruz A, Teixeira J. 2003. Transforming growth factor- β 1 down-regulates expression of chemokine stromal cell-derived factor-1: functional consequences in cell migration and adhesion. *Blood*. 102: 1978.
 107. Majka M, Ratajczak J, Villaire G, Kubiczek K, Marquez LA, Janowska-Wieczorek A, Ratajczak MZ. 2002. Thrombopoietin, but not cytokines binding to gp130 protein-coupled receptors, activates MAPK42/44, AKT, and STAT proteins in normal human CD34+ cells, megakaryocytes, and platelets. *Exp Hematol*. 30: 751.

108. Minamiguchi H, Kimura T, Urata Y, Miyazaki H, Bamba T, Abe T, Sonoda Y. 2001. Simultaneous signalling through c-mpl, c-kit and CXCR4 enhances the proliferation and differentiation of human megakaryocyte progenitors: possible roles of the PI3-K, PKC and MAPK pathways. *Br J Haematol.* 115: 175.
109. Ganju RK, Brubaker SA, Meyer J, Dutt P, Yang Y, Qin S, Newman W, Groopman JE. 1998. The alpha-chemokine, stromal cell-derived factor-1alpha, binds to the transmembrane G-protein-coupled CXCR-4 receptor and activates multiple signal transduction pathways. *J Biol Chem.* 273: 23169.
110. Wang J-F, Park I-W, Groopman JE. 2000. Stromal cell-derived factor-1 alpha stimulates tyrosine phosphorylation of multiple focal adhesion proteins and induces migration of hematopoietic progenitor cells: roles of phosphoinositide-3 kinase and protein kinase C. *Blood.* 95: 2505.
111. Kahn J, Byk T, Jansson-Sjostrand L, Petit I, Shvitiel S, Nagler A, Hardan I, Deutsch V, Gazit Z, Gazit D, Karlsson S, Lapidot T. 2004. Overexpression of CXCR4 on human CD34(+) progenitors increases their proliferation, migration, and NOD/SCID repopulation. *Blood.* 103: 2942.
112. Lapidot T, 2004.
<http://www.weizmann.ac.il/imm/LapidotPage.html>.
113. Yang FC, Atkinson SJ, Gu Y, Borneo JB, Roberts AW, Zheng Y, Pennington J, Williams DA. 2001. Rac and Cdc42 GTPases control haematopoietic stem cell shape, adhesion, migration, and mobilization. *Proc Natl Acad Sci USA.* 98: 5614.
114. Burger JA, Kipps TJ. 2002. Chemokine receptors and stromal cells in the homing and homeostasis of chronic lymphocytic leukemia B cells. *Leuk Lymphoma.* 43: 461.
115. Ishibe N, Albitar M, Jilani IB, Goldin LR, Marti GE, Caporaso NE. 2002. CXCR4 expression is associated with survival in familial chronic lymphocytic leukemia, but CD38 expression is not. *Blood.* 100: 1100.
116. Burger JA, Burger M, Kipps TJ. 1999. Chronic lymphocytic leukemia B cells express functional CXCR4 chemokine receptors that mediate spontaneous migration beneath bone marrow stromal cells. *Blood.* 94: 3658.
117. Shen W, Bendall LJ, Gottlieb DJ, Bradstock KF. 2001. The chemokine receptor CXCR4 enhances integrin-mediated in vitro adhesion and facilitates engraftment of leukemic precursor-B cells in the bone marrow. *Exp Hematol.* 29: 1439.
118. Spiegel A, Kollet O, Peled A, Abel L, Nagler A, Bielgorai B, Rechavi G, Vormoor J, Lapidot T. 2004. Unique SDF-1-induced activation of human precursor-B ALL cells as a result of altered CXCR4 expression and signaling. *Blood.* 103: 2900.
119. Akay C, Gazit Y. 2003. Mobilization of myeloma cells: reversible desensitization of the SDF-1/CXCR4 signaling and down regulation of VLA4. *Blood.* 102: 366b.
120. Kollet O, Petit I, Kahn J, Samira S, Dar A, Peled A, Deutsch V, Gunetti M, Piacibello W, Nagler A, Lapidot T. 2002. Human CD34(+)CXCR4(-) sorted cells harbor intracellular CXCR4, which can be functionally expressed and provide NOD/SCID repopulation. *Blood.* 100: 2778.
121. Durig J, Rosenthal C, Elmaagacli A, Heyworth C, Halfmeyer K, Kasper C, Novotny J, Duhrsen U. 2000. Biological effects of stroma-derived factor-1 alpha on normal and CML CD34 + haemopoietic cells. *Leukemia.* 14: 1652.
122. Salgia R, Quackenbush E, Lin J, Souchkova N, Sattler M, Ewaniuk DS, Klucher KM, Daley GQ, Kraeft SK, Sackstein R, Alyea EP, von Andrian UH, Chen LB, Gutierrez-Ramos JC, Pendergast AM, Griffin JD. 1999. The BCR/ABL oncogene alters the chemotactic response to stromal-derived factor-1alpha. *Blood.* 94: 4233.
123. Peled A, Hardan I, Trakhtenbrot L, Gur E, Magid M, Darash-Yahana M, Cohen N, Grabovsky V, Franitza S, Kollet O, Lider O, Alon R, Rechavi G, Lapidot T. 2002. Immature leukemic CD34(+)CXCR4(+) cells from CML patients have lower integrin-dependent migration and adhesion in response to the chemokine SDF-1. *Stem Cells.* 20: 259.
124. Xu M, Bruno E, Barosi G, Fruchtman SM, Prchal JT, Mahmud N, Rondelli D, Chao J, Hoffman R. 2003. Pathological mechanisms associated with CD34(+) cell mobilization in idiopathic myelofibrosis. *Blood.* 102: 147a.
125. Wang JC, Chen C, Novetsky AD. 2000. Myeloid metaplasia in agnogenic myeloid metaplasia (AMM) or idiopathic myelofibrosis (IMF) is related to inhibitory effects of the enhanced levels of growth factors on SDF-1 production in bone marrow. *Blood.* 96: 152b.
126. Wang JC, Chang TH, Hemavathy KC, Novetsky AD. 2003. Myeloid metaplasia in agnogenic myeloid metaplasia (AMM) or idiopathic myelofibrosis (IMF) is related to inhibitory effects of the enhanced levels of growth factors on SDF-1 production by bone marrow fibroblasts. *Blood.* 102: 343b.
127. Proudfoot AE, Power CA, Rommel C, Wells TN. 2003. Strategies for chemokine antagonists as therapeutics. *Semin Immunol.* 15: 57.
128. Ratajczak J, Reza R, Kucia M, Majka M, Allendorf DJ, Baran JT, Janowska-Wieczorek A, Wetsel RA, Ross GD, Ratajczak MZ. 2004. Mobilization studies in mice deficient in either C3 or C3a receptor (C3aR) reveal a novel role for complement in retention of hematopoietic stem/progenitor cells in bone marrow. *Blood.* 103: 2071.