Egress and mobilization of hematopoietic stem and progenitor cells*

Kfir Lapid¹, Yaron Vagima¹, Orit Kollet¹ and Tsvee Lapidot¹,§, ¹Department of Immunology, Weizmann Institute of Science, Rehovot 76100, Israel

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Abstract

Hematopoietic stem and progenitor cells continuously egress out of the bone marrow and into the circulation under homeostatic conditions. This process is dramatically enhanced during stress situations and induced mobilization regimens. Apart from cell autonomous mechanisms that affect cell motility and the breakdown of adhesion interactions that retain stem and progenitor cells attached to stromal cells, dynamic microenvironmental control plays a key role in releasing stem and progenitor cells from the bone marrow. Differentiating myeloid cells, bone remodeling by osteoblasts and osteoclasts, stimuli by the nervous system as well as integrity of the endothelial barrier form a complex network that regulates various aspects of stem cell function, including egress, recruitment, and mobilization. Hence, bone marrow stromal cells are ever-changing, dynamic vehicles for the residing reservoir of immature and maturing leukocytes as part of host defense and repair, rather than a static home from which stem and progenitor cells need to “escape”.


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§ To whom correspondence should be addressed. E-mail: Tsvee.Lapidot@weizmann.ac.il
1. Introduction

Hematopoietic stem cells (HSCs) reside in the bone marrow (BM), where they undergo proliferation and multi-lineage differentiation, giving rise to mature leukocytes and erythrocytes, which are in turn released to the blood in order to carry out their function (Orkin and Zon, 2008). In fact, not only do mature leukocytes circulate, but also small amounts of non-cycling hematopoietic stem and progenitor cells (HSPCs; Lapidot and Petit, 2002). Human umbilical cord blood (CB) contains relatively high amounts of CD34+ HSPCs (∼0.5%; Broxmeyer, 1996), however, postnatally their fraction in the peripheral blood (PB) decreases to ∼0.06% at steady-state conditions (Korbling and Anderlini, 2001). Although it is possible that steady-state PB HSPCs may reflect a leakage of the system, it is believed that they play a major role in homeostasis, such as in repopulation of damaged BM regions. Donor murine PB HSPCs are cleared very quickly, within a few minutes, from the intravascular space of intravenously transplanted congenic recipients (Wright et al., 2001). By pairing congenic mice and creating parabiotic mice with a shared blood system, it was revealed that HSCs rapidly and continuously migrate through the circulation, enabling functional re-engraftment of unconditioned BM (Wright et al., 2001; Abkovitz et al., 2003). These results demonstrate that release of HSCs into the circulation and their homing back to the BM are sequential or simultaneously occurring events that could also be important in steady-state homeostatic processes. Recently, it was found that HSCs with repopulation capacity also circulate in the lymphatic system (Massberg et al., 2007) as part of host defense mechanisms. Originating in the BM, HSPCs traffic via the blood and enter peripheral tissues, where they undergo differentiation into tissue-resident innate immune cells, in response to local stimuli, such as inflammation. Non-stimulated HSPCs re-circulate to the lymph, back to the PB, and eventually home to the BM (Massberg et al., 2007). HSPCs release into the circulation is dramatically augmented in a process termed cell egress, occurring during stress situations and upon demand of accelerated hematopoiesis (Lapidot and Petit, 2002). Proliferation (and differentiation) in the BM usually precedes enhanced progenitor cell egress (Morrison et al., 1997), and PB neutrophilia is associated with recruitment of HSPCs to the PB (Roberts et al., 1997; Levesque et al., 2007). Thus, the potential physiological role of cell egress is to replenish the blood and periphery with functional immune cells. Enhanced cell egress and recruitment occurs following various stress situations, such as exercise or adrenocorticotropic hormone administration (Barrett et al., 1978; Zaldivar et al., 2007), inflammation (observed upon LPS or endotoxin administration; Cline and Golde, 1977; Benner et al., 1981), bleeding (Kollet et al., 2006), administration of cytotoxic drugs (To et al., 1984; To et al., 1989), and even psychological anxiety (Elsenbruch et al., 2006). Since HSCs are strongly retained in specialized niches in the BM (see The hematopoietic stem cell niche (link to 10.3824/stembook.1.28.1)), the mere mechanistic concept of cell egress is deliberate detachment of HSCs and their active trafficking from the BM into the blood stream in order to accommodate blood cell replenishment on demand. Hence, the recruitment of HSCs to the PB is not simply a direct outcome of proliferation and passive release to the blood. Stress-induced HSPCs cell egress is a complex multi-step process, involving activities of various cytokines and chemokines, and proteolytic enzymes that enable detachment of HSPCs from their niches. The BM niches serve to prevent HSCs migration and proliferation via adhesion interactions, and thereby breakdown of these interactions is essential for the cell egress process. Growing evidence for the involvement of other cellular players, such as osteoclasts and neurons in regulation of the increased progenitor cell motility will be discussed. In this chapter, we will also discuss functional murine models for clinical HSCs mobilization, beginning with describing various mobilizing factors, deciphering pathways and molecular mechanisms promoting mobilization, continuing with a broader view on the microenvironmental regulation of this process and finishing with a brief clinical aspect.

2. Cytokine and chemokine induced mobilization

Since mobilized peripheral blood (MPB) contains relatively high yield of HSPCs, it serves as a valuable source for isolating repopulating stem cells for clinical transplantation. Mobilization of HSCs to the periphery of patients in response to chemotherapy or repeated cytokine stimulations was first documented in the late 1970s and early 1980s (To et al., 1997). Nowadays, a long array of mobilizing agents is used clinically or experimentally in animal models to induce mobilization, the concept of which is to mimic the process of physiological stress-induced cell egress and recruitment. Chemotherapeutic agents, such as cyclophosphamide (Cy) and paclitaxel (Verma et al., 1999), are potent mobilizing agents. However, in combination with hematopoietic growth factors, mobilization is significantly enhanced (Schwartzberg et al., 1992). This regimen is a standard protocol for mobilizing HSCs collected for autologous transplantsations. In case of allogeneic transplantations, donors receive hematopoietic growth factors alone. Granulocyte colony stimulating factor (G-CSF; Sica et al., 1992) and granulocyte/macrophage colony stimulating factor (GM-CSF; Gianni et al., 1989) are the most effective mobilizing agents used today, of which the first is the most commonly used cytokine. In humans, G-CSF administration leads to a ∼16 fold increase in immature CD34+ cells and an approximately 23 fold increase in the more primitive CD34+CD38− cells in the blood (Korbling and Anderlini, 2001). The advantages of mobilized HSPCs over steady-state BM HSPCs is also characterized by increased endogenous motility and homing rates, increased cellularity and higher levels of committed progenitor cells, therefore MPB has
higher engraftment capacities (Bonig et al., 2007). Usually, G-CSF is administered daily for 4–6 consecutive days in order to achieve optimal yields of HSPCs in the PB (Korbling and Anderlini, 2001). However, different mobilizing agents differ in their time frame to achieve mobilization. In mice, interleukin-1 (IL-1) mobilizes within hours (Fibbe et al., 1992); whereas a single injection of the chemokine interleukin-8 (IL-8) induces rapid mobilization, in which progenitors are detected in the blood, as soon as 20 minutes (Laterveer et al., 1995). Another CXCR2 ligand – GROα, is also known as a rapid mobilizer (Fukuda et al., 2007). Since CXCR2 is not expressed on HSPCs, the mechanism of action is indirect and is actually mediated by responsive neutrophils (further discussed under the “proteolytic activity” section). During G-CSF induced-mobilization, there is a massive production of neutrophils, in which IL-8 is produced (Watanabe et al., 1999). In addition, IL-1 by itself induces IL-8 secretion from BM stromal cells, suggesting that activated neutrophils mediate the mobilizing effect of IL-8 (Takashiba et al., 1992). The importance of neutrophils in driving mobilization is demonstrated in neutropenic mice. Generated either by depleting antibodies (Pruijt et al., 2002) or by knocking-out the G-CSF receptor (Liu et al., 1997), these mice fail to mobilize in response to IL-8, G-CSF or Cy. Nevertheless, mobilizing agents broadly differ in the type of cells mobilized and mechanism of action, thus neutrophils are not the sole players. Peptide analogs for the chemokines macrophage inflammatory protein 1α (MIP-1α; Lord et al., 1995) and stromal derived factor 1 (SDF-1; Pelus et al., 2005) cause rapid mobilization within an hour. MIP-1α’s course of action is unknown, however it is known that SDF-1 directly acts on HSPCs via binding to its receptor CXCR4, which is expressed by many types of white blood cells (WBCs; Wright et al., 2002; Mohle et al., 1998). In combination with G-CSF, chemokine administration synergistically enhances mobilization (Pelus and Fukuda, 2008). The effects of direct mobilization on HSPCs can also be mediated by administration of stem cell factor (SCF), which binds to c-Kit expressed on primitive cells (de Revel et al., 1994). SCF was also tested clinically in combination with G-CSF or chemotherapy (To et al., 2003). However, it should be noted that SCF induced mobilization is associated with adverse effects, such as skin darkening and severe allergic reactions mediated by activation of mast cells (Stiff et al., 2000). FLT3 ligand (FLT3-L) is a growth factor of early hematopoiesis, and thrombopoietin (TPO) is essential for thrombopoiesis, but also affects primitive cells. Both FLT3-L and TPO are modest mobilizing agents, however, in combination with G-CSF, they exhibit a synergistic effect (Linker et al., 2003; Molineux et al., 1997). Vascular endothelial growth factor (VEGF), which is a potent pro-angiogenic factor that plays a major role in regulating HSCs as well (Gerber et al., 2002), is also able to mobilize HSPCs (Hattori et al., 2001), the mechanism of which is unknown. VEGF may act directly on HSPCs, since they express its receptor, or indirectly by remodeling the BM vasculature. Obviously, in order to evaluate the efficacy of a mobilizing agent, one has to test the mobilized HSCs repopulation capacity, apart from measuring their numbers in the blood. Generally, transplantation of human MPB has advantages over human BM aspirates, since it demonstrates faster hematopoietic recovery (examined by calculating neutrophils and platelets content in the blood; Korbling and Anderlini, 2001). It should be noted that this relatively rapid engraftment results from increased doses of transplanted mobilized progenitor cells. In spite of these advantages, qualitative comparison shows that primitive human HSPCs (i.e. immature Lin-CD34+ or primitive CD34+CD38- ) from MPB are inferior to equivalent cells from steady state BM, in terms of proliferation, differentiation, long-term repopulation potential and serial transplantsations in nonobese diabetic severe combined immune-deficient (NOD/SCID) transplanted mice or pre-immune sheep (Ramirez et al., 1998; van der Loo et al., 1998; Verfaillie et al., 2000). Nonetheless, these results should be interpreted with care, due to the limitations that xenotransplantation models have and the striking success of clinical protocols for MPB transplantation. Another characteristic of mobilized HSPCs is their non-cycling state (Morrison et al., 1997; Uchida et al., 1997). It has been suggested that following G-CSF administration, HSPCs enter the cell cycle but egress to the blood only after the M phase (Wright et al., 2001), thus remaining in a non-cycling state. Interestingly, under in vitro conditions, non-cycling HSCs retain higher long-term repopulation potential in comparison to their cycling counterparts (Szilvassy et al., 2000). It is yet unknown whether the non-cycling status is related to a better engraftment capacity of HSCs or whether motile cells are rather non-cycling de facto.

### 3. The SDF-1/CXCR4 pathway

SDF-1 (also termed CXCL12) is the only chemokine able to strongly attract human and murine HSPCs (Wright et al., 2002; Peled et al., 1999). In addition to its chemotactic function, SDF-1 has important roles in homing, retention, survival and cell cycle. SDF-1 is highly expressed by endosteal bone lining osteoblasts (Ponomaryov et al., 2000), BM endothelium (Cerdini et al., 2004) and other stromal cell types, including CXCL12 abundant reticular (CAR) cells (Sugiyama et al., 2006), whereby attracting circulating CXCR4+ HSPCs to their niches.Blocking CXCR4 function by neutralizing antibodies impairs homing of transplanted human CD34+ HSPCs to the BM of transplanted immunodeficient mice (Kollet et al., 2001), accompanied by impaired engraftment (Peled et al., 1999). Impairment of SDF-1 or CXCR4 in murine embryos results in multiple lethal defects including lack of stem cell seeding of the BM (Nagasawa et al., 1996; Zou et al., 1998). In order to circumvent lethality, conditional knock-out (KO) models were established. Induced deletion of CXCR4 in the hematopoietic system of the adult mouse, leads to severely reduced BM cellularity and HSCs numbers as well as impaired repopulation capacity (Sugiyama et al., 2006). Of interest,
CXCR4 KO HSCs are pushed into the cell cycle, resulting in increased numbers of more mature progenitors, but loss of function in general. These observations implicate CXCR4 in promoting quiescence of HSCs, explaining why CXCR4 conditional KO mice have reduced survival following myeloablative treatment (Sugiyama et al., 2006) or irradiation (Foudi et al., 2006). Conditional deletion of CXCR4 also results in increased HSPCs numbers in the spleen and PB (Nie et al., 2008), suggesting that blocking CXCR4 hampers their retention in the BM. The essential role of SDF-1 in retention can be inferred from the observations that following G-CSF administration, SDF-1 levels in the BM are transiently increased followed by their downregulation at both protein (Petit et al., 2002; Levesque et al., 2003) and mRNA (Semerad et al., 2005) levels, enabling transient and local SDF-1 gradients towards the blood. In addition, CXCR4 upregulation is observed on immature human CD34+ cells and primitive CD34+CD38- cells resident in the BM of G-CSF treated chimeric mice (Petit et al., 2002). Of note, immediately following after G-CSF injection, the levels of SDF-1 in the BM are transiently increased prior to their downregulation. Blocking CXCR4 or SDF-1 reduces G-CSF induced mobilization, thus demonstrating an active role for SDF-1 in mobilization of murine progenitors (Petit et al., 2002). Since SDF-1 plays a major role in retention of HSPCs in the BM, it is presumed that increased SDF-1 levels in the PB may attract HSPCs and thereby increase cell egress to the circulation. For instance, artificially elevating SDF-1 plasma levels, by adenoviral vector expressing SDF-1, causes HSPC mobilization (Hattori et al., 2001). Likewise, repetitive daily administrations of SDF-1 for five consecutive days trigger mobilization (Kollet et al., 2006). Administration of the sulfated polysaccharide fucoidan leads to mobilization in mice and non-human primates (Sweeney et al., 2000). In a follow-up study, it was found that fucoidan competes with SDF-1 for binding heparan sulfate proteoglycans in the BM, consequently releasing SDF-1 into the blood (Sweeney et al., 2002). Interestingly, neutralizing SDF-1 with antibodies in fucoidan treated mice, reduced mobilization of HSPCs, but not mature WBCs, revealing higher dependence on SDF-1 among undifferentiated cells. It is logical to assume that hampering SDF-1/CXCR4 signaling in the BM would result in loss of retention. Indeed, when the Met-SDF-1/β analog, which induces prolonged CXCR4 desensitization, was given to mice, it resulted in modest mobilization (Shen et al., 2001). CXCR4 has selective chemical antagonists, among which are the bicyclam AMD3100 and T-140. Upon AMD3100 administration, mouse, human and non-human primate HSPCs undergo rapid mobilization (Liles et al., 2003; Broxmeyer et al., 2005; Larochelle et al., 2006) and the mobilized HSCs demonstrate increased SDF-1 induced migration in vitro and in vivo repopulation potential. Similarly, T-140 induces mobilization of primitive murine repopulating cells (Abraham et al., 2007). Both AMD3100 and T-140 synergistically augment G-CSF induced mobilization. AMD3100 is the only chemokine receptor antagonist utilized clinically for inducing mobilization, either alone (Devine et al., 2008) or in combination with G-CSF administration (Liles et al., 2005). Notably, preliminary results reveal that blocking the SDF-1/CXCR4 axis using neutralizing antibodies to either SDF-1 or CXCR4 in AMD3100 treated mice, reduced mobilization of HSPCs, but not mature WBCs (Dar et al., 2006), further strengthening the hypothesis that this pathway is relatively more selective for HSPCs. It is stated that AMD3100 disrupts SDF-1/CXCR4 interactions in vitro and therefore its administration in vivo results in loss of HSPC retention (Broxmeyer et al., 2005). Nonetheless, the observations with anti-SDF-1 and anti-CXCR4 antibodies complicate this theory, suggesting that active SDF-1/CXCR4 is also necessary for AMD3100 induced mobilization. Apart from being an antagonist for CXCR4, as demonstrated by its ability to block ligand binding to its receptor on leukocytes (Gerlach et al., 2001), AMD3100 is able to weakly signal, serving as a partial agonist (Zhang et al., 2002). In a particular preclinical study, weekly injections of AMD3100 reduced the tumor burden of NOD/SCID mice engrafted with a human non-Hodgkin’s lymphoma, while continuous infusion of AMD3100 led surprisingly to an increase of tumor growth (Paul et al., 2002). Our preliminary experiments reveal that AMD3100 does not affect only hematopoietic cells, but also BM stromal cells, which express CXCR4 as well (Dar et al., 2006; Dar et al., 2005). It was found that in response to AMD3100, SDF-1 secretion from BM endothelial cells and osteoblasts in vitro and in vivo to the circulation is increased, further contributing to transient, local SDF-1 gradients towards the blood, after which motile HSPCs may follow. It is possible that a similar mechanism enables increased recruitment of lymphoma cells in the continuous AMD3100 infusion model, as mentioned earlier (Paul et al., 2002). Altogether, this data adds more complexity to the AMD3100 induced mobilization process, which will be further discussed later.

4. Adhesion molecules and loss of retention

HSCs reside in specialized niches in the BM (see The hematopoietic stem cell niche [link to 10.3824/stem-book.1.28.1]), supported by niche forming stromal cells to which they tightly adhere. Many adhesion molecules are implicated in this adhesion machinery, directed at both cell-cell contact and adhesion to extracellular matrix (ECM) components. During cell egress, breakdown of this tight adhesion is a principal mechanism for releasing HSCs from the BM, allowing them to egress to the blood. For example, very late antigen-4 (VLA-4 or integrin α4β1) binds to vascular adhesion molecule-1 (VCAM-1), which is expressed by BM stromal cells (Ryan et al., 1991; Simmons et al., 1992). Administration of either VLA-4 or VCAM-1 neutralizing antibodies leads to mobilization of HSPCs into the PB in both mice and primates (Papayannopoulou and Nakamoto, 1993; Papayannopoulou et al., 1995). In a
follow-up study, it was found that anti-VLA4/VCAM-1-induced mobilization requires cooperative signaling through the c-Kit/SCF pathway, demonstrated by the inability of mutant mice to mobilize (Papayannopoulou et al., 1998). In addition, anti-VLA-4 administration augments G-CSF and SCF induced mobilization of long-term repopulating HSCs (Craddock et al., 1997). Expectedly, induced deletion of either integrin α4 or VCAM-1, results in accumulation of HSPCs in the PB (Scott et al., 2003; Ulyanova et al., 2005). It is reasonable to assume that modulation of the VLA-4/VCAM-1 pathway occurs in a more physiological manner than administration of neutralizing antibodies. Indeed, G-CSF mobilized human CD34+ HSPCs express less active VLA-4 in comparison to steady-state BM cells (Lichterfeld et al., 2000), and plasma levels of soluble cleaved VCAM-1 markedly increase following G-CSF induced mobilization (Sudhoff and Sohngen, 2002; Levesque et al., 2001), suggesting reduced numbers of intact VCAM-1 molecules in the BM, to which HSPCs could adhere. Fucoidan induced mobilization was enhanced in integrinαM deficient mice (Hidalgo et al., 2004) and neutralizing antibodies against this integrin enhance G-CSF induced mobilization (Velders et al., 2002), suggesting that macrophage antigen-1 (MAC-1 or integrin αMβ2) is another integrin involved in retention. Yet, since MAC-1 is expressed at later stages of differentiation than HSCs, the mechanism is unclear. Very late antigen-5 (VLA-5 or integrin α5β1) and lymphocyte function-associated antigen-1 (LFA-1 or integrin αLβ2) are additional integrins that are implicated in homing and engraftment of HSCs (van der Loo et al., 1998; Peled et al., 2000; Asaumi et al., 2001). The BM abundant ECM component fibronectin (FN) is a common ligand for the VLA-5 and VLA-4 integrins. However, interference with the VLA-4/FN interaction, by either continuous administration of competitive FN fragment or anti-VLA-5 antibodies, did not cause cell egress (Craddock et al., 1997). LFA-1 binds to intercellular cell adhesion molecule-1 (ICAM-1), which is expressed by many stromal cells. Administration of anti-LFA-1 antibodies was ineffective in causing cell egress by itself (Papayannopoulou et al., 2001), although in combination with anti-VLA-4 antibodies, G-CSF (Velders et al., 2002) or IL-8 (Pruijt et al., 2002), a synergistic effect on mobilization was observed. These results suggest that homing and cell egress (or mobilization) are not mirror-image processes. Thus, some of the adhesion molecules that are involved in the mechanism of homing, are not necessarily as important for cell egress out of the BM, and vice versa. Selectins are another set of adhesion molecules, which are involved in initial steps of HSPCs homing to the BM (Frenette et al., 1998). Mice deficient in both E-Selectin and P-Selectin, which are expressed by the endothelium, harbor higher numbers of circulating HSPCs in PB, and inhibition of P-Selectin function in E-Selectin deficient mice by either administration of fucoidan or anti-P-Selectin antibodies, results in rapid mobilization (Frenette and Weiss, 2000). Nevertheless, inhibition of selectins function as an inducer of mobilization remains controversial, due to the observation that fucoidan is also able to induce mobilization in mice lacking selectins (Sweeney et al., 2000). The mechanism of which was explained by competition with SDF-1 for binding heparin sulfate in the BM, leading to its subsequent release to the blood (Sweeney et al., 2002). CD44 is an important adhesion molecule that interacts with several ECM components, including hyaluronan, which is essential for in vitro culture and homing of immature human CD34+ cells (Miyake et al., 1990; Avigdor et al., 2004). Blocking CD44 function results in mobilization (Vermeulen et al., 1998), and combination with G-CSF or anti-VLA-4 administration, enhances their induced HSPCs mobilization (Christ et al., 2001). Furthermore, CD44 was shown to be downregulated on mobilized immature human CD34+ cells (Lee et al., 2000). SCF/c-Kit interaction has been shown to promote retention via activation of VLA-4 and VLA-5, in addition to its roles in HSCs self-renewal and hematopoiesis (Levesque et al., 1995). Hence, mice carrying inactivating mutations of c-Kit were shown to be poor mobilizers (Papayannopoulou et al., 1998; Cynnshi et al., 1991), and administration of soluble c-Kit, which competes with endogenous c-kit for binding SCF, resulted in mobilization (Nakamura et al., 2004). HSCs bind to various ECM components, such as fibronectin and osteopontin (OPN). OPN serves as a negative regulator of the stem cell pool size (Stier et al., 2005; Nilsson et al., 2005). Since it is implicated in directing cells towards the endosteme (Nilsson et al., 2005), it is plausible to assume that inhibition of this specific integrin β1/OPN interaction would release HSCs from the BM at some extent. It was found that OPN deficient mice accumulate HSPCs in the spleen in addition to the BM (Stier et al., 2005), however, it is yet to be determined whether this results from increased cell egress or rather increased proliferation. In summary, adhesion molecules play a major role in retention and therefore loss of their function results in loss of retention and consequently HSPCs egress, recruitment and mobilization. It remains to be asked, how their activity is regulated in vivo during the progenitor cell motility process.

5. Proteolytic activity

In chimeric mice, harboring mixed BM from G-CSF receptor (GCSFR) −/− and +/+ origin, GCSFR −/− HSPCS mobilize equally, suggesting that GCSFR expression on HSPCs is not required for their mobilization and other GCSFR expressing cells indirectly mediate it (Liu et al., 2000). The observations that following G-CSF administration, neutrophils undergo proliferation and activation (Falanga et al., 1999), pinpoint a possible role for neutrophils in the process. Indeed, it was found that neutrophil elastase (NE) and cathepsin G (CG), enzymes secreted by neutrophils, are capable of cleaving VCAM-1, a major player in retention, both in vitro and during G-CSF induced mobilization (Levesque et al., 2001). Other retention factors that were shown to be cleaved by these serine proteases include
c-Kit (Levesque et al., 2003), SDF-1 (Petit et al., 2002) and CXCR4 (Levesque et al., 2003; Valenzuela-Fernandez et al., 2002). Thus, inhibition of neutrophil elastase (NE) reduced G-CSF induced mobilization (Petit et al., 2002). Intriguingly, the natural serine protease inhibitors serpina1 and serpina3 are downregulated in the BM, following G-CSF administration, enabling the enhanced proteolytic activity (Winkler et al., 2005). Matrix metalloproteinases (MMPs) are a family of proteases that play a significant role in transendothelial migration and ECM degradation, one of which is MMP-9, which is secreted by neutrophils, but also by other cells in the BM (Page-McCaw et al., 2007). Active MMP-9 plasma levels are increased following mobilization induced by IL-8 (Pruijt et al., 1999), G-CSF, GROβ (Pelus et al., 2004) or VEGF (Rafii et al., 2002), and its inhibition subsequently results in reduced mobilization. MMP-9 was found to cleave c-Kit (Levesque et al., 2003) and membrane bound SCF (Heissig et al., 2002), releasing a soluble SCF. Soluble SCF, in turn, enhances cell motility and egress. In spite of these observations, mice lacking both NE and CG, MMP-9 or dipetidyl peptidase IV, is expressed by HSPCs and is able to cleave SDF-1, the truncated form of which, acts as an antagonist for CXCR4 dependent chemotaxis (Christopherson et al., 2003). As a consequence, G-CSF induced mobilization is reduced either upon CD26 inhibition (Christopherson et al., 2003) or in mice lacking CD26, compared to their wild-type counterparts (Levesque et al., 2003). It should be noted that SDF-1 upregulates the expression of MMP-2, MMP-9, and membranal type I MMP (MT1-MMP or MMP-14) on human CD34+ cells (Janowska-Wieczorek et al., 2000; Shirvaikar et al., 2008). The latter has been shown to be involved in mobilization as well, mediating cell egress by cleavage of CD44 (Vagima et al., 2009). Recently, it was found that upon G-CSF administration, carboxypeptidase M, which is expressed by many BM resident cells, is significantly increased and is also capable of cleaving SDF-1 (Marquez-Curtis et al., 2008). Due to the importance of proteases in the process of mobilization, mediating cell egress by degrading various retention promoting factors, other yet undiscovered proteases may also participate. A surprising finding was the ability of cathepsin K (CTK), which is expressed by bone degrading osteoclasts, to also cleave the endosteal niche components SDF-1, OPN and membrane bound Kit ligand (Kollet et al., 2006). Its role is discussed later in the chapter. Alongside proteolytic activity, regulatory mechanisms may assist in modulating cell egress. A low dose of total body irradiation (TBI) prevents G-CSF or IL-8 induced mobilization of HSPCs (van Pel et al., 2006). An elevated expression of the endogenous protease inhibitor serpin1 (also known as α1-antitrypsin) at both mRNA and protein levels was detected in BM extracts, but not in the plasma. Blocking serpin1 by neutralizing antibodies rescued mobilization in the low dose TBI treated mice, whereas administration of serpin1 prior to IL-8 administration in healthy mice inhibited it. Preliminary data reveals that RECK, which is the endogenous inhibitor of MT1-MMP, MMP-2 and MMP-9, is inversely regulated on human CD34+ HSPCs and mouse BM cells (Vagima et al., 2009). Upon G-CSF treatment, MT1-MMP expression is increased, while RECK expression is decreased, emphasizing the importance of balanced proteolytic activity in the BM.

6. Bone remodeling and HSC egress

Bones are dynamic and continuously undergo formation by bone lining osteoblasts and resorption by the stem cell derived fused-monoocytes, termed osteoclasts, throughout life. Osteoblasts are involved in maintaining quiescent HSCs at the endosteal niches and play a major role in supporting BM hematopoiesis (Porter and Calvi, 2008). Following G-CSF administration, BM SDF-1 mRNA levels are reduced, and it was discovered that reduction in numbers and activity of osteoblasts, which produce SDF-1, is one of the major reasons (Semerad et al., 2005). Decrease in BM SDF-1 levels is correlated with degree of mobilization (Petit et al., 2002). The reduction in osteoblast numbers results from stimulation of the sympathetic system, during G-CSF treatment (Katayama et al., 2006; further discussed under “the nervous system” section). Furthermore, osteoblasts isolated from Cy/G-CSF treated mice could expand HSPCs in vitro at higher rates than osteoblasts from non-treated mice (Mayack and Wagers, 2008). One of the factors promoting HSC self-renewal is interleukin-10, whose secretion by endosteal osteoblasts is upregulated following irradiation induced stress (Kang et al., 2007). Thus, it is suggested that BM niches are undergoing dynamic changes during stress induced mobilization to allow proliferation and egress of HSPCs. Communication between osteoblasts and osteoclasts activities constantly occurs. For instance, osteoclasts precursors seeded on top of osteoblast monolayers in vitro induce osteoblasts retraction in MMPs dependent manner (Perez-Amodio et al., 2004). This retraction enables the osteoclasts to exert their resorpive activity on the bone matrix below. Following G-CSF administration, osteoclasts activity is upregulated, leading to increased bone degradation measured by urine levels of deoxypyridinoline, explaining why G-CSF mobilized PB donors show reduced bone mass (Takamatsu et al., 1998; Watanabe et al., 2003). Osteoclasts express both SDF-1 and CXCR4, and SDF-1 is involved in recruitment of osteoclast precursors to the bone surface.
and promotes immature osteoclast development and survival as well (Yu et al., 2003; Wright et al., 2005). In addition, G-CSF, SDF-1 or hepatocyte growth factor (HGF) administration for five consecutive days induces both increase in osteoclast numbers and PB progenitors (Kollet et al., 2006). Stress-induced situations, such as mild bleeding and LPS treatment, also trigger HSPCs mobilization concomitantly with osteoclast activity. Indeed, we have demonstrated that osteoclasts are directly involved in inducing progenitor cell egress by CTK mediated degradation of niche components, such as SDF-1, OPN and SCF (Kollet et al., 2006), linking bone remodeling with mobilization of HSPCs. Osteoclast activation by receptor activator of nuclear factor-κB ligand (RANKL) preferentially increased mobilization of immature cells, whereas osteoclasts inhibition by the hormone calcitonin decreased induced mobilization as well as homeostatic release of progenitors. Of note, mobilization induced by repeated stimulations with RANKL, HGF or SDF-1 was selective in terms of mobilizing HSPCs to the PB, but not mature leukocytes (Kollet et al., 2006). Utilization of protein tyrosine phosphatase epsilon (PTPe) deficient young female mice, which have transient and mild impairment of osteoclast adhesion and resorption (Chiusaroli et al., 2004), also demonstrated reduced numbers of circulating progenitors and capacity to mobilize, even in response to repeated RANKL stimulations, despite normal levels of immature cells in the BM (Kollet et al., 2006). Interestingly, osteoclasts secrete other factors, which have shown to be involved in mobilization, such as MMP-9 (Pruijt et al., 1999; Heissig et al., 2002), CG (Goto et al., 2003) and IL-8 (Laterveer et al., 1995; Rothe et al., 1998). Another example for the involvement of osteoclasts in the mobilization process is presented by CD45 KO mice, which demonstrate defective osteoclast activity, altered metaphysial trabecular bone structure and reduced BM pool of primitive HSPCs (Shivtiel et al., 2008). RANKL and suboptimal G-CSF induced mobilization are consequently impaired in those mice. Parathyroid hormone (PTH) maintains calcium homeostasis by accelerating bone remodeling, demonstrated by proliferation of osteoblasts that express its receptor, which is next followed by indirect osteoclasts activation. The direct positive effect of PTH on osteoclasts has been shown to increase the HSCs pool size via the Notch pathway (Calvi et al., 2003), however, PTH administration also results in HSC mobilization (Brunner et al., 2008; Adams et al., 2007). Moreover, pre-treatment of mice with PTH five weeks prior to administration of G-CSF, augments mobilization (Adams et al., 2007). It is yet to be determined whether osteoclasts are involved or not in PTH induced mobilization. Altogether, bone remodeling has a strong impact on the stem cell function and the degree of both steady-state progenitor cell egress and stress-induced mobilization, by shaping the dynamic BM microenvironment and stem cell niches. Nonetheless, other microenvironmental cellular components have recently emerged as key regulators of HSPCs recruitment to the PB.

7. The nervous system

Psychological stress mediated by neuronal signals may affect HSCs function and even exert their release to the PB. For example, anxiety induced by speaking in public raises the numbers of circulating leukocytes (Elsenbruch et al., 7). The nervous system activation by receptor activator of nuclear factor-κB ligand (RANKL) preferentially increased mobilization of immature cells, whereas osteoclasts inhibition by the hormone clacitonin decreased induced mobilization as well as homeostatic release of progenitors. Of note, mobilization induced by repeated stimulations with RANKL, HGF or SDF-1 was selective in terms of mobilizing HSPCs to the PB, but not mature leukocytes (Kollet et al., 2006). Utilization of protein tyrosine phosphatase epsilon (PTPe) deficient young female mice, which have transient and mild impairment of osteoclast adhesion and resorption (Chiusaroli et al., 2004), also demonstrated reduced numbers of circulating progenitors and capacity to mobilize, even in response to repeated RANKL stimulations, despite normal levels of immature cells in the BM (Kollet et al., 2006). Interestingly, osteoclasts secrete other factors, which have shown to be involved in mobilization, such as MMP-9 (Pruijt et al., 1999; Heissig et al., 2002), CG (Goto et al., 2003) and IL-8 (Laterveer et al., 1995; Rothe et al., 1998). Another example for the involvement of osteoclasts in the mobilization process is presented by CD45 KO mice, which demonstrate defective osteoclast activity, altered metaphysial trabecular bone structure and reduced BM pool of primitive HSPCs (Shivtiel et al., 2008). RANKL and suboptimal G-CSF induced mobilization are consequently impaired in those mice. Parathyroid hormone (PTH) maintains calcium homeostasis by accelerating bone remodeling, demonstrated by proliferation of osteoblasts that express its receptor, which is next followed by indirect osteoclasts activation. The direct positive effect of PTH on osteoclasts has been shown to increase the HSCs pool size via the Notch pathway (Calvi et al., 2003), however, PTH administration also results in HSC mobilization (Brunner et al., 2008; Adams et al., 2007). Moreover, pre-treatment of mice with PTH five weeks prior to administration of G-CSF, augments mobilization (Adams et al., 2007). It is yet to be determined whether osteoclasts are involved or not in PTH induced mobilization. Altogether, bone remodeling has a strong impact on the stem cell function and the degree of both steady-state progenitor cell egress and stress-induced mobilization, by shaping the dynamic BM microenvironment and stem cell niches. Nonetheless, other microenvironmental cellular components have recently emerged as key regulators of HSPCs recruitment to the PB.

7. The nervous system

Psychological stress mediated by neuronal signals may affect HSCs function and even exert their release to the PB. For example, anxiety induced by speaking in public raises the numbers of circulating leukocytes (Elsenbruch et al., 2006). Early in the 20th century, researchers found a marked increase in the number of circulating leukocytes following injection of adrenaline (epinephrine), a neurotransmitter produced by the sympathetic system, into healthy human volunteers (Benschop et al., 1996). In rats, epinephrine administration stimulates the release of leukocytes from the spleen, BM and lymphatic organs, contributing significantly to leukocytosis (Iversen et al., 1994). Recently, sympathetic system induced mobilization of primitive cells was documented (reviewed in Spiegel et al., 2008). The BM is highly innervated and sympathetic nerve endings localize especially in proximity to endothelial cells and the endosteum in the epiphysis and metaphysis, regions known to be enriched with stem cell niches, implying indirect control over HSC function (Artico et al., 2002). Indeed, immature and primitive progenitor cells, among other leukocytes, express β2 adrenergic receptors as well as dopamine receptors (Spiegel et al., 2007). These receptors are upregulated in G-CSF mobilized immature human CD34+ cells, and more pronouncedly in primitive CD34+CD38− cells, suggesting a role for sympathetic system stimulation in inducing mobilization of progenitor cells. β2 adrenergic stimulation by norepinephrine administration in mice, resulted in increased numbers of circulating HSPCs, while administration of propranolol, which is a β2 adrenergic antagonist, resulted in their reduced PB numbers (Spiegel et al., 2007). In addition, catecholamines (i.e. dopamine and norepinephrine) facilitate enhanced HSPCs motility, which is a key characteristic of mobilized cells. The importance of the nervous system for the mobilization process was determined by establishing galactosyltransferase deficient mice that display aberrant nerve conduction, without affecting the BM resident HSC pool during steady state homeostasis. Strikingly, G-CSF or fucoidan administration in these mice had no effect on HSPC mobilization (Katayama et al., 2006). Mice that underwent catecholaminergic ablation induced by 6-hydroxydopamine or mice lacking β-hydroxylase that cannot synthesize norepinephrine, similarly exhibited inability to induce mobilization (Katayama et al., 2006). Our preliminary data demonstrate that norepinephrine administration stimulates release of SDF-1 from BM stromal cells to the PB, after which progenitors follow, consequently increasing their numbers in the circulation (Dar et al., 2006). Interestingly, this norepinephrine induced mobilization was selective, since no increase in circulating mature leukocytes was observed. Recently, it was revealed that neuronal stimulation plays a role in steady-state egress of HSPCs and not only during stress situations. HSPCs exhibit robust daily oscillations, peaking 5 hours after the initiation of light and reaching a nadir 5 hours after darkness (Mendez-Ferrer
et al., 2008). In fact, β3 adrenergic stimulations (and not β2 adrenergic receptors that play a role in G-CSF induced mobilization) were affected by circadian rhythmic control over norepinephrine release, leading to downregulation of SDF-1 transcription in BM stromal cells. As a result, SDF-1 synthesis fluctuates, triggering circadian rhythmic oscillations of PB circulating progenitor cells (Mendez-Ferrer et al., 2008). Furthermore, it seems that G-CSF-induced leukocytosis is also dependent on the timing of G-CSF injection in both mice (Ondo et al., 1998) and human patients (Sato et al., 2002). Unexpectedly, adrenergic stimulation suppressed bone-lining osteoblasts, causing a significant reduction in SDF-1 production (Semerad et al., 2005; Katayama et al., 2006) and thereby enabling cell egress. It was found that G-CSF induced mobilization requires adrenergic signals, since G-CSF administration in mice lacking catecholaminergic activity did not result in osteoblast suppression, SDF-1 downregulation in the BM or subsequent progenitor cell mobilization (Katayama et al., 2006). Osteoblasts as well as BM resident neurons do not express GCSFR, indicating that another cellular component mediates this suppressor mechanism. It was suggested that GCSFR expression in the brain, which exerts neuroprotection and neural tissue repair, triggers adrenergic stimulation in response to G-CSF treatment, conducting its message all the way down to the BM endostium (Katayama et al., 2006; Schneider et al., 2005). Adrenergic stimulation positively affects osteoclasts in addition to suppression of osteoblasts, leading to loss of bone mass (Kondo et al., 2005). It is yet to determine whether adrenergic stimulation exert its effect on HSCs mobilization via osteoclasts as well. Apart from showing direct induction of mobilization by the sympathetic system, these new findings broaden the concept of complex microenvironmental regulation of progenitor cell egress, orchestrating bone remodeling processes, dynamic nervous system and immune system interactions with stromal cells, regulating stem cell activity. The nervous system’s influence over HSCs is not restricted to their egress, affecting hematopoiesis in general (Maestroni, 1998), and therefore additional functions are waiting to be discovered.

8. The endothelial barrier

The BM is highly vascularized, and cells that home to the BM or egress out of it have to actively cross endothelial and ECM barriers. Thus, this barrier regulates progenitor cell motility. For example, while the levels of progenitor cells in the circulation and spleen are equally distributed in parabiotic mice, the BM progenitors are mostly host type due to the presence of the BM endothelial barrier (Abkowitz et al., 2003). These low levels of partner derived progenitors are dramatically increased after G-CSF mobilization, documenting that mobilization and homing back to the BM are sequential events with physiological roles (Abkowitz et al., 2003). Transendothelial migration, which is dependent on various adhesion molecules, chemotactic activity as well as ECM degradation machinery, is a key step in both homing and egress processes (Laird et al., 2008). The BM vasculature undergoes striking changes upon stress, such as increased vascular permeability and active angiogenesis, both of which may affect HSPCs egress and mobilization. Vascular trauma results in plasma elevation of VEGF, which promotes recruitment of endothelial cells to the site of active angiogenesis. Upregulation of VEGF expression in the BM is observed during G-CSF treatment due to increased hypoxia in vascular regions (Levesque et al., 2007). VEGF is also capable of mobilizing hematopoietic progenitors in addition to endothelial progenitors, both of which express its receptor (Hattori et al., 2001). Angiopoietin-1 (Ang-1) is another pro-angiogenic factor that is capable of mobilizing HSPCs, but in a delayed fashion (Hattori et al., 2001). Another support for mobilization, which is dependent on changes in BM vasculature, comes from recent observations in mice lacking the gene Nf2/Merlin (Larsson et al., 2008). Those mutant mice exhibited expansion of BM endothelial cells, increased circulating HSPCs as well as increased VEGF expression. Nevertheless, apart from the interesting context, the mechanism is currently unknown. Despite the fact that HSPCs express receptors for both VEGF and Ang-1, it is possible that active angiogenesis in the BM promotes indirectly hematopoiesis and subsequent progenitor cell egress (Rafii et al., 2002). Upon irradiation, chemotherapy or G-CSF administration, the permeability of BM sinuses increases, thus disrupting the BM endothelial barrier (Shirotta and Tavassoli, 1992; Narayan et al., 1994; Szumilas et al., 2005). Pre-conditioning of recipient mice by irradiation for instance, enhances the ability of intravenously injected cells to durably engraft the BM. Likewise, stress-situations can improve the permissiveness of the endothelium. MMP-9, which is highly produced in the BM following G-CSF treatment (Heissig et al., 2002), is able to degrade occludin, one of the components that comprise the endothelial tight junctions (Giebel et al., 2005; Caron et al., 2005). It is yet to be determined whether other components of the endothelial tight junctions are downregulated during stress-induced mobilization or whether other unknown mechanisms controlling endothelial integrity are triggered. The role of the endothelium does not end at broadening the gaps and letting cells out. Endothelial cells, like leukocytes, express CXCR4, and it was found that it has a unique additional function. Bound SDF-1 is internalized and transcytosed via clathrin-coated pit vesicles. Then, it is translocated to the abdominal side of the endothelium and secreted to the BM. The released SDF-1 is functionally able to support and increase the homing of human CD34+ progenitors to the BM of NOD/SCID mice (Dar et al., 2005). This translocation of SDF-1 is CXCR4 dependent and is prevented when CXCR4 inhibitors are introduced. It is not clear yet whether an opposite transcytosis of SDF-1 towards the blood stream occurs upon cell recruitment to the circulation. Surprisingly, preliminary results reveal that addition of AMD3100 to BM stromal cells in culture or its administration in vivo, trigger SDF-1 secretion (Dar et al., 2006). In vivo, this results
in SDF-1 release to the PB, after which motile HSPCs may follow. The mechanism that enables secretion of SDF-1 from the endothelium to the PB is poorly understood and it is possible that SDF-1 transcytosis plays a role in it as well (Dar et al., 2005). Another role for the BM endothelium might be suggested from another direction. It is highly supported today that BM sinusoids serve as a vascular niche for HSPCs. The vascular niche is believed, according to some investigators, to support ongoing self-renewal of HSCs as well as myeloid differentiation in contrast to HSCs quiescence, which is preferentially maintained by the endosteal niche (Sugiyama et al., 2006; Aveillan et al., 2004; Kiel et al., 2005; Wilson and Trumpp 2006). It is intriguing to suppose that the vascular niche can serve also as a selective gateway for HSPCs egress. Does the vascular niche behave differently during stress-situations, regulating hematopoiesis and making the resident HSCs more prone to egress? The BM endothelium is relatively a new player in the field of hematopoiesis and mobilization and therefore much remains to be investigated.

9. The clinical aspect

One of the major aims of the stem cell mobilization research field is to develop improved procedures to harvest HSCs from mobilized blood together with improved reconstitution capacity. Nowadays, G-CSF administration, based on repetitive stimulations, is the predominant clinical procedure to harvest transplantable healthy donor HSCs (Desikan et al., 1998). The engraftment capacity of G-CSF MPB is higher than CB or BM, characterized with faster neutrophil and platelet recoveries, fewer transfusions, faster lymphocyte reconstitution, fewer febrile complications and lower mortality rates (Korbling and Anderlini, 2001; To et al., 1997; Pelus, 2008; Heldal et al., 2000). Furthermore, MPB collection is less invasive than BM harvest and avoids risks associated with general anesthesia. In principle, one has to distinguish between protocols utilized to harvest HSCs for autologous vs. allogeneic transplantations. While human leukocyte antigen (HLA) matched donors undergo G-CSF administration alone, self-donors undergo additional chemotherapeutic treatment in order to eradicate the malignant cells and to synergistically increase circulating transplantable HSCs. Obviously, myeloablative treatment also causes side effects and leads to febrile complications. Yet, many self-donors are poor mobilizers, particularly patients with advanced hematological disorders and malignancies (Pelus, 2008). One of the options to overcome poor mobilization in patients with malignant diseases is by utilizing allogeneic instead of autologous transplantations. From an immunological point of view, autologous transplantation manifests lower incidence of graft versus host disease, leading to sufficient leukocyte recovery and durable reconstitution, but also lack of graft versus cancer cells effects. Moreover, patients require higher doses of mobilized cells to overcome genetic barriers in cases of haploidentity. Since lower cell doses correlate with higher risk of graft failure, improved clinical procedures are sought after. A pegylated form of G-CSF was developed to increase its half-life and reduce its clearance from the blood (Kroschinsky et al., 2008). Moreover, combination of different mobilizing agents together with G-CSF may assist, achieving additive or synergistic effect on the quantities of cells mobilized. One of those mobilizing agents is AMD3100, an antagonist for the CXCR4/SDF-1 pathway (Liles et al., 2003; Liles et al., 2005), which holds a great promise, since it has less severe adverse effects, no impact on the BM population, it induces rapid mobilization in contrast to G-CSF, and acts in a more selective fashion in mobilizing primitive cells. Recently, AMD3100 has been shown clinically to induce mobilization of repopulating cells, in both healthy donors (Devine et al., 2008) and patients (Devine et al., 2004), without the combinational administration of G-CSF. It has recently been approved for clinical usage. T-140 is a novel potent CXCR4 antagonist, which also exhibits rapid mobilization and a synergistic effect when given together with G-CSF in mice (Abraham et al., 2007). In addition to CXCR4 antagonists, another approach that hampers HSC retention and may be useful in the future is the utilization of neutralization antibodies, such as administration of anti-VLA-4 (Papayannopoulou and Nakamoto, 1993) and anti-Kit (Czechowicz et al., 2007). Targeting the niche can be also applicable from the micro-environmental perspective. Notably, studies in mice have shown that both RANKL and norepinephrine selectively mobilize HSPCs into the circulation (Kollet et al., 2006; Katayama et al., 2006; Spiegel et al., 2007). Interestingly, PTH treatment protects the HSC pool from exposure to cytotoxic chemotherapy, in addition to enhancement of G-CSF induced mobilization (Adams et al., 2007). MPB from PTH +G-CSF treated mice has higher engraftment capacity, in comparison to MPB from G-CSF only treated mice. Thus, PTH has beneficial characteristics in terms of the stem cell harvest quality in addition to its quantity. These observations may have a positive impact on designing future clinical protocols, which will target the microenvironment (e.g. bone remodeling and neuronal stimulation), in order to improve the quality of the donor cells in addition to quantitative improvement.

10. Concluding remarks

Today, it is obvious that mobilization of HSPCs does not reflect a leakage of the system in response to stress. During mobilization processes, various mechanisms actively mediate cell egress from the BM to the PB, by breaking retention of HSCs or altering chemotactic gradients, in addition to reinforcement of cell motility. Proteolysis of adhesion molecules or ECM components that promote adherence to the BM niches is a key general mechanism that
Egress and mobilization of hematopoietic stem and progenitor cells enables recruitment of cells to the PB, so as elevating plasma SDF-1 levels concomitantly with decreasing its levels in the BM. Mimicry of one or more mechanisms by each one of the long array of existing mobilizing agents, may lead to mobilization, and therefore many procedures and approaches can be utilized to clinically harvest repopulating progenitor cells from the blood. It should be noted that normal levels of circulating progenitors in some genetic deficiency models (Pruijt et al., 2002) highlight the redundancy in the system. It will be difficult to generate viable mice with multiple deficiencies in order to examine necessity of specific mechanisms in the cell egress and mobilization process. Pinpointing at specific signaling pathways or establishing chimeras could be better approaches to investigate different players in mobilization at details. For example, inhibition of Rac-1 (Cancelas et al., 2005) or PKCζ (Petit et al., 2005), which are known to be activated downstream to CXCR4, leads to mobilization of HSPCs. Another example is the establishment of CD45 KO to wild-type chimeras, in which CD45 deficient cells demonstrate impaired mobilization in comparison to wild-type host cells (Shivtiel et al., 2008). Nevertheless, cell autonomous ability to mobilize is only one side of the story. The growing evidence for the involvement of the microenvironment in the cell egress and mobilization process is an emerging field in stem cell research. Various cellular players, including neutrophils, osteoclasts, osteoblasts, and neurons, as well as BM endothelial cells, play significant roles in mediating cell egress. Each cellular player may affect another cellular player (e.g., adrenergic stimulation suppresses osteoblasts activity), resulting in a complex microenvironmental network of regulatory control over HSC function. Hence, the BM niches should not be regarded as static “homes” for stem cells, in which they reside, but rather dynamic niches that undergo changes on demand, directly affecting hematopoiesis and motility. A broader view on cell egress and mobilization than in the past that includes microenvironmental control opens doors to intriguing theories and brand new conceptual studies.

11. Acknowledgements

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12. References


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