ELSEVIER

EXPERIMENTAL HEMATOLOGY

Experimental Hematology 30 (2002) 973–981

Current understanding of stem cell mobilization: The roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells

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Mobilization of hematopoietic stem and progenitor cells from the bone marrow into the circulation by repetitive, daily stimulations with G-CSF alone, or in combination with cyclophosphamide, is increasingly used clinically; however, the mechanism is not fully understood. Moreover, following mobilization stem cells also home back to the bone marrow, suggesting that stem cell release/mobilization and homing are sequential events with physiological roles. Previously, a role for cytokines such as G-CSF and SCF, and adhesion molecules such as VLA-4 and P/E selectins, was determined for stem cell mobilization. Recent results using experimental animal models and samples from clinical mobilization protocols demonstrate major involvement of chemokines such as stromal derived factor-1 (SDF-1) and IL-8, as well as proteolytic enzymes such as elastase, cathepsin G, and various MMPs in the mobilization process. These results will be reviewed together with the central roles of SDF-1 and CXCR4 interactions in G-CSF or G-CSF in combination with cyclophosphamide-induced mobilization. Furthermore, the central role of this chemokine in stem cell homing to the bone marrow as well as retention of undifferentiated cells within this tissue will also be discussed.

Definitive, repopulating stem cells

During embryonic development, blood-forming stem cells migrate from the fetal liver via the blood circulation, home to the bone marrow (BM), and repopulate this tissue with high levels of immature and maturing blood cells of all lineages, which in turn are released into the circulation while maintaining a small pool of undifferentiated stem cells within the bone marrow [1]. Prior to their localization in the murine fetal liver, hematopoietic stem cells can be isolated from the yolk sac and aorta-gonad-mesonephros (AGM) region even before completion of the circulatory system [2]. The vast majority of these stem cells, however, are not definitive stem cells (i.e., cannot migrate in the circulation and home to and repopulate the host bone marrow in order to rescue lethally irradiated recipients), since they require an additional maturation step in the form of in vitro incubation with stromal cells and cytokines before they develop from predefinitive into definitive (i.e., functional migrating and repopulating) stem cells [3–5]. Despite their inability to home to the BM, stem cells isolated from the murine yolk sac can give rise to hematopoietic progeny if they are directly injected into the liver of newborn mice that were preconditioned as fetuses with low-dose chemotherapy [6]. These results demonstrate that stem cell motility (i.e., migration, homing, retention, and release) is crucial for hematopoietic development and bone marrow repopulation.

Circulating stem cells in peripheral blood

Immediately after birth, human umbilical cord blood (CB) cells contain relatively high levels of immature $CD34^+$ progenitor cells ($\sim$0.5%), including a minority of more primitive, undifferentiated $CD34^+/CD38^{-/low}$ cells, suggesting high levels of hematopoietic stem cell migration via the circulation during late-stage embryonic development [7]. A small pool of hematopoietic stem cells within the bone marrow continuously produces high levels of immature and maturing myeloid and lymphoid blood cells with a limited lifespan, which are released into the circulation, while maintaining undifferentiated stem cells within the bone marrow throughout life [1]. However, very low levels of noncycling quiescent progenitor cells, including primitive stem cells, are also released into the peripheral blood. The physiological role of circulating stem cells, their turnover rate, their destination, and their fate are currently unknown. It is, however, believed that the physiological role of circu-

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0301-472X/02 $–see front matter.
PII S0301-472X(02)00883-4

lating stem cells is to repopulate areas of damaged bone marrow and possibly also to continuously repopulate the thymus [1,8]. Alternatively, the possibility that circulating stem cells do not have a physiological role but may instead reflect a leakage of the system (i.e., that the mechanisms that restrict stem cells to the bone marrow are not perfect) also needs to be considered. Recently, Weissman et al. elegantly demonstrated that immature murine progenitor cells isolated from the circulation of donor mice are cleared very quickly, within a few minutes, from the circulation of intravenously transplanted congenic recipients. In addition, by pairing congenic mice and creating parabiotic mice with shared blood circulations, this study further revealed that hematopoietic stem cells rapidly and constitutively migrate through the circulation and suggest that they play a physiological role, i.e., the functional reengraftment of unconditioned bone marrow [9]. However, inflammation and trauma as a result of the surgery and the nonphysiological parabiotic joining of the mice may well result in enhanced levels of circulating stem cells, particularly given the wide range of stimuli that have previously shown to elicit stem cell release [10]. These results demonstrate that release of stem cells into the circulation and their homing back to the bone marrow are sequential events that could also be important in steady-state homeostatic processes. Thus, the concept of stem cell niches occupied by quiescent, undifferentiated cells in the bone marrow which are stationary may be an oversimplification of a more dynamic situation in which there is a higher migratory turnover of stem cells involving their release, migration in the circulation, homing, and reengraftment of the bone marrow as part of their developmental program (i.e., proliferation and differentiation). Moreover, we suggest that in addition to hematopoietic osteoclasts, which continuously destruct the bone surface by forming absorption pits and stromal osteoblasts that remodel new bone formation, there are other vital players in the bone marrow destruction/restructure cycle. These include different types of hematopoietic and stromal cells within the bone marrow that secrete a wide array of proteolytic enzymes in response to stimulation with cytokines or chemokines. These proteolytic enzymes degrade and cleave the bone marrow extracellular matrix, adhesion molecules, cytokines, and chemokines and also facilitate transendothelium cell migration [11]. These processes could also mediate stem cell release and remodeling of the bone marrow microenvironment, followed by stem cell migration via the circulation, homing back to the bone marrow, and repopulation of damaged/restructured sites in this organ as part of the continuous replenishment of the blood with new immature and maturing cells while maintaining undifferentiated stem cells. In support of our hypothesis, by applying cDNA array technology two recent studies reveal high expression of proteinase 3 and low expression of a proteinase 3 inhibitor in human bone marrow $CD34^+$ cells compared to mobilized $CD34^+$ cells isolated from healthy donors treated with granulocyte colony-stimulating factor (G-CSF). These results suggest that the ability to cleave connective tissue by proteinase 3 in the bone marrow is needed for maintaining a niche for hematopoeitic progenitors [12,13]. Interestingly, G-CSF stimulation induces both stem cell mobilization and osteoclast-mediated bone resorption and calcium release. In addition, soluble calcium causes detachment of immature progenitor cells from fibronectin [14]. Since osteoclasts are derived from hematopoietic stem cells and since immature osteoblasts lining the endosteum region secrete cytokines and chemokines and are in proximity to hematopoietic stem and progenitor cell niches, external and internal bone and bone marrow destruction followed by remodeling could be orchestrated. These processes could be sequential events that regulate new bone formation as well as stem cell self-renewal and steady-state hematopoiesis.

Stem cell mobilization

Release of hematopoietic stem cells into the periphery of patients in response to chemotherapy or cytokine stimulation was first documented in the late 1970s and early 1980s [15]. This process, termed mobilization, has been shown to be induced clinically or experimentally in animal models by a wide number of molecules: cytokines such as G-CSF, granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-7, IL-3, IL-12, stem cell factor (SCF), and flt-3 ligand; chemokines like IL-8, Mip-1α, Groβ, or SDF-1; and the chemotherapeutic agents cyclophosphamide (Cy) and paclitaxel. These molecules differ in their time frame to achieve mobilization, the type of cells mobilized, and efficiency. G-CSF is the most commonly used agent, usually administrated daily at a dose of 5–10 μg/kg for 5–10 days, sometimes in combination with cyclophosphamide [15]. However, some patients, and also a minority of healthy individuals, are poor mobilizers [10].

Human stem cell mobilization and positive selection of immature $CD34^+$ cells have become the preferred source of repopulating stem cells for clinical transplantation because of the higher cell yield of immature cells, shorter time frame needed to reach successful repopulation, and technical reduced intervention and pain advantages compared to harvested bone marrow cells [16]. However, the mechanism of stem cell mobilization is not fully understood. Mobilization of hematopoietic cells from the bone marrow into the circulation seems to be a multistep process in which adhesion interactions, which mediate anchorage of hematopoietic progenitors to the bone marrow stromal cells in response to stimulation with cytokines and chemokines, need to be disrupted [8]. Mobilized progenitor cells differ from their BM counterparts by several features. A significantly higher percentage of mobilized $CD34^+$ cells are noncycling quiescent cells [17,18]. Recent results with enriched human $CD34^+$ cells from G-CSF-mobilized peripheral blood leukocytes (PBL) compared to bone marrow $CD34^+$ cells from healthy

volunteers using cDNA array technology reveal that in addition to increased quiescence, the mobilized progenitors also express higher levels of proapoptotic genes such as caspases 3, 4, and 8 and lower levels of antiapoptotic genes such as antiproteinase 2, which may explain the sensitivity of mobilized progenitors to 5-fluorouracil (5-FU) despite their increased quiescent cell-cycle status [12,13]. Hematopoietic stem cell proliferation before emigration is believed to occur and play an important role in this process. G-CSF and Cy induces proliferation of hematopoietic cells within the bone marrow [19], since most mobilized $CD34^+$ cells in the blood are quiescent [17,18]. It has been suggested that all BM cells enter cell cycle but that cells egress towards the blood only after the M phase by a process that may be related to cell-cycle status [20] Combined stimulation with SCF and G-CSF leads to improved mobilization. In the murine system the addition of SCF induces higher levels of mobilization of long-term repopulating stem cells. In dog, baboon, and human transplantations, cells mobilized with G-CSF and SCF lead to faster recovery in transplanted recipients. These results suggest that a combination of cytokines such as SCF and G-CSF increase the levels of proliferation in the bone marrow prior to mobilization and could also increase the yield of long-term repopulating stem cells in clinical protocols [21]. Mobilized PBL cells are increasingly used clinically since the hematopoietic recovery in transplanted recipients (i.e., neutrophils and platelets) are much faster compared to patients transplanted with bone marrow cells. The more rapid engraftment is due to the increased cell dose of transplanted mobilized cells, in particular increased numbers of committed progenitor cells (including $CD34^+$ cells, GM-CSF, and Mk-CFC). More importantly, there is evidence for qualitative differences between primitive, undifferentiated human progenitors obtained from mobilized PBL compared to bone marrow cells. The proliferation and differentiation potential of undifferentiated $CD34^+Lin^-$ or $CD34^+CD38^-$ cells from mobilized PBL is inferior to that of undifferentiated bone marrow cells. In addition, higher cell doses of mobilized cells are needed to highly repopulate immune-deficient NOD/SCID mice. Most importantly, mobilized $CD34^+$ cells serially transplanted in preimmune sheep were also inferior to bone marrow cells [21]. These results should be carefully interpreted due to the surrogate xenogeneic limitations of these assays and the superior repopulation potential of mobilized cells in clinical protocols [22].

The expression of the very late antigen-4 (VLA-4) integrin and the cytokine receptor c-kit are also reduced on mobilized progenitor cells, while the levels of the metalloproteinase (MMP) enzymes MMP-2 and MMP-9, which are needed for degradation of the extracellular matrix and for transendothelial migration, are increased [23,24]. Mechanistic insights into stem cell mobilization were provided by Papayannopoulou et al., using primate and murine animal models. This group demonstrated that VLA-4 plays a critical role in stem cell mobilization since anti-VLA-4 antibodies (Ab) induce mobilization of primate and murine stem/progenitor cells, which also involves signaling via the c-kit receptor [25]. Recently, Levesque et al. showed increased release of the proteolytic enzymes elastase and cathepsin G from murine BM neutrophils during Cy and G-CSF mobilization, reaching peak levels on days 4 and 5, correlating with stem cell egress [26]. These enzymes cleaved VCAM-1 from the bone marrow stromal cells, preventing VLA-4 antigens expressed on the surface of hematopoietic progenitors to bind to their ligand VCAM-1 expressed by stromal cells. Lastly, sulphated polysaccharides such as fucoidan induce increase of SDF-1 in the blood and concomitant murine and primate stem cell mobilization. This compound can still induce stem cell mobilization in double P and E selectin ligand knockout (KO) mice, suggesting that the mechanism is not only by preventing selectin interactions [27,28]. Taken together, these results demonstrate a major role for disrupting adhesion interactions between hematopoietic stem cells and the bone marrow stromal microenvironment during mobilization. These interactions involve integrins and selectins; however, there are additional players involved in stem cell mobilization (i.e., chemokines such as SDF-1 and IL-8, other cytokines such as SCF, and proteolytic enzymes such as the MMP superfamily).

SDF-1 and CXCR4 interactions

The essential roles of SDF-1 and CXCR4 interactions in murine embryonic development and adult stem cell hematopoiesis

KO experiments revealed that murine embryos that lack the chemokine SDF-1 (also named CXCL-12) or its receptor CXCR4 have multiple defects that are lethal, including impaired bone marrow lymphoid and myeloid hematopoiesis [29–31]. However, while B lymphopoiesis in the fetal liver was also impaired due to the fact that this chemokine is a pre–B cell growth factor, T-cell development in the embryonic thymus and myeloid development in the fetal liver were not affected. These results demonstrate an essential role for this chemokine and its receptor in definitive fetal liver stem cell homing to the bone marrow and/or hematopoietic retention and repopulation of this organ during embryonic development. Unexpectedly, fetal liver cells isolated from $CXCR4^{null}$ embryos can engraft the marrow of transplanted adult wild-type mice, preconditioned with total-body irradiation. However, stem cell self-renewal, retention of maturing hematopoietic cells within the bone marrow, and migration patterns of both myeloid and lymphoid progenitor cells, including thymic T-cell development, were defective [30,32,33]. While the levels of maturing myeloid and lymphoid cells in the bone marrow remained low even when a 10-fold increase of fetal liver cells was transplanted, the levels of immature $CXCR4^{null}$ cells (i.e., $c\text{-}kit^+$Sca-

$1^{+}Lin^{-/low}$ cells with a stem cell phenotype) were normal [34]. However, transplantation of $CXCR4^{null}$ cells recovered from the bone marrow of these mice into serially transplanted secondary recipients resulted in additional reductions in the levels of engraftment compared to the levels obtained with serially transplanted wild-type cells. These results also demonstrate an essential role for SDF-1 and CXCR4 interactions in definitive stem cell repopulation and development in transplanted adult mice. Therefore, $CXCR4^{null}$ progenitor cells recovered from the murine fetal liver do not qualify as hematopoietic stem cells. In addition to their inability to home and repopulate the bone marrow of the developing embryo, these cells also fail to give rise to high levels of multilineage myeloid and lymphoid cells in the bone marrow and peripheral blood of primary and serially transplanted secondary murine recipients, which is essential for a repopulating cell in order to qualify as a pluripotent stem cell with self-renewal potential. These results are of significance since transplantation of stem cells from the bone marrow of p21 KO mice required five consecutive serial transplantations in order to fully reveal defects in stem cell self-renewal and repopulation potential [35], while $CXCR4^{null}$ serial transplantations revealed significant defects in engraftment potential already in the primary recipients, which were further intensified in secondary transplanted recipients! In addition, while $CXCR4^{null}$ cells do not migrate to a gradient of SDF-1 in vitro in transwell assays, preliminary results from our experiments demonstrate SDF-1-mediated adhesion and migration in three-dimensional ECM-like gels of $CXCR4^{null}$ cells, suggesting that these cells can partially compensate for the absence of CXCR4 [36]. Moreover, microparticles derived from activated platelets that express functional CXCR4 adhere to bone marrow endothelium-bound SDF-1, and also have the capacity to bind to murine stem cells and to immature human $CD34^{+}$ cells via their platelet-binding sialo-mucin P-selectin (CD162) and integrin Mac-1 (CD11b–CD18) antigen. These interactions increase adhesion of human and murine progenitors to endothelium-bound SDF-1 and, more importantly, also increase in vivo murine stem cell homing and repopulation [37]. These results suggest that $CXCR4^{+}$ platelets in the circulation of adult wild-type mice transplanted with $CXCR4^{null}$ fetal liver cells could also increase homing and engraftment of $CXCR4^{null}$ progenitor cells in response to SDF-1 signaling. Murine T cells that overexpress human CXCR4 and CD4 are mostly localized within the bone marrow of transgenic mice, demonstrating a central role for this chemokine in homing and retention of hematopoietic cells within the bone marrow microenvironment, most probably by chemotaxis, by activation of adhesion molecules, and also by influencing their cell-cycle status [38]. Mice reconstituted with progenitor cells infected with SDF-1 intrakine, preventing surface CXCR4 expression, suffered from impaired myeloid and lymphoid (both B- and T-cell) hematopoiesis, demonstrating a key role for this chemokine, as well, in release of maturing cells from the bone marrow into the circulation in adult hematopoiesis. Finally, transplantation of infected murine progenitor cells that overexpress SDF-1 resulted in increased myeloid and B-lymphoid hematopoiesis while T-cell development was impaired, most probably due to the antiapoptotic survival properties of this chemokine [39–41]. A recent publication by Wright et al. demonstrated that purified adult murine hematopoietic stem cells migrate to SDF-1 and not to any other known chemokine revealing a central role for SDF-1/CXCR4 interactions in adult murine hematopoiesis [42]. Taken together, these results demonstrate essential roles for SDF-1 and CXCR4 interactions both in murine embryonic development and in steady-state, adult hematopoiesis (i.e., regulation of leukocyte trafficking and stem cell self-renewal. In addition, these results suggest that SDF-1 is a key regulator of murine stem cell migration, homing, and anchorage of repopulating cells to the bone marrow, as well as release of maturing cells into the blood circulation.

The roles of IL-8, SDF-1, and CXCR4 in stem cell mobilization and homing

Fibbe et al. have demonstrated that the CXC chemokine IL-8, which is a chemoattractant and activator of neutrophils, can rapidly induce stem cell mobilization in primates and in mice within a few hours; however, the total amounts of mobilized cells are low compared to mobilization induced by daily stimulations with G-CSF [43]. IL-8-induced mobilization involves activation of MMP-9 and the β2 integrin LFA-1 (CD11a), since anti-MMP-9 or anti-LFA-1 antibodies significantly reduced mobilization [44]. LFA-1 expression is dynamic and bone marrow $CD34^{+}$ stem cells can express LFA-1 within 24 hours [45]. In addition, neutralizing anti-LFA-1 Ab significantly reduced homing of murine progenitor cells to the host BM [46]. Alternatively, anti-LFA-1 treatment could also inhibit IL-8-induced mobilization indirectly via LFA-1-dependent activation of neutrophils. The role of MMP-9 in stem cell mobilization remains unclear, since results (also by Fibbe et al.) demonstrate normal IL-8-induced mobilization in $MMP\text{-}9^{-/-}$ mice [47]. Other chemokines such as Groβ and MIP-1α were also shown to induce modest mobilization of progenitors [48,49]. Aiuti et al. found very low levels of surface CXCR4 expression on mobilized human $CD34^{+}$-enriched cells and reduced migration in vitro toward a gradient of SDF-1 in transwells [50]. We reported that while surface CXCR4 levels on freshly isolated human bone marrow or CB $CD34^{+}$-enriched cells are fairly constant (about 40–50%), as is their migration toward SDF-1 in transwells (about 25%), the expression of CXCR4, and migration toward a gradient of this chemokine, are highly variable on mobilized $CD34^{+}$-enriched cells obtained from G-CSF-treated healthy donors. These results strongly suggest involvement of SDF-1/CXCR4 interactions, as well, in mobilization of immature human $CD34^{+}$ cells [51]. Efficient mobilization of murine stem/progenitor cells was observed following elevated levels of SDF-1 in the blood of immune-deficient SCID mice due to the injec-

tion of adenovirus expressing SDF-1 [52], or of met-SDF-1 [53]. Sweeney et al. reported a rapid increase in SDF-1 levels in the circulation of mice and primates treated with sulfated polysaccharides, followed by SDF-1-dependent mobilization of murine stem/progenitor cells [54]. In this report, a transient reversal of the SDF-1 gradient from the bone marrow into the circulation was rapidly formed. They demonstrate that fucoidan and Des X compete for SDF-1 binding to the bone marrow endothelium via its nonsignaling C terminus, leading to release of this chemokine into the circulation, followed by rapid mobilization of stem and progenitor cells. Interestingly, treatment with neutralizing anti-SDF-1 antibodies significantly reduced stem and progenitor cell mobilization in fucoidan-treated mice; however, the levels of mature leukocytes were not affected. These results reveal different sensitivities to the levels of SDF-1 between immature, undifferentiated cells and mature cells, demonstrating a higher dependence on this chemokine for cell migration among progenitor cells compared to mature white blood cells [54]. Finally, a correlation between lower levels of SDF-1 and CXCR4 expression in the periphery and higher mobilization rates of immature $CD34^+$ cells in patients treated with Cy and G-CSF has been reported [55,56]. However, the mechanism, which regulates stem cell mobilization and the specific role of SDF-1/CXCR4 interactions in G-CSF-induced mobilization, are currently unclear.

Reduction of bone marrow SDF-1 levels and CXCR4 upregulation mediate G-CSF-induced mobilization of human and murine stem cells

In recent reports proteolytic enzymes, including elastase, cathepsin G, MMP-2, and MMP-9, were found to inactivate SDF-1 by cleaving a few amino acids at the signaling N-terminus of this chemokine, creating a truncated chemokine that is devoid of chemotaxis [57,58]. Preliminary results by Levesque and Simmons et al. demonstrate that in addition to cleavage of VCAM-1 in the murine BM by elastase and cathepsin G during G-CSF- and Cy-induced mobilization, these enzymes also inactivate and degrade SDF-1 in the murine bone marrow in a gradual manner, as part of the mobilization process [26,60]. In parallel, results from our experiments demonstrate that G-CSF induces gradual proteolytic degradation of human and murine bone marrow SDF-1 via elastase, accompanied by a gradual increase in CXCR4 expression on bone marrow cells [61–63]. In parallel, the levels of this chemokine and its receptor in the circulation were less affected. Unexpectedly, G-CSF increased SDF-1 mRNA expression by osteoblasts, leading to transient elevations of bone marrow SDF-1 levels and transient decreases of CXCR4 expression after each stimulation [63]. Most importantly, inhibition of neutrophil elastase in vivo in BALB/c mice stimulated with G-CSF led to reduction of SDF-1 degradation in the murine bone marrow and also prevented mobilization of both mature and immature progenitor cells, demonstrating an essential role for elastase in optimal stem cell mobilization [63]. Of interest, two defective forms of the human elastase gene due to genetic mutations are the cause of two infantile neutropenias, i.e., severe congenital neutropenia and cyclic hematopoiesis (also known as Kostman disease), suggesting a role for elastase-induced degradation of SDF-1, adhesion molecules, and ECM components, also in the constitutive homeostatic release of maturing hematopoietic cells into the human blood circulation [64]. Of interest, neutralizing anti-CXCR4 or anti-SDF-1 antibodies significantly reduced stem cell mobilization, demonstrating a role for SDF-1/CXCR4 signaling in cell egress as well [63]. It appears that although a dramatic reduction of SDF-1 levels within the bone marrow is needed for stem cell mobilization, concentrations of this chemokine are still higher in the bone marrow than in the circulation. Our data are not in disagreement with a mechanism of egress involving SDF-1 signaling, since it has been reported that hematopoietic T cells can migrate in a SDF-1-dependent manner without the formation of a gradient when the chemokine is presented by fibronectin [65] or by human endothelial cells under shear flow [66]. The ability of human SDF-1 to mediate transendothelial migration could also explain the inhibition of murine and human mobilization in our experiments by neutralizing anti-CXCR4 and anti-SDF-1 antibodies. Moreover, human T cells are also able to migrate away from a gradient of SDF-1 [67].

Lastly, results obtained by the groups of Rafii and Moore et al. reveal that SDF-1-, G-CSF-, and VEGF-mediated mobilization in mice is dependent on activation of MMP-2 and MMP-9. Treatment of mice with the MMP inhibitor MPI prevented both murine stem cell mobilization as well as homing to the bone marrow [68]. Additional results by this group show that MMP-9 causes the release of SCF from the bone marrow to the circulation, which is essential for SDF-1, and G-CSF-mediated mobilization in mice [69]. Figure 1 illustrates a model for G-CSF-induced mobilization.

SDF-1 and CXCR4 interactions mediate immature human cell homing and repopulation in transplanted NOD/SCID and $B2m^{null}$ NOD/SCID mice: relevance for clinical autologous and allogeneic $CD34^+$-enriched cell transplantation

Human and murine SDF-1 are cross-reactive and differ in one amino acid, enabling human CXCR4 to respond to murine SDF-1 signaling and vice versa. We demonstrated that SDF-1/CXCR4 interactions tightly regulate homing and repopulation of human SCID repopulating cells (SRC)/stem cells from BM, CB, and mobilized $CD34^+$-enriched cells by chemotaxis and activation of the major integrins LFA-1, VLA-4, and VLA-5 under shear flow [51,70,71]. The importance of these adhesion molecules in homing of both human and murine progenitor cells has been demonstrated by the use of neutralizing antibodies [46,71]. Short-term 24- to 48-hour prestimulation of human $CD34^+$-enriched cells

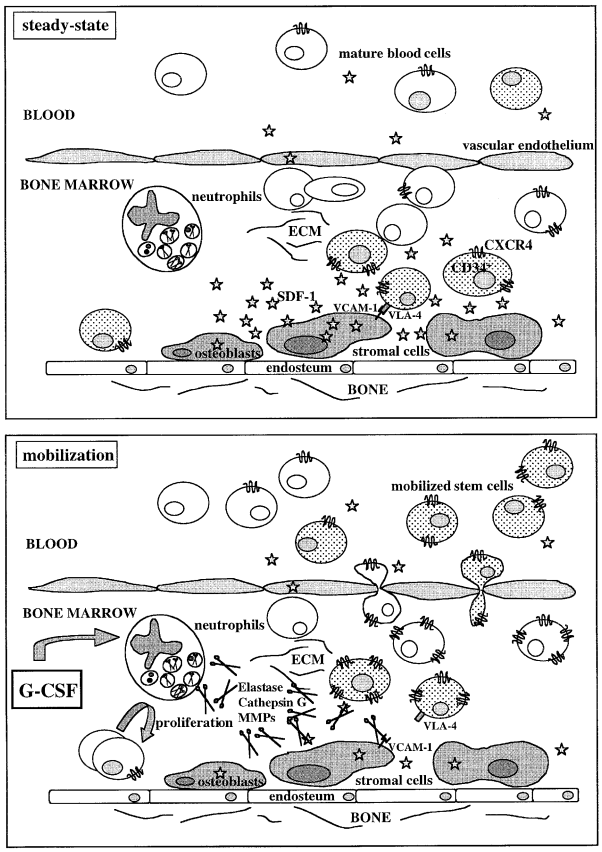

Figure 1. A model for stem cell mobilization by G-CSF. In steady state (upper panel), stem cells are localized in close proximity to stromal cells. Retention is mediated by adhesion molecules such as VCAM-1/VLA-4 and through SDF-1/CXCR4 interactions. During mobilization (lower panel), G-CSF induces both cell proliferation and release of neutrophil proteases (elastase, cathepsin G, and MMPs), which participate in cell egress by degrading retention signals (VCAM-1 and SDF-1) and by remodeling the extracellular matrix. Upregulation of CXCR4 on BM cells during G-CSF-induced mobilization suggests active participation of SDF-1/CXCR4 interactions in the migration of cells towards the blood.

with SCF and IL-6 in vitro before transplantation increases surface CXCR4 expression ans in vitro migration towards a gradient of SDF-1, as well as in vivo homing and repopulation in transplanted NOD/SCID mice and serially transplanted $B2m^{null}$ NOD/SCID mice. In summary, we have recharacterized human SRC/stem cells as $CD38^{-/low}/CXCR4^{+}$ cells with the potential to home and repopulate the bone marrow of transplanted NOD/SCID mice and serially transplanted $B2m^{null}$ NOD/SCID mice with high levels of both myeloid and lymphoid cells while maintaining undifferentiated cells, in response to stimulation with SDF-1. Interestingly, a recent report demonstrated equal levels of NOD/SCID engraftment by CB $CD34^{+}/CD38^{-}/CXCR4^{-}$ and $CXCR4^{+}$ sorted cells with neutralizing anti-CXCR4 Ab. These engraftment levels, however, are very low compared to the entire sorted CB $CD34^{+}/CD38^{-}$ cell population [72,73]. More importantly, preliminary results from our studies demonstrate that sorted CB $CD34^{+}/CXCR4^{-}$ cells harbor functional intracellular CXCR4, which can rapidly be expressed on the cell surface within 24 hours, and rescue low SDF-1-dependent engraftment in transplanted NOD/SCID mice. Both homing to the murine bone marrow and repopulation of transplanted NOD/SCID mice by sorted CB $CD34^{+}/CXCR4^{-}$ cells and the entire $CD34^{+}$-enriched cell population were blocked by co-injection with neutralizing anti-CXCR4 Ab [74]. Recent results with enriched human $CD34^{+}$ progenitor cells demonstrated that the more differentiated $CD38^{+}$ cells also secrete low levels of SDF-1 in an autocrine manner [75–77]. These results could explain the reduced surface CXCR4 expression, reduced in vitro migration toward a gradient of SDF-1, and reduced in vivo homing and repopulation potential in transplanted NOD/SCID mice by the more mature $CD34^{+}/CD38^{+}$ cells [51,71,78]. Autocrine secretion of SDF-1 could interfere with exogenous BM SDF-1 signals and reduce its chemotactic/homing potential, as compared to primitive $CD34^{+}/CD38^{-/low}$ cells within the $CD34^{+}$ population, since the more primitive cells do not secrete this chemokine [75,76]. In other reports, Eaves et al. demonstrated that high doses of this chemokine induce quiescence of undifferentiated human $CD34^{+}$ cells including LTC-IC and CRU stem cells and that short stimulation in vitro with low levels of this chemokine increase NOD/SCID repopulation potential of human cord blood $CD34^{+}$ enriched cells previously stimulated with other cytokines [77,79]. Stem and progenitor cell niches are mostly localized along the endosteum region in proximity to immature bone-forming osteoblasts and also in periartherial sites [80,81]. The highest levels of SDF-1 production in adult human bone marrow were detected along the endosteum region, secreted by immature osteoblasts. In addition, this chemokine was highly expressed by both human and murine bone marrow endothelium [82,83]. Interestingly, Nilsson et al. demonstrated that primitive, undifferentiated murine stem cells preferentially home to the endosteum region while the more differentiated progenitors do not [84]. Furthermore, total-body irradiation and chemotherapy increased SDF-1 production that correlated with increased human stem cell repopulation of transplanted immune-deficient mice [83]. Higher SDF-1 levels may induce an improved SDF-1 gradient between the periphery and the bone marrow as well as have an increased antiapoptotic effect on repopulating stem cells leading to improved homing and repopulation in clinical protocols.

The relevance of SDF-1 and CXCR4 interactions in clinical human $CD34^{+}$-enriched cell transplantation is currently being investigated. Preliminary results in autologous transplantation of positive CD34 selection after chemotherapy and G-CSF-induced mobilization show a correlation between the motility of the cells in vitro both in spontaneous and SDF-1-mediated migration and the rate of repopulation in patients [56]. These results are important since they demonstrate that homing and repopulation in transplanted patients are not random and that they correlate with the migra-

tion potential of the progenitor cells. In another report, allogeneic transplantation of $CD34^+$-enriched cells in matched recipients demonstrated significantly faster platelet recovery in patients transplanted with immature $CD34^+$ cells that express higher levels of CXCR4 as compared to those that received immature cells with lower levels of CXCR4 expression [85]. A growing number of manuscripts demonstrate decreased CXCR4 expression on mobilized human $CD34^+$ cells compared to bone marrow $CD34^+$ cells, which correlate with improved mobilization. These results suggest that manipulation of SDF-1/CXCR4 interactions could improve the outcome of stem cell mobilization for clinical transplantation [12,13,55,56]. At present, patients transplanted with allogeneic or autologous cells have low levels of primitive progenitor cells (about 10% of LTC-IC) and increased shortening of telomeres in different hematopoietic cells compared to healthy individuals. Therefore, improving the levels of stem cell homing and repopulation could also improve the success and cure rates of transplantation protocols [86]. Preliminary results with enriched $CD34^+$ cell transplantation in patients [56,85] suggest that our approach of increased CXCR4 expression, migration towards a gradient of SDF-1, homing, and repopulation following short-term ex vivo stimulation with SCF and IL-6 could also improve the outcome of clinical stem cell transplantation.

Concluding remarks

Regulation of hematopoietic stem cell release, migration, and homing to the bone marrow, as well as the mechanism of different mobilization pathways, involve a complex interplay between adhesion molecules, chemokines, cytokines, proteolytic enzymes, stromal cells, and hematopoietic cells; however, the mechanism is not fully understood. In the current review we have discussed recent findings and have focused on the major roles of the chemokine SDF-1 and its receptor CXCR4 in stem cell mobilizations, including G-CSF and G-CSF with Cy-induced mobilization, as well as in stem cell homing to the bone marrow.

We suggest that SDF-1 and CXCR4 interactions are key players in stem cell homing to the bone marrow and anchorage (i.e., activation of adhesion interactions in order to retain stem cells within this organ). We also suggest that in addition to SDF-1 degradation and inactivation within the bone marrow by proteolytic enzymes such as neutrophil elastase, which is essential for optimal stem cell mobilization, interactions between this chemokine and its receptor are also needed for stem cell release and mobilization. For example, we suggest that IL-8, which is secreted in response to SDF-1 stimulation, and MMP-2 and MMP-9, which are mostly secreted by neutrophils but are also secreted by immature human $CD34^+$ progenitor cells in response to stimulation with this chemokine, can also lead to migration away from the bone marrow across the endothelium into the circulation also in the absence of or against a gradient of SDF-1 under shear flow forces within the extravascular space of the bone marrow [66]. Lastly, we suggest that in order to maintain stem cells in the circulation low levels of surface CXCR4 are required and may be achieved by factors in the blood plasma such as proteolytic enzymes that can also cleave CXCR4 in addition to SDF-1 [59]. Furthermore, increase in the levels of CXCR4 expression on the surface of stem cells in the circulation will mediate their homing and reengraftment of the bone marrow as part of homeostatic regulation of leukocyte trafficking as well as steady-state hematopoiesis and stem cell self-renewal, which go hand in hand with bone destruction and bone remodeling. However, this hypothesis is also an oversimplification of a much more complex and dynamic situation with physiological steady-state homeostatic as well as stress-induced mobilization situations in which the mechanisms and mode of regulation are still poorly understood.

A significant number of studies in the past few years have revealed insights into regulation of hematopoietic stem cell release, migration, and homing as well as the mechanism of different mobilization pathways. However, the exact sequence of events involving many different molecules is still not clear. More importantly, in addition to results from clinical mobilization protocols using Cy and G-CSF, which demonstrate a role for SDF-1 and CXCR4 interactions in immature human $CD34^+$ cell mobilization, two recent reports also demonstrate a role for these interactions in autologous and allogeneic $CD34^+$ cell homing and repopulation. Taken together, these results suggest that stem cell homing and release or mobilization are mirror images utilizing a similar mechanism and suggest manipulation of SDF-1/CXCR4 interactions in order to improve stem cell mobilization or to target migration of transplanted cells to specific organs. Furthermore, these results strongly support the idea that increasing the migration potential of immature human $CD34^+$ cells prior to transplantation, either by short-term stimulation with SCF and IL-6 and/or by cotransplantation with accessory cells, could also increase homing and repopulation in transplanted patients, leading to improved treatment efficiencies and cure rates in clinical protocols.

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