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The Ins and Outs of Hematopoietic Stem Cells: Studies to Improve Transplantation Outcomes

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Abstract

Deciphering the mechanisms of hematopoietic stem/progenitor cell (HSPC) mobilization and homing is important for the development of strategies to enhance the efficacy of HSPC transplantation and achieve the full potential of HSPC-based cellular therapy. Investigation of these mechanisms has revealed interdependence among the various molecules, pathways and cellular components involved, and underscored the complex nature of these two processes. This review summarizes recent progress in identifying the specific factors implicated in HSPC mobilization and homing, with emphasis on our own work. Particularly, we will discuss our studies on stromal cell-derived factor-1 and its interaction with its receptor CXCR4, proteases (matrix metalloproteinases and carboxypeptidase M), complement proteins (C1q, C3a, C5a, membrane attack complex), sphingosine-1-phosphate, and pharmacologic agents such as the histone deacetylase inhibitor valproic acid and hyaluronic acid.

Keywords

Hematopoietic stem cells; Mobilization; Homing; Transplantation

Introduction

Hematopoietic stem/progenitor cell (HSPC) transplantation, a clinical procedure in which cells capable of reconstituting normal bone marrow (BM) function are administered to a patient, has been successfully performed for decades to treat various cancers and diseases of the blood and immune system [1]. Traditionally, HSPC for use in both autologous and allogeneic transplantation were collected by multiple aspirations of BM, but this harvesting procedure has now been almost completely replaced by the collection of peripheral blood (PB). This was made possible by the early discovery that HSPC can be coaxed out of the BM and into circulation in response to stimuli such as stress [2], exposure to myelosuppressive chemotherapy [3], and many other factors [4] in a process referred to as mobilization. Upon transplantation, intravenously administered HSPC seek out niches in the medullary cavity of the BM in a process referred to as homing. It was previously suggested that HSPC mobilization and homing are mirror-image processes regulated by similar molecules and utilizing similar signalling pathways [5]. It is true that HSPC mobilization is characterized by a downregulation of adhesive contacts between HSPC and stromal cells and a desensitization of chemotactic responses, and conversely, HSPC homing is accompanied by upregulation of cell adhesion molecules and activation of signals for chemotaxis. However, both mobilization and homing are more complex than previously envisioned and in fact, accumulating evidence indicates that HSPC mobilization is not the exact reverse of homing. Current perception of these processes derives from our better understanding of the dynamic interactions between HSPC and the BM microenvironment.

The BM Niche: “Home Sweet Home” of HSPC

The maintenance and survival of HSPC in the BM are regulated by signals emanating from their local microenvironment, often referred to as the stem cell niche. The concept of niches was first proposed more than 30 years ago to define fixed anatomical compartments in the BM where stem cells reside and are maintained [6]. Mounting evidence revealed later that the BM niche provides not only a simple static structural support but also topographical information and the appropriate physiological cues to control the dynamic balance of stem cell quiescence, self-renewal, differentiation and apoptosis, as well as HSPC localization and migration [7,8]. Significant breakthroughs in identifying the cellular constituents and structure of the BM niche as well as the interactions between HSPC and the niche have been achieved with the development of realtime imaging techniques in murine models and by tracking the movement of HSPC during their mobilization or homing [9–11]. It is now apparent that HSPC are not randomly distributed in the BM but are rather localized along the endosteal surface of bone in close proximity to the osteo-progenitors and osteoblasts and around blood vessels [11]. HSPC home to BM through the vascular system and have been found to localize preferentially in perivascular regions [10]. By real-time imaging it has been shown that the endosteum is well-vascularized and the vasculature is frequently located near pre-osteoblastic cells [11]. Although the evidence on the role of osteoblasts in the BM niche have been mainly derived from in vivo and in vitro murine models, osteoblasts isolated from human marrow trabecular bone were also shown to stimulate the growth of human BM progenitor cells [[12], reviewed in [13]]. Moreover, findings from other studies substantiate the notion that BM niches in humans are organized in a manner similar to mice [reviewed in [14]].

Different HSPC subsets are distributed to distinct locations according to their stage of differentiation, with the most dormant and primitive stem cells residing in niches characterized by poor blood perfusion [15]. Whereas the endosteal zone is thought to favour the maintenance of cells in an undifferentiated state, the centrally located vascular niche in the BM allows for differentiation and ultimately mobilization to the circulation [16,17]. HSPC mobilization is accompanied by a suppression of endosteal osteoblasts leading to

decreased expression of factors required for HSPC retention and self-renewal [18]. Furthermore, depletion of endosteal macrophages (osteomacs) that form a canopy over mature osteoblasts at sites of bone formation and support osteoblast function elicited robust mobilization of HSPC, suggesting that BM macrophages play a critical role in the maintenance of endosteal HSPC niches [18]. On the other hand, it was recently demonstrated that nestin-expressing perivascular mesenchymal stem cells (MSC), which outnumber HSPC in a ratio of 10:1, are either in direct contact with HSPC or in clusters around them [19]. These nestin⁺ MSC express HSPC maintenance genes and upon their deletion significant reduction in BM HSPC is observed, owing at least in part to their mobilization towards extramedullary sites [19].

Aside from the cellular components of the BM microenvironment, the niche comprises a complex and dynamic structure that transmits and receives signals through soluble factors (growth factors, cytokines and chemokines, hormones), cell adhesion molecules, extracellular matrix (ECM), neural inputs and the vascular network. Stromal cell-derived factor (SDF)-1 (also termed CXCL12) is a chemokine that strongly attracts HSPC, which express its receptor CXCR4 [20,21]. SDF-1 is constitutively expressed at high levels by osteoblasts [22] and endothelial cells [23], and represents a potent retention signal for CXCR4-expressing HSPC in their niches. HSPC are also retained in the niche by adhesion molecules directed at both the levels of cell-to-cell contact and attachment to ECM components.

With the HSPC firmly entrenched in the BM niches, it can be envisioned that their mobilization to peripheral blood requires the breaking down of chemotactic and adhesive interactions that tether them there, as well as the traversing of basement membrane and endothelial barriers. On the other hand, homing occurs when transplanted HSPC travel to and establish residence within the BM niche. In this review, we discuss these two processes independently; the first section highlights the mechanisms involved in HSPC mobilization and provides an update on developments in the quest for novel targets for this process, and the second section discusses the factors involved in HSPC homing and emphasizes putative strategies to improve the homing ability of HSPC.

Mobilization

Many reviews on the biology of HSPC mobilization [24–33] and strategies to improve HSPC collections have been published recently [34–42]. The need for elucidating the mechanisms of HSPC mobilization is underscored by the fact that in as many as 5 to 40% of autologous patients clinical regimens fail to mobilize sufficient HSPC for transplantation, necessitating additional mobilization attempts or precluding HSPC transplantation as therapy [27,34]. In a recently reported retrospective study of 2177 patients diagnosed with onco-hematological diseases, up to 60% of patients did not mobilize enough HSPC for an autologous transplant procedure [43]. Based on a review of clinical trials using chemotherapy, cytokines or combination regimens for mobilization of HSPC it was concluded that the optimal mobilization strategy is still unknown [41]. Thus, improving the strategies to boost HSPC mobilization could result in better transplantation outcomes.

How Cytokines and Growth Factors Promote the Mobilization of HSPC

Under steady-state conditions, a small percentage of HSPC (0.01–0.05%) circulate in the peripheral blood, but this number is significantly increased upon administration of hematopoietic growth factors [16,29,44]. Granulocyte colony stimulating factor (G-CSF, Filgrastim) and granulocyte macrophage (GM)-CSF (Sargramostim) are currently the only cytokines approved by the Food and Drug Administration (FDA) for HSPC mobilization, with G-CSF being the standard of care for initial mobilization therapy [40,41]. HSPC from

G-CSF-mobilized peripheral blood (mPB) engraft better than those from BM or umbilical cord blood (CB) and with fewer adverse reactions. The mechanisms by which G-CSF triggers HSPC mobilization are still incompletely understood, but evidence suggests that G-CSF acts indirectly on HSPC, as G-CSF receptor expression on HSPC is not required for their mobilization by G-CSF [45,46]. G-CSF-induced HSPC mobilization is accompanied by massive production of neutrophils, the importance of which has been demonstrated in neutropenic mice, which do not mobilize [47]. HSPC proliferation and differentiation in the BM usually precedes their egress and, in fact, neutrophilia accompanies recruitment of HSPC to the circulation [48]. In addition to stimulating neutrophil production, G-CSF induces the release of proteases that weaken the interactions that anchor HSPC in the BM niches [49,50].

One problem with the clinical use of G-CSF as a mobilizing agent is that in its recombinant unglycosylated form it is rapidly metabolized and cleared from the plasma, thereby necessitating daily injections. To overcome this the polyethylene glycol (PEG) moiety has been attached to G-CSF, masking proteolytic cleavage sites and resulting in elevated serum G-CSF levels which are sustained for up to 14 days after a single injection [51]. It has also been shown that G-CSF binds non-covalently but with high affinity and specificity to PEGylated liposomes, which extends its circulation time and results in higher G-CSF levels several hours after both subcutaneous and intravenous injections [52].

Despite the efficacy of G-CSF, poor mobilization still occurs in a considerable percentage of patients, particularly those who are older or in the advanced stages of malignancy and who have been heavily exposed to radiation and chemotherapy. Alternative regimens have been suggested based on pre-clinical findings; for example, the chemokine GRO β mobilizes more murine Sca-1⁺-c-kit⁺-Lin⁻ HSPC when used alone and synergizes with G-CSF when used in combination [53]. GRO β mobilizes HSPC within 15 min suggesting that a readily accessible large pool of HSPC likely associated with sinusoidal endothelium can be rapidly mobilized. Combining G-CSF with other growth factors has also been attempted [29]. For example, recombinant human stem cell factor (SCF, Ancestim) shows promising synergy when used in combination with G-CSF and provides an alternative option for patients who mobilize poorly or who exhibit refractory BM failure [54]. Also, in another study co-administration of IL-8 and Flt3-ligand led to a synergistic enhancement of HPC mobilization on days 3 and 5, compared to either used alone [55]. Elevated Flt3-ligand levels before mobilization negatively correlate with the absolute number of BM HSPC (CD34⁺ cells) before G-CSF treatment, and with the number of G-CSF-mobilized CD34⁺ cells on days 3–5 of treatment. Thus Flt3-ligand concentration in the blood reflects BM status before mobilization, and could be used as a predictive indicator of the number of CD34⁺ cells recovered after G-CSF-induced mobilization [56].

We found elevated levels of hepatocyte growth factor (HGF) in the plasma of patients mobilized with G-CSF. The HGF level positively correlated with the number of CD34⁺ cells and WBC in the PB, being low in patients who were poor mobilizers [57]. The HGF receptor c-Met was highly expressed on mPB CD34⁺ cells but not expressed on steady-state BM CD34⁺ cells. We suggested that during G-CSF-induced mobilization, c-Met expression increases on BM HSPC and myeloid cells which renders them more responsive to the HGF gradient in the circulating PB, thus promoting their egress [57]. Consistent with this, we found that HSPC have higher trans-Matrigel migration towards HGF, which is inhibited by a c-Met antagonist (K-252a). Moreover, G-CSF, together with HGF, increased the secretion of matrix metalloproteinase (MMP)-9 and expression of membrane type 1- MMP (MT1-MMP) on the surface of BM leukocytes [57]. This contributed to the degradation of the ECM and inactivation of SDF-1, further facilitating the egress of HSPC from the BM into circulation. Enhanced c-Met expression on the surface of murine BM cells, PMN cells and immature

HSPC treated with G-CSF reported recently [58] confirmed our findings, indicating the importance of c-Met signalling in mobilization.

How Modulation of the SDF-1/CXCR4 Axis Influences HSPC Mobilization

SDF-1 is the major chemokine involved in HSPC migration [20,59]; in fact, most of the cytokines and growth factors mediate HSPC mobilization via modulation of SDF-1 or its receptor CXCR4 [60]. It was demonstrated that neutralizing antibodies to CXCR4 or to SDF-1 significantly reduced HSPC mobilization [61]. Following G-CSF administration, SDF-1 levels transiently increase in the BM, followed by downregulation at the gene [62] and protein [63] levels. Moreover, G-CSF downregulates CXCR4 expression in human BM myeloid cells which attenuates their response to an SDF-1 gradient [64]. Thus disruption of SDF-1/CXCR4 signalling is a key step in the release of HSPC from the BM to the PB during mobilization.

Human SDF-1 is composed of three distinct structural regions: an N terminus, a central core region of three antiparallel β -sheets, and a C-terminal helix. Previous studies have demonstrated the essential role of the N terminus, particularly the first nine residues, as the major site for direct interaction with the CXCR4 receptor and subsequent signal transduction [65]. Modulation of this ligand-receptor interaction has been harnessed for application in mobilization.

AMD3100 or Plerixafor (Mozobil, Genzyme), a bicyclam molecule that selectively and reversibly antagonizes the binding of SDF-1 to its receptor CXCR4, was approved in December 2008 by the FDA for use in patients with lymphoma and multiple myeloma undergoing autologous transplantation. Alone or in combination with G-CSF AMD3100 enhances HSPC mobilization [66]. In a clinical trial 35 patients who failed previous mobilization attempts were mobilized with plerixafor plus G-CSF and underwent autologous transplantation with an average dose of 3.1×10^6 CD34⁺ cells/kg. All patients engrafted (neutrophil: 12 d; platelet: 15 d) and no severe drug-related adverse events were observed [67]. In another phase 3, multi-center, randomized, double-blind, placebo-controlled study of multiple myeloma patients mobilized with G-CSF (10 μ g/kg daily for 8 days) and plerixafor (240 μ g/kg daily starting from day 4 and continuing for 4 days), apheresis was started at day 5 and continued until 6×10^6 CD34⁺ cells/kg had been collected. The target was reached after one apheresis in 54% of patients mobilized with plerixafor plus G-CSF, whereas 56% of patients on placebo plus G-CSF required 4 aphereses to reach target [68]. Thus, plerixafor is an effective alternative for the collection of sufficient numbers of CD34⁺ cells for autologous transplantation in patients who fail conventional mobilization protocols. A continuous infusion of saturating doses of AMD3100 was also found to be an efficacious mobilization regimen [69].

Apart from targeting the CXCR4 receptor, we have directed our investigations at ways to inactivate the SDF-1 ligand. The cleavage of SDF-1 at the N terminus is effected by various enzymes such as MMPs [70], CD26/dipeptidyl-peptidase IV [71], and the serine proteases cathepsin G [72] and elastase [73], generating distinct truncated forms of SDF-1 that are functionally inactive. In addition, the major bone-resorbing proteinase, cathepsin K, also cleaves SDF-1 [74]. These enzymes are thought to be released during G-CSF-induced HSPC mobilization in the BM microenvironment, particularly by neutrophils [50,75]. Interestingly, it was demonstrated that the C-terminus of SDF-1 may also have a functional role as its cleavage by serum carboxypeptidase N (CPN) reduced the ability of SDF-1 to bind to heparin and to stimulate chemotaxis [76,77]. We investigated whether the membrane-bound carboxypeptidase M (CPM), which is ideally situated to elicit a more targeted activity at local tissue sites than soluble CPN, is also able to cleave the C-terminal of SDF-1. We found that CPM mRNA is broadly and ubiquitously expressed by CD34⁺ HSPC, hematopoietic

progenitors (CFU-Meg, BFU-E and CFU-GM), mononuclear cells (MNC), polymorphonuclear cells (PMN), endothelial cells, fibroblasts and mesenchymal stromal cells, while the CPM protein is expressed at higher levels by more mature myeloid precursors as well as monocytes and granulocytes [78]. We showed that CPM cleaves the C-terminal lysine residue of the α isoform of SDF-1 resulting in a decrease of its chemoattractant activity towards CD34⁺ HSPC and found that G-CSF strongly upregulates CPM expression at the gene and protein levels in MNC and PMN [78]. As it has been suggested that the C-terminal lysine of SDF-1 α contributes to tethering of SDF-1 to heparin on the cell surface and preserving its activity [77], we proposed that cleavage of lysine by CPM facilitates the release of SDF-1 from the cell surface, and exposes it to further degradation by other proteases [78]. Truncated SDF-1 has diminished chemotactic attraction for HSPC allowing for their egress to peripheral circulation. Degrading the CXCR4 receptor is another way by which chemoattraction can be abrogated. Leukocyte elastase is able to cleave the N-terminal of CXCR4 [73], thereby disrupting binding with its ligand SDF-1. Surprisingly, the G-CSF-induced downregulation of SDF-1 and CXCR4 expression on HSPC was still detected in mice deficient in neutrophil elastase (NE) and cathepsin G (CG) [79]. Moreover, G-CSF-induced HSPC mobilization was normal in MMP-9-deficient mice, NE and CG-deficient mice or in mice lacking dipeptidyl peptidase I, an enzyme required for the functional activation of neutrophil serine proteases. This observation may be partly explained by the existence of other proteases that have overlapping and therefore compensatory activities.

How Matrix Metalloproteinases and Other Proteases Contribute to HSPC Mobilization

Neutrophils activated after G-CSF treatment release proteolytic enzymes capable of degrading various components of the BM microenvironment that are implicated in HSPC mobilization. An enzyme abundantly secreted by neutrophils is MMP-9, whose substrates extend from the conventional ECM components (e.g., collagens, fibronectin, laminins, proteoglycans) to non-matrix molecules [80–83]. MMPs have been traditionally considered to facilitate cell migration by breaching basement membrane barriers comprised of matrix proteins. In particular, we have shown that the gelatinases MMP-2 and/or MMP-9 are expressed by normal BM MNC and mobilized CD34⁺ HSPC as well as stromal cells (fibroblasts, endothelial cells and long-term marrow culture cells) [84–86]. We also found that steady-state BM CD34⁺ cells, unlike circulating PB CD34⁺ cells, did not express MMP-2 and MMP-9 mRNA transcripts and proteins [49]. However, various cytokines and growth factors, including IL-3, IL-6, G-CSF, GM-CSF, M-CSF, SCF and TNF- α , which are endogenously produced in the BM microenvironment [87], could induce the secretion of MMP-2 and MMP-9 by BM CD34⁺ cells and upregulate their trans-migration across reconstituted basement membrane by 50 to 150%. Moreover, the trans-Matrigel migration of CD34⁺ cells was reduced by antibodies to MMP-2 and MMP-9, tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) and the synthetic protease inhibitor, *o*-phenanthroline [49]. Furthermore, we provided evidence that the chemokines IL-8 and MIP-1 α also directly stimulate the secretion of both MMP-2 and MMP-9 in BM CD34⁺ cells [88]. Our findings suggest that various exogenously administered mobilizing agents and also those growth factors, cytokines and chemokines that are endogenously secreted within the BM microenvironment may facilitate the release of CD34⁺ HSPC into PB by signalling through MMP-2 and MMP-9.

Because as mentioned above, mice deficient in MMP-9 have normal, unimpaired mobilization [79], we investigated the role in HSPC mobilization of another MMP, MT1-MMP, the best characterized among the membrane-bound MMPs. MT1-MMP degrades ECM macromolecules, cytokines, chemokines and adhesion molecules and mediates tumour cell migration. For example, MT1-MMP cleaves CD44 and SDF-1, resulting in detachment

and reduced chemoattraction of HSPC to stroma [89–91]. Previously, we reported that MT1-MMP facilitates the migration of MSC [92], CB CD34⁺ cells and ex vivo-expanded megakaryocytic progenitors [93]. More recently, we demonstrated the role of MT1-MMP in the mobilization of HSPC from BM [94]. First, we found that steady-state BM leukocytes and CD34⁺ cells have low MT1-MMP expression at both the mRNA and protein levels, in contrast to the high expression in their G-CSF-mobilized counterparts. Next, we showed that G-CSF stimulation in vitro upregulates the transcription and translation of MT1-MMP in CD34⁺ cells and BM leukocytes, activation of proMMP-2 in co-cultures of HSPC with BM stromal cells and trans-Matrigel migration which is reduced significantly by siRNA-mediated silencing of MT1-MMP [94]. Our findings suggested an additional mechanism by which an upregulation of MT1-MMP expression in HSPC could contribute to their reduced retention in the BM and their enhanced egress into the PB. Consistent with our results, G-CSF-induced mobilization of CD34⁺ cells correlated with high expression of MT1-MMP, and G-CSF enhanced the cleavage of the cell surface glycoprotein CD44 by MT1-MMP, resulting in reduced adhesion of CD34⁺ cells and their release into circulation [95]. Taken together, these studies underscore the important contribution of MMP-2, MMP-9 and MT1-MMP in the mobilization of HSPC.

In addition to MMPs, CD26, which is also expressed by HSPC, has been shown to cleave and inactivate SDF-1 and, consequently, mobilization is reduced upon CD26 inhibition or in CD26-deficient mice [96]. Drugs affecting CD26 that could be used in the clinic to augment the mobilizing effects of G-CSF have been reviewed [97]. Other proteases have also been implicated in HSPC mobilization. For example, genetic loss of the plasminogen activator inhibitor Pai-1 or of the plasmin inhibitor α 2-antiplasmin increases HPC mobilization in response to G-CSF [98]. Moreover, thrombolytic agents enhance HPC mobilization in mice and humans, indicating a possible role for plasmin in augmenting G-CSF-induced HPC mobilization. Recently, the expression, secretion and function of the cysteine protease cathepsin X by cells of the human BM were examined with interesting results. Osteoblasts and stromal cells secrete cathepsin X, whereas HSPC do not. Activated cathepsin X is able to reduce cellular adhesive interactions between CD34⁺ HSPC and osteoblasts, and it also digests SDF-1/CXCL12, thus enhancing mobilization [99].

How Innate Immunity Orchestrates HSPC Mobilization

The complement system is an essential component of innate immunity that we recently postulated to be a major player in HSPC mobilization [31,100]. G-CSF-induced mobilization is impaired in patients suffering from severe combined immunodeficiency disease (SCID), i.e., those who lack functional B and T lymphocytes [101], supporting the notion that the egress of HSPC from the BM occurs as part of the immune response. Activation of the complement system triggers a cascade of reactions generating various bioactive peptides such as C3 and C5 cleavage fragments. Our studies using C3-deficient (C3^{-/-}) and C3a receptor-deficient (C3aR^{-/-}) mice revealed that the C3a-C3aR axis protects HSPC from their uncontrolled egress from the BM and that blockade of this axis with the C3aR antagonist SB 290157 increases HSPC mobilization [102].

On the other hand, we found C5- and immunoglobulin-deficient mice respond poorly to G-CSF-induced mobilization in contrast to their wild type counterparts [103]. An explanation of this phenomenon might be that C5 cleavage fragments are potent anaphylatoxins that increase the permeability of the BM vasculature as well as activate granulocytes to release proteolytic enzymes. Thus, to better elucidate the role that C5 cleavage fragments play in mobilization of human HSPC we evaluated plasma levels of the cleavage fragment ^{desArg}C5a. We found that ^{desArg}C5a levels are significantly higher in patients who are good mobilizers and correlate with CD34⁺ cell and WBC counts in mPB [104]. C5 cleavage fragments did not chemoattract myeloid progenitors (CFU-GM) but ^{desArg}C5a did

strongly chemoattract mature nucleated cells. Consistently, the C5a receptor CD88 is not detected on CD34⁺ cells, but appears on more mature myeloid precursors, monocytes and granulocytes. Moreover, G-CSF-mobilized MNC and PMN from PB have a significantly higher percentage of cells expressing CD88 than these cells from non-mobilized PB. Furthermore, C5a stimulation of granulocytes and monocytes decreases CXCR4 expression and chemotaxis towards an SDF-1 gradient, and increases secretion of MMP-9 and expression of MT1-MMP and CPM by these cells [104]. Thus, we proposed that C5 cleavage fragments facilitate the egress of HSPC into mPB, by i) increasing the secretion of proteolytic enzymes from granulocytes thereby attenuating the function of the SDF-1/CXCR4 axis, and ii) chemoattracting granulocytes to these cleavage fragments and promoting their egress through the endothelial barrier, which “paves the way” for subsequent egress of HSPC. In addition, granulocytes activated by C5a in PB secrete some cationic peptides that increase the responsiveness of HSPC to the SDF-1 gradient in the blood [105].

Surprisingly, we observed that the plasma SDF-1 levels do not correlate with the mobilization efficiency of CD34⁺ HSPC in patients [106]. We discovered that normal and mobilized plasma chemoattracts HSPC in a manner independent of SDF-1. In our quest for identifying the major chemoattractant present in PB that is responsible for the egress of HSPC, we found that at physiologically relevant concentrations sphingosine-1-phosphate (S1P) is several magnitudes more potent than SDF-1. The S1P receptor has been previously shown to participate in the egress of HSPC from extramedullary tissues to lymph [107]. As the complement cascade is activated during mobilization and the membrane attack complex (MAC) is generated at the final step of the cascade, interaction of the MAC with erythrocytes (the major reservoir of S1P in PB) induces the release of S1P creating high levels of it in the PB. High S1P levels chemoattract HSPC and cause their egress into the circulation [31,106]. Interestingly, G-CSF has also been recently demonstrated to be a stronger chemoattractant for HSPC than SDF-1 [108].

We also have evidence that mobilization induced by the CXCR4 antagonist AMD3100, similarly to G-CSF, depends on activation of the complement cascade [109]. However, in contrast to G-CSF, AMD3100 activates the complement system directly at the C5 level, resulting in the release of C5a cleavage fragments and facilitating the final steps of HSPC egress [109]. At the same time AMD3100 stimulates the secretion of neutrophil proteases, including MMP-9, which perturb the adhesive interaction between the very late antigen (VLA-4 or integrin $\alpha_4\beta_1$)-expressing HSPC and its ligand, vascular cell adhesion molecule (VCAM)-1, expressed by stromal cells and osteoblasts.

How Adhesion Molecules and Other Factors Participate in HSPC Mobilization

Integrins are a major family of adhesion molecules that mediate cell-to-cell and cell-to-matrix interactions. The binding of HSPC through VLA-4 with BM stromal cells through VCAM-1 was found to be essential for the homeostatic distribution of HSPC in the body [110] and it was recently shown that the alpha cytoplasmic domain plays a critical role [111]. The monoclonal anti-VLA-4 antibody (Natalizumab) has been shown in the clinic to mobilize CD34⁺ HSPC [112]. Furthermore, disruption of the VCAM-1/VLA-4 axis with a small molecule inhibitor of VLA-4 (BIO5192) resulted in a 30-fold increase in the mobilization of murine HSC over basal levels [113]. An additive effect (3-fold) on HSPC mobilization was observed with AMD3100 when it was combined with BIO5192. Furthermore, the combination of G-CSF, BIO5192 and AMD3100 enhanced mobilization 17-fold compared to G-CSF alone. Blockade or genetic ablation of VLA-4 in mice provided reproducible but modest mobilization [113]. Similarly, CXCR4 inhibition with small molecule antagonists resulted in a modest mobilization efficiency; however, targeting both VLA-4 and CXCR4 resulted in additive mobilization effects [114].

CD44 is the cell-surface glycoprotein receptor for hyaluronic acid (HA) and can also interact with other ligands, such as osteopontin, collagens and MMPs [115]. HSC bind to various ECM components and blocking CD44 has been shown to result in mobilization of murine HSPC [116]. In clinical trials, a significant decrease in CD44 expression was observed during G-CSF-induced mobilization [117].

Other factors have also been implicated in HSPC mobilization, including hormones and neurotransmitters. As mentioned previously, HSPC are localized in close proximity to osteoblastic cells lining the endosteal surface of the bone, and osteoblasts are known to respond to parathyroid hormone (PTH), the endocrine regulator of calcium concentration in extracellular fluid [9,118]. Injecting mice with PTH resulted in a significant increase in the stem cell-enriched subpopulation ($\text{Lin}^- \text{Sca-1}^+ \text{c-kit}^+$) in the BM [9]. Consequently, a clinical trial has been initiated to determine whether PTH can facilitate HSPC mobilization in patients [119]. In phase I of the trial 20 patients received an escalating dose of PTH (from 20 μg to 100 μg) and 47% of patients who had failed a prior mobilization attempt were able to meet the collection criteria after 14 days of treatment.

Another cellular signal that has emerged as a regulator of HSPC mobilization derives from the sympathetic nervous system (SNS). Knockout of UDP-galactose:ceramide galactosyltransferase (Cgt), which is essential for the synthesis of galactocerebroside (the major components of the myelin sheaths that surround nerve fibers and facilitate nerve impulse conduction), resulted in impaired mobilization in a murine model [120,121]. Moreover, mice whose SNS function is chemically damaged by 6-hydroxydopamine and have reduced production of the neurotransmitter norepinephrine failed to respond to HSPC mobilization by G-CSF. The lack of mobilization with G-CSF in these mice could be reversed by administration of a β_2 adrenergic agonist drug, suggesting that adrenergic stimulation could be a method of HSC mobilization [120]. More recently, regulation of bone formation and HSC traffic was shown to depend on β -adrenergic receptors (β -AR) expressed on HSC, osteoblasts and MSC. Circadian HSC release from the BM follows rhythmic secretion of norepinephrine from nerve terminals, β_3 -AR activation and CXCL12 down-regulation, which is a contributing mechanism to HSC egress [122].

Homing

Over the last several years, there has been a number of comprehensive reviews on HSPC homing [123–131]. Homing is defined as the early event that lodges and firmly retains HSPC in the BM prior to their proliferation and expansion [128]. Clearly, the success of clinical HSPC transplantation relies on the inherent ability of transplanted HSPC to home efficiently to the BM niche and engraft. Migratory events that lead to HSPC homing are fairly rapid, taking place within 5 to 15 h and not later than 2 days post-transplantation [127,130]. Homing occurs when intravenously administered HSPC recognize and interact with the microvascular endothelial cells of the BM, adhere to the vessel wall with sufficient strength to overcome the considerable shear stress exerted by the flowing blood, and then extravasate across the vascular wall along an SDF-1 gradient generated by osteoblasts in the endosteal niche [125,126]. Early on, the homing process was graphically described as the “rolling, crawling and nesting” of HSPC into the marrow stromal space [132]. Even then, seminal studies had already identified the significant contributions of a wide variety of adhesion molecules and their receptors in mediating cell-to-cell and cell-to-matrix interactions [129,133,134]. The role of the SDF-1/CXCR4 axis in the retention of HSPC in the BM niche was then also beginning to be established [20,135–137]. It soon became evident that proteolytic enzymes capable of degrading ECM components could conceivably play a role in the migration of HSPC [49,138]. It is now recognized that homing is a complex multi-step process that involves signalling through adhesion molecules,

chemotactic molecules and their receptors, innate immunity components, proteases and other factors. In addition, cytokines such as GM-CSF, IL-3 and SCF that activate VLA-4 and VLA-5 and increase the adhesiveness of HSPC, as well as Flt3-ligand, SCF, IL-3, IL-6 and HGF that upregulate the expression of CXCR4 on HSPC, are factors that play a role in the homing of HSC [131,139]. A flexible hierarchy of cooperating homing pathways in which the dominant players are characterized by significant functional overlap and are constantly repositioned within changing cytokine milieu has been postulated [123]. We will present an update on studies pertaining to the role of adhesion molecules, the SDF-1/CXCR4 axis, proteases and complement cascade proteins in HSPC homing, with particular emphasis on our recent investigations of priming agents that could confer to HSPC increased homing potential.

How Adhesion Molecules Tether and Retain HSPC in Their Niches

A number of adhesion molecules such as P- and E-selectins, integrin/receptor pairs, immunoglobulins and other adhesion receptors mediate the anchoring of hematopoietic cells within the BM [132]. A functional redundancy of the adhesion molecules was recognized based on the failure of inhibition of a single ligand/receptor interaction to abolish seeding [140]. Novel non-integrin adhesion receptors have also been identified. For example, HSC were shown to express a glycoform of CD44 known as hematopoietic cell E-/L-selectin ligand, a very potent adhesion molecule that allows blood-borne cells to brake, contact and roll along the endothelium [141].

Data are accumulating that indicate the participation of the CD34 antigen in adhesion or perhaps the homing of lympho-hematopoietic progenitors. This has been attributed to the presence of a large number of carbohydrate moieties attached within the extracellular region that provide not only antigenicity but also contribute toward attaining the mucin-like structure of an adhesion molecule [142]. Moreover, it was reported that sialomucin endolyn (CD164), an adhesion receptor that regulates the adhesion of CD34⁺ cells to BM stroma and their recruitment into the cycle, associates with CXCR4, an interaction that follows the association of CXCR4 with the integrins VLA-4 and VLA-5 [143].

In another study, inhibition of the integrin $\alpha 4$ receptor virtually abrogated the homing of HPC to BM [144]. On the other hand, blockade of $\alpha 6$ integrin enhanced the homing of human BM-derived HSPC and led to significantly improved engraftment [145]. Aside from the established role of the $\alpha 4\beta 1$ integrin receptor VCAM-1 in the homing of hematopoietic cells to the BM, the importance of MAdCAM-1 and its ligand, $\alpha 4\beta 7$ integrin, in the homing of BM-derived hematopoietic cells to both the spleen and the BM has been recently postulated [146].

Additionally, Annexin II, expressed at high levels by osteoblasts and endothelial cells was shown to mediate adhesive interactions between HSC and osteoblasts mainly through its N-terminal [147]. Hyaluronan-mediated adhesion of HSPC to stabilin-2-transfected cells suggests that stabilin-2 also contributes to the adhesion and homing of circulating stem and progenitor cells to BM [148]. N-cadherin-mediated cell adhesion was shown to be required for the establishment of hematopoiesis in the BM niche after transplantation [149]. While the tethering role of adhesion molecules has been proven, the directional cues that guide HSPC towards the BM niche continue to be an area of active investigation.

How the SDF-1/CXCR4 Axis Directs the Migration of HSPC to Their Niches

SDF-1 is secreted in abundance by BM osteoblasts and stromal cells under physiological conditions and after myeloablative therapy given before transplantation. SDF-1 strongly chemoattracts HSPC, which express CXCR4 on their surfaces [20], and the role of the

SDF-1/CXCR4 axis in the homing of human HSPC has been demonstrated in non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice [136].

It has been suggested that for optimal signalling CXCR4 must be incorporated into membrane lipid rafts [150,151]. We found that molecules such as fibronectin, fibrinogen fragments, soluble VCAM-1, platelet-derived microparticles (PMPs), C3a anaphylatoxin and HA, which are present in the supernatants from leukapheresis products of patients mobilized with G-CSF, increase the chemotactic responsiveness of HSPC to an SDF-1 gradient [152]. By themselves these molecules do not chemoattract HSPC but they prime the chemotactic response of HSPC to a low or threshold dose of SDF-1, and this chemotaxis is dependent on the cell membrane cholesterol content and the incorporation of CXCR4 into membrane lipid rafts [152]. Based on these, we inferred that the responsiveness of HSPC to an SDF-1 gradient may predict their ability to home to BM niches and engraft. Although number of HSPC plays a large part in determining the ultimate function of the graft, HSPC quality is likewise important for engraftment results. Graft characteristics, defined as the cell types found in the graft, their gene expression profiles, and their chemotactic reactivity to an SDF-1 gradient, all influence BM homing, engraftment, immune reconstitution and other post-transplant outcomes [153,154].

In many studies a correlation has been shown between the number of HSPC infused and the rate of engraftment [155–158]. Thus it has been the practice of HSPC transplant centres to collect a sufficient number of CD34⁺ cells, preferably about 2–3×10⁶/kg, in order to attain a favourable clinical outcome [159]. Interestingly, in a study of 225 patients with amyloidosis who underwent autologous PB HSPC transplantation, no differences in the rate of neutrophil engraftment were observed when CD34⁺ cell doses >5.0×10⁶/kg were used [160]. Consistent with this finding, we reported in 35 patients transplanted with a relatively high CD34⁺ cell dose (median: 7.7×10⁶/kg, ranging from 3.9 to 41×10⁶ CD34⁺ cells/kg) no correlation between cell dose and neutrophil recovery, and only a weak correlation between CD34⁺ cell dose and platelet recovery [161]. We then evaluated whether in vitro HSPC migration potential correlated with rate of hematopoietic engraftment and found that the CD34⁺ cell dose was inversely related to the chemotactic response towards SDF-1α. We suggested that in achieving a fast hematopoietic engraftment a higher responsiveness of CD34⁺ cells to SDF-1α could compensate for a lower CD34⁺ cell dose. As CXCR4 expression levels on CD34⁺ cells isolated from mPB correlated with their in vitro chemotactic response to an SDF-1 gradient, a high CXCR4 expression would predict a greater propensity to be attracted to the BM niche. This could explain why neutrophil recovery in patients transplanted with a lower dose of CD34⁺ cells (but having a high CXCR4 expression and in vitro responsiveness to SDF-1) was achieved within the same period of time as in those patients in whom a higher number of CD34⁺ cells with a low CXCR4 expression and chemotactic response was transplanted [161].

In further support of the pivotal role of CXCR4 in HSPC homing, engraftment and retention within the BM, it was shown that overexpression of CXCR4 by lentiviral gene transfer in human CD34⁺ HSPC increased the ability of transduced cells to respond to lower, physiologic levels of SDF-1 when compared to control cells [162]. The enhanced SDF-1–induced migration, proliferation and survival resulted in significantly higher levels of in vivo repopulation of NOD/SCID mice [162].

Gene expression can be regulated by chromatin remodelling, and histone deacetylase (HDAC) is one enzyme that participates in the regulation of chromatin structure [163]. Valproic acid (VPA) is a specific and potent short-chain HDAC inhibitor that has been shown to stimulate proliferation and self-renewal of normal HSPC [164]. In a recent work we reported that chromatin remodelling by VPA increases CXCR4 expression in HSPC, and

CD34⁺ cells treated with VPA have increased chemotactic responses towards an SDF-1 gradient [165]. We suggested that VPA could be used in vivo for enhancing the homing/engraftment of HSPC because it is safe and well-tolerated by patients. More importantly, due to its effect on CXCR4 expression in both the progenitor (CD34⁺ CD38⁺) population as well as the more primitive HSPC (CD34⁺ CD38⁻) we proposed that VPA could be beneficial for both early short-term and long-term engraftment [165]. However, this needs to be further investigated and verified.

It is known that HSPC derived from mPB have superior homing and engraftment capabilities compared to those derived from BM [166,167]. We have provided evidence that the improved engraftment of mobilized HSPC could be attributed to components such as PMPs which are found in elevated levels in the supernatants of leukapheresis products to which HSPC have been exposed [168]. Microparticles were previously regarded as mere cellular debris but have recently been recognized to play a role in many cellular functions [169–172]. PMPs are released upon activation of platelets and express several functional membrane receptors including CXCR4. We hypothesized that PMPs released by activated platelets could subsequently bind to the membranes of CD34⁺ HSPC and transfer onto their surface PMP-derived antigens. First, we found that human CD34⁺ cells isolated from mPB, in contrast to those obtained from non-mobilized PB, highly express CD41 and CD61, which are characteristic of platelets, yet no intact platelets were detected on their surfaces by electron microscopy [168]. We showed that murine BM cells pre-treated with PMPs engraft lethally irradiated mice significantly faster than those not covered with PMPs. Platelets are exposed to many platelet agonists during mobilization, and we found that CD34⁺ cells isolated from leukapheresis products are more highly covered with PMPs. Hence we suggested that pre-treatment of CD34⁺ cells with PMPs may prove to be a strategy for accelerating engraftment of BM or CB HSPC [168]. Furthermore, we found that CD41 is efficiently transferred by PMPs to hematopoietic precursors, resulting in their increased adhesion to fibrinogen [173]. PMPs were also shown by us to enhance the in vitro invasive potential of breast cancer cells lines and induce metastasis in lung cancer by upregulating the expression of the proteolytic enzymes MMP-2, MMP-9 and MT1-MMP [174,175].

Other molecules present in leukapheresis products that could contribute towards priming of the chemotactic responses of HSPC towards SDF-1 are HA and thrombin. HA through interactions with its receptor CD44 and the SDF-1-CXCR4 axis promoted HSPC homing to BM and their retention in the BM niches [176]. On the other hand, thrombin, through proteolytic activation of its receptor PAR-1, elicits numerous cellular responses in platelets and endothelial cells such as induction of adhesion molecules, production of chemokines, activation of proMMP-2, cytoskeletal reorganization and cell migration [177]. Recently, we showed that the priming effects of HA and thrombin are modulated through the proteolytic enzyme MT1-MMP as discussed below [178].

How Proteases Participate in HSPC Homing

In our recently published studies, we provide new evidence that HA and thrombin prime homing-related responses of HSPC towards SDF-1 by upregulating MT1-MMP expression at both the mRNA and protein levels, which in turn leads to proMMP-2 activation [178]. Activation of proMMP-2 is important not only because active MMP-2 plays a crucial role in matrix remodeling but also because it initiates a cascade of activation of other MMPs [179]. Previously we showed that MT1-MMP mediates trans-Matrigel chemoinvasion of CB CD34⁺ cells and megakaryocytic progenitors [93]. We presented further proof of the role of MT1-MMP in CB HSPC migration by showing that specific inhibition of MT1-MMP by siRNA significantly diminished their migration across reconstituted basement membrane [178].

It has been demonstrated that intracellular crosstalk and positive feedback loops between phosphatidylinositol 3-kinase (PI3K)-AKT [180] and the Rho-family GTPases including Rac, Rho and Cdc42 regulate actin cytoskeletal dynamics and F-actin polymerization [181–184]. Rac-1 GTPase signalling pathways lead to localized signal amplification which in turn results in enhanced F-actin polymerization and chemotaxis [185,186]. We demonstrated that in CD34⁺ cells, both PI3K and Rac-1 GTPase pathways are activated by HA and thrombin [178]. Intracellular crosstalk occurs between these signalling pathways since inhibition of PI3K attenuated Rac-1 activation, and inhibition of Rac-1 GTPase diminished phosphorylation of AKT [178]. Moreover, we reported that SDF-1-CXCR4 signalling during HSPC homing occurs in the lipid rafts where CXCR4 and Rac-1 assemble together, thus allowing an optimal chemotactic response to SDF-1 [152]. Hence we suggest that HA and thrombin cooperate with the SDF-1-CXCR4 axis by activating PI3K and Rac-1, thus allowing signal amplification of a weak SDF-1 gradient. This signal amplification also recruits MT1-MMP to the cell surface where several functions are elicited, including proMMP-2 activation and pericellular proteolysis. Overall, these signals could lead to enhanced homing-related responses of HSPC and potentially speed up engraftment.

It was shown that HSPC from mPB, which engraft faster than their BM counterparts, are characterized by enhanced SDF-1-dependent chemotaxis and low CD26 expression [166]. As the major function of CD26 is to cleave the N-terminal of SDF-1, inhibition of CD26 on HSPC increases their SDF-directed chemotaxis in vitro and their in vivo homing and engraftment [187]. In fact, sitagliptin, an oral inhibitor of CD26, has been used clinically and has resulted in speedy engraftment of HSPC [97].

Other enzymes that have been reported to play putative roles include the membrane-anchored plasminogen activator urokinase receptor (MuPAR), which marks a subset of HSPC in the BM. Loss of MuPAR increased HSPC proliferation and impaired their homing, engraftment and adhesion to the BM microenvironment [188]. In addition, PIM1 serine/threonine kinase activity was also shown to be essential for proper CXCR4 surface expression and migration of cells toward a CXCL12 gradient [189]. Likewise, the role of phosphatase 2A in SDF-1-mediated migration and adhesion of CD34⁺ cells was revealed when its inhibition led to reduced chemotaxis, impairment of adhesion to fibronectin and impaired ability to repopulate NOD/SCID mice [190].

How Complement Component Proteins Prime Homing-Related Responses of HSPC

Another component of leukapheresis products that contributes to the enhanced engraftment of mobilized HSPC is complement cleavage fragments. The complement system is activated during mobilization and leukapheresis [191] and because of this, mobilized HSPC are exposed to cleavage fragments of complement activation such as C3a anaphylatoxin. We found that HSPC express a functional C3a receptor and that stimulation of human lympho/hematopoietic cell lines, CD34⁺ and lineage-committed myeloid, erythroid and megakaryocytic progenitors with C3a potentiates CXCR4-dependent responses to low doses of SDF-1, primes SDF-1-dependent migration across reconstituted basement membrane and stimulates MMP-9 secretion and adhesion to VCAM-1 [192]. Furthermore, priming of murine BM cells with C3a enhanced their engraftment after transplantation into lethally irradiated mice [192]. Our findings suggest that C3a-mediated sensitization of the responsiveness of HSPC to an SDF-1 gradient might be one of the key factors responsible for the retention of HSPC in the BM microenvironment. We obtained further support for this notion when we used mice deficient in C3a or C3aR mobilized with G-CSF, and found that mobilization was significantly accelerated and enhanced compared to their wild type counterparts [102]. Interestingly, on investigating C1q, the first subcomponent of the C1 complex of the classical pathway of complement activation, we found that CD34⁺ cells from CB and steady-state BM do not express it, while CD34⁺ cells from G-CSF-mPB expressed it

strongly [193]. Moreover, stimulation of BM cells with G-CSF induced the expression of C1q in the CD34⁺ cells. C1qRp (receptor for complement component 1, subcomponent q phagocytosis, also known as human CD93) is present on HSPC from human BM, mPB and CB CD34⁺ cells and on myeloid, megakaryocytic and erythroid progenitors. C1q by itself was not found to be a chemoattractant for CD34⁺ cells, but it primed the SDF-1-dependent chemotaxis as well as SDF-1-dependent trans-Matrigel migration of CD34⁺ cells [193]. The presence of functional C1qRp on human CD34⁺ cells could allow these cells to respond better to SDF-1 gradient and so be retained within BM niches. Having shown that C1q enhances the responsiveness of CD34⁺ cells to an SDF-1 gradient in vitro, we subsequently used a C1q-deficient mice for in vivo studies and found that they are more sensitive to mobilization induced by G-CSF than wild type mice, suggesting that C1q is involved in the retention of HSPC in the BM niches and that perturbation of this axis facilitates egress of HSPC into the blood [193]. Given that HSPC derived from mPB engraft faster than those from BM, and because the complement system is activated during mobilization and leukapheresis, it is most likely that during mobilization CD34⁺ cells are exposed to C1q which facilitates their homing and engraftment after transplantation.

How the HSPC Graft Can Be Improved

Because not all HSPC home with absolute efficiency, attempts to enhance the homing and engraftment of transplanted cells have been at the forefront of research investigations. Here we summarize our findings that the homing-related responses of HSPC can be enhanced by priming agents such as valproic acid, PMPs, C3a, C1q, HA and thrombin. Other investigators have also explored various ways by which the homing ability of HSPC can be augmented. Putative strategies to boost homing of cells to target tissues include pre-treatment of cells (genetically modified or incubated with small molecules such as soluble E-selectin to enhance adhesion) or pre-activation of target tissue by induction of chemokines [124]. Recently, it was reported that AMD3100 administered shortly after transplantation increases donor stem cell homing efficiency by expanding the available marrow niches as well as relieving the inhibitory effect of SDF-1 on primitive HSPC and allowing for engrafted HSPC to proliferate [194].

It was also reported that short-term ex vivo exposure to prostaglandin E2 (PGE2) of human HSC which express PGE2 receptors increased CXCR4 gene and surface expression, HSC in vitro migration to SDF-1 and in vivo homing to the BM, and enhanced their survival and entry into and progression through the cell cycle [195]. The interaction between the PGE2 and the Wnt signalling pathways controls HSC engraftment and, in fact, PGE2 is currently being tested in clinical trials as a potential therapy to enhance HSC engraftment following transplantation [196].

Murine HSC mobilized by GRObeta restore neutrophil and platelet recovery faster than G-CSF-mobilized PB HSC because they adhere better to VCAM⁺ endothelial cells and they show significantly lower levels of apoptosis and superior repopulation kinetics [53,197] and clinical trials are indicated.

Summary and Conclusions

Figure 1 presents the salient findings of our investigations of the mechanisms involved in mobilization (left panel) and homing (right panel). Other components of the BM niche and the conditions and mechanisms that have been implicated in these processes have been summarized in other most recent reviews (see [14] and [31]). The main objective of research on HSPC mobilization is to develop clinical protocols for optimal harvest of HSPC from PB with the ultimate aim of improving hematopoietic recovery after transplantation. We discuss in this review the molecules, pathways and mechanisms that actively mediate the egress of

HSPC from the BM into circulation and thus could be targeted to mobilize HSPC. The use of the CXCR4 antagonist AMD3100 as a mobilization agent is a clear demonstration of how basic mechanistic studies on mobilization can be harnessed for clinical application. The discovery of CXCR7, another receptor for SDF-1 [198,199], has changed our view that CXCR4 is the exclusive receptor for SDF-1. The role of CXCR7 in the metastasis of tumor cells is emerging [200]; however, its role in the migration of normal HSPC has not been demonstrated. Because CXCR7 functions as a sink for SDF-1 it may indirectly play a role in SDF-1-directed cell migration [201] and warrants further investigation. We believe that novel mobilization protocols will employ a variety of agents that mobilize HSPC with different kinetics and efficiencies and whose effects are additive or synergistic when used in combination.

We also discuss newly identified molecules that could confer an enhanced homing capacity on HSPC, and could be particularly useful in CB transplantation. In recent years the use of CB as a source of HSPC has rapidly increased because of many favourable factors, but the low number of HSPC in a single CB unit causes significant delays in hematopoietic recovery in most patients [202–204]. The principal strategies that have been proposed to hasten the rate of engraftment include augmenting the cell dose (by ex vivo expansion of CB HSPC or infusion of two CB units) and improving the homing ability of transplanted cells (by co-transplantation with MSC, direct intra-bone delivery, modulation of CD26 and administration of PTH) [205,206]. We suggest that in the future this obstacle could also be overcome through the application of some of the priming agents discussed in this review.

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Fig. 1. Mobilization (left panel): Mobilizing signals (e.g., G-CSF, AMD3100, chemotherapy, stress, infection, injury, etc.) expands the number of myeloid cells in the bone marrow and activates the complement cascade. C5 cleavage fragments activate myeloid cells to express and secrete proteolytic enzymes (including MMP-2, MMP-9, MT1-MMP and CPM) that disrupt interactions that retain the HSPC in the BM (e.g., SDF-1/CXCR4, VCAM-1/VLA-4, kit ligand/c-kitR, ECM). Subsequently, permeabilization of the endothelial barrier occurs with granulocytes “paving the way” for HSPC. Chemoattractants in the blood (primarily bioactive lipids such as S1P released from red blood cells via MAC, and HGF) further potentiate egress into PB of C5aR-expressing granulocytes and c-Met expressing HSPC. Homing (right panel): Agents priming SDF-1 responses (e.g., complement proteins C1q and C3a, hyaluronic acid and PMPs) enhance the incorporation of CXCR4 into the lipid rafts of the cell membrane, complement receptor expression (C1qRp, C3aR), and the accumulation of MT1-MMP on the cell surface. HSPC then attach/tether to and extravasate the sinusoid endothelium. MT1-MMP promotes the activation of MMP-2 that degrades ECM barriers. HSPC are chemoattracted to their BM niches by amplification of the chemotactic response via CXCR4 towards an SDF-1 gradient produced by osteoblasts and stromal cells, which leads to HSPC lodgement in the BM niches