Review

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Innate immune cell trafficking

Hematopoietic stem and progenitor cell trafficking

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Migration of hematopoietic stem cells (HSCs) is essential during embryonic development and throughout adult life. During embryogenesis, trafficking of HSCs is responsible for the sequential colonization of different hematopoietic organs by blood-producing cells. In adulthood, circulation of HSCs maintains homeostasis of the hematopoietic system and participates in innate immune responses. HSC trafficking is also crucial in clinical settings such as bone marrow (BM) and stem cell transplantation. This review provides an overview of the molecular and cellular signals that control and fine-tune trafficking of HSCs and hematopoietic progenitor cells in embryogenesis and during postnatal life. We also discuss the potential clinical utility of therapeutic approaches to modulate HSC trafficking in patients.

Hematopoietic stem cells: characteristic features

In the hematopoietic system, self-renewal capacity is the privilege of rare multipotent cells named hematopoietic stem cells (HSCs). Their closest progeny, hematopoietic progenitor cells (HPCs), can be multipotent, oligopotent or unipotent. Although HPCs lack significant self-renewing capacity, they are capable of further differentiation into mature blood cells of all hematopoietic lineages. HSCs are responsible for the development, maintenance and regeneration of all blood-forming tissues in the body. They are also crucial for long-term engraftment and reconstitution in the setting of bone marrow transplantation (BMT) [1].

HSCs and HPCs are not only crucial to maintain hematopoiesis, but also can contribute to tissue leukocyte homeostasis. Thus, knowing how the cells migrate between bone marrow (BM), blood and peripheral tissues is of great significance. In particular, clinical applications such as BMT and regenerative medicine could benefit from strategies that enhance, inhibit or modulate migration. Here, we examine emerging techniques that can be used to study HSC and HPC migration, and review current knowledge of the mechanisms that control HSC and HPC trafficking throughout the body. A number of *in vitro* and *in vivo* engraftment assays are available by which HSCs and HPCs can be discriminated and characterized both at a phenotypic and functional level [2]. However, when it comes to analysis of *in vivo* HSC migration and the underlying molecular mechanisms, large numbers (several million) of cells are usually required, which are difficult to obtain, especially in mice that each harbor only a few thousand *bona fide* HSCs [3]. As a consequence, most studies that address trafficking do not differentiate between true HSCs and HPCs, but rather analyze cell populations enriched in both stem and progenitor cells. Thus, this review focuses on the combined population of hematopoietic stem/progenitor cells (HSPCs).

Techniques to dissect the mechanisms of HSPC migration

Various experimental tools are currently available to provide information on how HSPCs find their way in the body (Table 1). Using a combination of several techniques, one can obtain a detailed in-depth description of where, when and how HSPCs seed various tissues. Some approaches (such as adoptive transfer experiments, engraftment studies and studies in parabiotic mice) treat the mouse as a 'black box', and answer questions about which cell populations target a specific organ. Other techniques based on single cell visualization address the dynamics of cell movement and enlighten us of the mechanism utilized by migrating cells to seed a particular organ. The latter utilize intravital microscopy (IVM), which involves microscope-based imaging of a microsurgically prepared tissue in a live anesthetized animal. A traditional IVM approach employs epifluorescence illumination and video technology, which is useful to study molecular and biophysical mechanisms of HSPC adhesion to endothelial cells and to characterize intravascular cell behavior. IVM strategies that incorporate a laser light source for multiphoton (MP) excitation, provide 3D imaging of single cells in living tissue and analyze cell–cell interactions in the extravascular space (reviewed in [4,5]).

The anatomical inaccessibility of BM cavities in long bones has long made IVM imaging of undisturbed BM challenging. Early attempts date back many decades, when several IVM models in long bones of rabbits were developed. Later on, BM windows were also placed into the mouse femur [6]. The surgical procedures required to gain access to femur BM in long bones [6,7] are associated with considerable trauma, which raises the possibility that

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Table 1. Techniques to dissect the mechanisms of HSPC migration.

Approach	Principle	Advantage	Disadvantage
Adoptive transfer (homing) experiments	Genetically marked or fluorescently labeled cells are transfused into the circulation of recipient mice. After a relatively short time (up to 72 h), cells are identified by fluorescence-activated cell sorting (FACS) or immunohistology.	Generates information on cell trafficking and retention at the cell population level and identify multiple organs of preferential HSPC accumulation. Provides information on short-term kinetics of HSPC migration into different tissues.	Cannot distinguish between intra- and extravascular events and does not resolve the dynamics of cell movement. Because cells are transfused i.v., many of them are trapped in lungs, so relatively large numbers of cells have to be injected.
Engraftment studies	Genetically marked cells are transfused into the circulation of recipient mice. The read-out is performed at later time points (after several weeks) by FACS-based identification of transfused HSPC progeny.	Generates information on cell ability to proliferate and differentiate in a host. Indirectly, provides information on niche availability and functional capacity.	Cannot distinguish between intra- and extravascular events and does not resolve the dynamics of cell movement.
Parabiosis	Congenic mice are surgically joined so that their circulatory systems fuse and allow exchange of blood-borne cells between partners. Partner-derived cells are analyzed e.g. by FACS.	Can analyze physiological trafficking and engraftment of physiologically low numbers of partner-derived HSCPs. Kinetics of HSCP presence and differentiation can be studied in the peripheral blood of the same animal.	Cannot distinguish between intra- and extravascular events and does not resolve the dynamics of cell movement.
Conventional (epifluorescence illumination) intravital microscopy (IVM) of BM	A microsurgically prepared mouse is placed on a suitable stage under a microscope objective, and BM within long or flat bones is visualized with the help of epifluorescence illumination. Real-time video recording is performed.	Provides information on a single cell level. The setup allows us to study rapid intravascular events at high temporal (i.e. video-rate) resolution. Useful to dissect molecular and biophysiological mechanisms of HSPC adhesion to endothelial cells	Limited ability to observe extravascular events because: (i) cannot provide accurate spatial information in three dimensions; (ii) its ability to image fluorescent events that occur below the surface of a solid organ is restricted by scattering and absorption of excitation and emission light. Visible areas of BM might not represent the state of affairs in the total organ.
Multiphoton (infrared laser light source for excitation) of BM	A microsurgically prepared mouse is placed on a suitable stage under a microscope objective, and skull or femur BM is visualized by laser scanning microscopy.	Provides information on a single cell level. Characterizes extravascular events, such as cell behavior and cell–cell interactions in the interstitial compartment. Generates quantitative information for multiple parameters (e.g. space, time, fluorescence and fluorescence intensity).	Limited ability to observe intravascular events and limited temporal resolution. Analysis is restricted to anatomically accessible BM areas, which might not represent the state of affairs in the total organ.

observations are skewed by a local inflammatory response that might not represent physiological conditions. An alternative IVM technique that maintains tissue integrity visualizes BM in flat bones of the mouse skull calvarium, which is sufficiently transparent to allow observation of BM cavities without requiring surgical manipulation, except for a small skin incision [8,9]. The approach has proved useful to observe cell behavior both within BM vessels [8–10] and in the extravascular space [11,12].

Characteristic features of HSPC migration at different developmental stages

HSPC circulation between tissues is intimately connected with the establishment and maintenance of hematopoiesis, which starts early in embryonic development and continues after birth. Table 2 summarizes trafficking molecules expressed on HSPCs during different developmental stages and their role in HSPC migration.

HSPC migration in fetal life

The development of embryonic hematopoiesis and the multiple tissues that demonstrate hematopoietic activity at early stages of gestation have recently been reviewed [13]. Mammalian hematopoiesis in mice is first represented by nucleated erythrocytes in the yolk sac (Figure 1). This primitive hematopoiesis is then replaced by adult-type definitive hematopoiesis and HSPCs capable of engrafting upon transfer to another recipient are first found in the yolk sac and the aorta–gonad–mesonephros (AGM) region. The placenta is another source of HSPCs in the developing mouse embryo. However, it is not clear whether placental HSPCs colonize the organ via the circulation or arise through *de novo* generation [14], or both. Also, the exact contribution of HSPCs from the above tissues to definite hematopoiesis remains a matter of debate. HSPCs are first found in the fetal liver (FL), the major site of fetal hematopoiesis at E10, where they expand and differentiate. Soon after FL colonization, more differentiated HSPCs colonize the thymus [15] and spleen [16]. Shortly before birth, HSPCs eventually start to seed the BM [17], which is the site of permanent adult hematopoiesis (Figure 1). At that point, FL HSPCs become quiescent and exhibit only limited proliferative activity [18].

HSPC trafficking in adulthood

Lodging in the BM by no means indicates the final stop in the journey of HSPCs. In fact, at least some HSPCs maintain a migratory phenotype throughout postnatal life. Several studies have shown a constant exchange of HSPCs between BM and peripheral blood [12,19] (Figure 1). It has

Table 2. Trafficking molecules expressed by fetal and adult murine HSPCs.

Trafficking molecule	Role in HSPC migration	Developmental stage of expression
Adhesion receptor		
N-cadherin (CD325)	Retention of quiescent HSPCs within the BM niche	Quiescent adult HSPCs [28]
VE-cadherin (CD144)	HSPC trafficking to and from fetal hematopoietic tissues	Fetal HSPCs (yolk sac, AGM and placenta) [44,122]
αIIb integrin (CD41, glycoprotein IIb (GPIIb))	HSPC trafficking to and from fetal hematopoietic tissues, trafficking of adult HSPCs to the BM compartment	Fetal HSPCs (yolk sac, AGM and placenta) [47,123] Subset of adult HSPCs
α4 integrin (CD49d)	Retention of adult HSPCs within BM (α4β1), homing to BM microvessels via α4β1 and α4β7	Adult BM-derived HSPCs [8,54,86]
β1 integrin (CD29)	Colonization of FL, retention of adult HSPCs within BM (α4β1)	Fetal and adult HSPCs [48]
β2 integrin (CD18)	Retention of adult HSPCs within BM	Adult BM-derived HSPCs [60,61]
CD44 and HCELL (hematopoietic cell E- and L-selectin ligand)	Homing to BM microvessels	Adult BM-derived HSPCs [88,124]
PSGL-1	Homing to BM microvessels and to peripheral tissues (skin)	Adult BM-derived HSPCs [8]
Chemoattractant receptors		
CaR	Receptor for calcium, lodgment of BM HSPCs in endosteal niche	Adult HSPCs [72,73]
cKit (tyrosine kinase receptor)	Receptor for SCF, retention in the FL, interaction with stromal cells in adult BM	Fetal and adult HSPCs [63–65]
CXCR4	Receptor for CXCL12 (stromal cell-derived factor-1), Major guidance signal for BM colonization, retention in BM hematopoietic niche, migration to peripheral organs in response to tissue injury	FL HSPCs during late fetal development [50] Adult HSPCs [53]
S1P1	Receptor for the signaling lipid S1P, exit of HSPCs from peripheral non-hematopoietic tissues	Fetal and adult HSPCs [12]

been estimated that up to 400 HSPCs circulate in the blood of a mouse at any one time. These cells constantly re-engraft the BM and are continuously replenished by HSPCs that are newly mobilized from the BM [19]. Such BM re-engraftment occurs under physiological conditions in the absence of myelosuppression and does not involve or require inflammation. In fact, the recirculation of HSPCs between BM and blood is believed to be important for the maintenance of hematopoietic homeostasis [19].

The efficiency of BM re-engraftment by HSPCs depends on the availability of certain anatomical/morphological structures in the BM known as niches [20] (Figure 2). The highly specialized niche microenvironment controls self-renewal and lineage differentiation of HSPCs (reviewed in [21,22]). In unmanipulated animals, the number of available niches is low (50–500 per mouse) [23]. To empty niches for newly arriving HSPCs, hematoablative treatments (such as irradiation, myelosuppressive drugs, and cytokines) are routinely used. Alternatively, hematopoietic engraftment in unirradiated mice can be enhanced by antibody-mediated depletion of the endogenous HSPCs of the recipient [24].

Migration of adult HSPCs occurs not only to and from the BM, but also within the BM [25]. Most primitive HSPCs are located in the subendosteal region of the BM cavity of long bones where osteoblasts are thought to produce essential growth factors [26,27] and provide regulatory signals for primitive stem cells [28–30]. In addition to osteoblasts, nestin$^+$ mesenchymal stem cells have been recently identified to contribute another essential component of the HSC niche in the BM [31]. Upon maturation, primitive HSPCs lose their quiescence and migrate towards the center of the BM cavity to proliferative niches [32–34]. The existence of specialized niches to which HSPCs migrate during their differentiation might explain the differential distribution of myeloid and lymphoid populations in the BM. For example, one of the cellular components of the niche, endothelial cells, appears to play key roles in megakaryopoiesis, because mature megakaryocytes are almost exclusively localized near thin-walled sinusoids [35]. By contrast, lymphoid progenitors are found in close contact with osteoblasts [32]. In addition, even phenotypically identical HSCs obtained from different anatomical regions of the BM have recently been found to differ with regards to their biological potential [36].

Although it had been known for a long time that HSPCs travel within the BM and from the BM into the blood and *vice versa*, an additional route of HSPC recirculation has recently been identified [12]. BM-derived HSPCs travel from the blood into multiple peripheral tissues, from the tissues into the lymph, and from the lymph via the thoracic duct, the main draining lymph vessel in the body, back into the blood. Once in the circulation, HSPCs can either re-enter the BM or repeat the peripheral migration cycle (Figure 1). It is still unclear whether the subset of HSPCs that embark on this voyage are somehow different from the bulk of HSPCs, which are found in the BM–blood compartment, or whether all circulating HSPCs have the choice and potential to patrol peripheral tissues. The recirculating HSPCs can differentiate into immune and inflammatory effector cells under certain conditions, such as distress signals that activate HSPC-expressed Toll-like receptors (TLRs), during infection or tissue damage. This differentiation can occur in peripheral tissues and is thought to provide a local source of needed immune cells, such as dendritic cells (DCs) and other myeloid cell types (Figure 3). Recent evidence has suggested a role for TLR2 and TLR4 in the differentiation of mouse HSPCs into myeloid lineage cells [37]. In addition, it has been demonstrated that TLR7 and TLR8 are expressed on

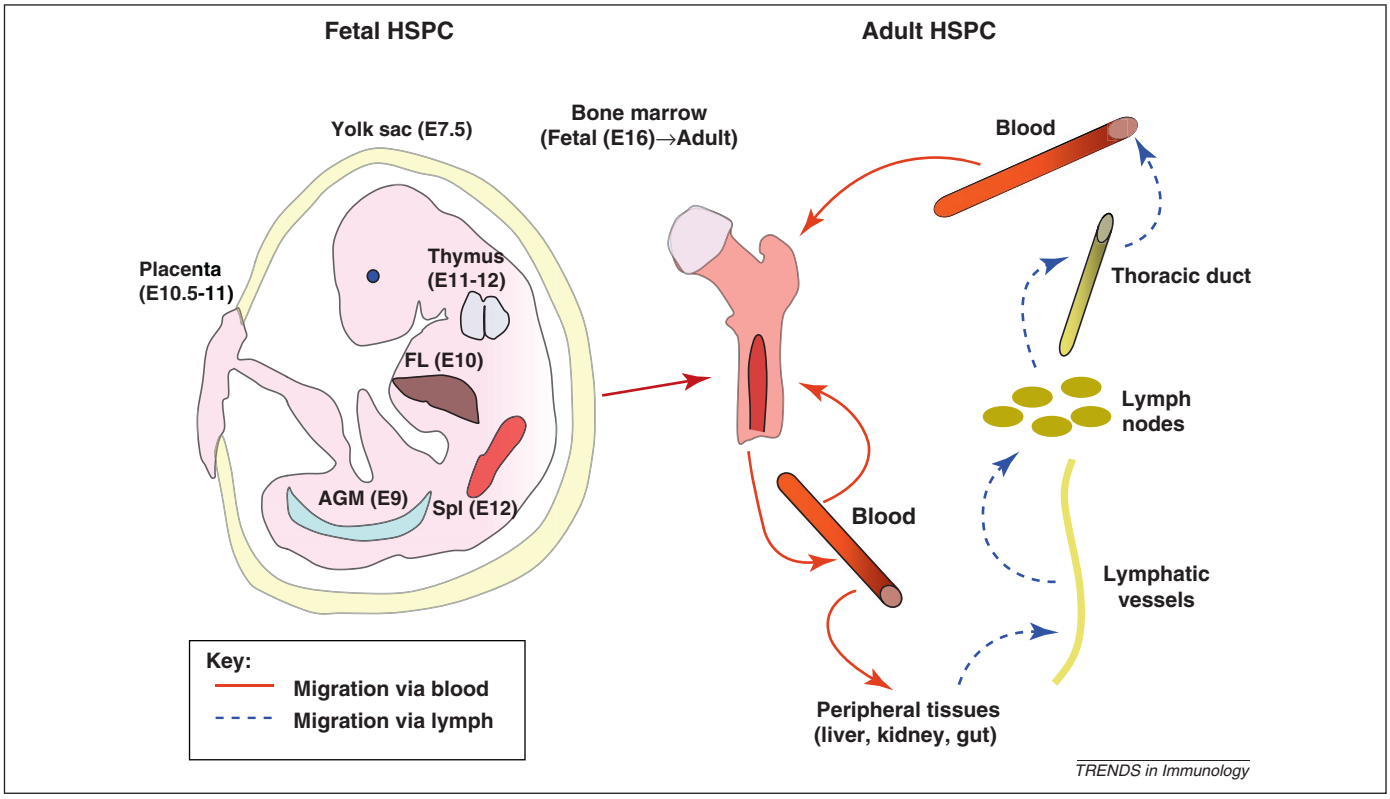

Figure 1. Trafficking of HSPCs in fetal life and during adulthood. Left panel: fetal HSPCs originate in the yolk sac and migrate to the AGM region and placenta. Alternatively, placental HSPCs originate *de novo*. Very soon after that, HSPCs from early embryonic hematopoietic sites colonize the FL, which becomes the main hematopoietic organ during fetal development. Subsequently, FL emigrants inhabit the thymus, spleen and BM. BM becomes the main organ of adult HSPC development (right panel). The majority of HSPCs reside in the BM where they undergo self-renewal and give rise to differentiated hematopoietic cells. However, some HSPCs continuously leave the BM and enter the blood. Circulating HSPCs either return to the BM or migrate to peripheral organs, which they exit via lymphatics. The major lymph vessel in the body, the thoracic duct, drains into the venous circulation, therefore, HSPCs can reach the BM from the periphery via the blood. Spl, spleen; numbers in parenthesis, day of gestation when a fetal organ is colonized with HSPC.

human BM $CD34^+$ progenitors, and activation of these TLRs causes differentiation of HSPCs into macrophages and monocytic DC precursors [38]. The locally generated HSPC-derived leukocytes can participate in classical innate immune responses and contribute to early eradication of local infection, elimination of dead cells, and replenishment of tissue-resident DCs lost during infection due to cell death or migration into draining lymph nodes (reviewed in [39]). HSPCs have also been claimed to give rise to hepatocytes in a liver injury model [40]. Although this so-called transdifferentiation of HSPCs is controversial, the finding raises the intriguing possibility that migratory HSPCs might have a role during the repair of hematopoietic, as well as non-hematopoietic tissues.

How HSPC migration is controlled in fetal life

Migration of fetal HSPCs between tissues is essential to establish the hematopoietic system during embryonic development. As a result of limitations in the intrauterine accessibility of embryos, it is technically challenging to visualize directly fetal HSPCs in their native environment, therefore, only a few studies have managed to observe and track fetal HSPCs directly *in vivo* [41–43]. Moreover, the placenta poses a barrier that hinders the passage of cells, antibodies, fluorescent dyes and other compounds, making it difficult to dissect the molecular mechanisms of cell movement in the fetal circulation. Thus, our knowledge regarding the molecular mechanisms that orchestrate fetal HSPC migration is currently based mostly on differential expression of trafficking molecules on HSPCs isolated from various embryonic organs. In addition, analysis of fetal hematopoiesis in genetically modified mice as well as in *in vitro* migration assays has provided valuable information on how HSPCs find their way within the fetus.

In murine fetal yolk sac, AGM and placenta, HSPCs express CD144, also known as vascular endothelium (VE) cadherin [44], a cell-surface glycoprotein that mediates Ca^{2+}-dependent homophilic cell–cell adhesion. Upon transition to the FL, HSPCs downregulate CD144 expression and become $CD144^-$ by E16.5, at which point they leave the FL and colonize the BM [45]. The surface expression of CD144 is a unique feature of fetal HSPCs, which is not shared by their adult counterparts. However, whether CD144 directly controls fetal HSPC trafficking remains unclear.

Integrin-mediated adhesion is important for HSPC movement in embryogenesis and adulthood (see later discussion). HSPCs in the yolk sac, AGM and placenta express CD41 (GPIIb integrin encoded by the gene *Itga2b*). Expression gradually decreases during development (reviewed in [46]), and adult HSPCs express little or no CD41 [47]. It has been shown that inactivation of the *Itga2b* allele results in increased numbers of HSPCs in various embryonic sites; an effect that might be explained, at least in part, by loss of binding to fibronectin [47], suggesting a potential role of CD41 in retention of HSPCs in their niche (reviewed in

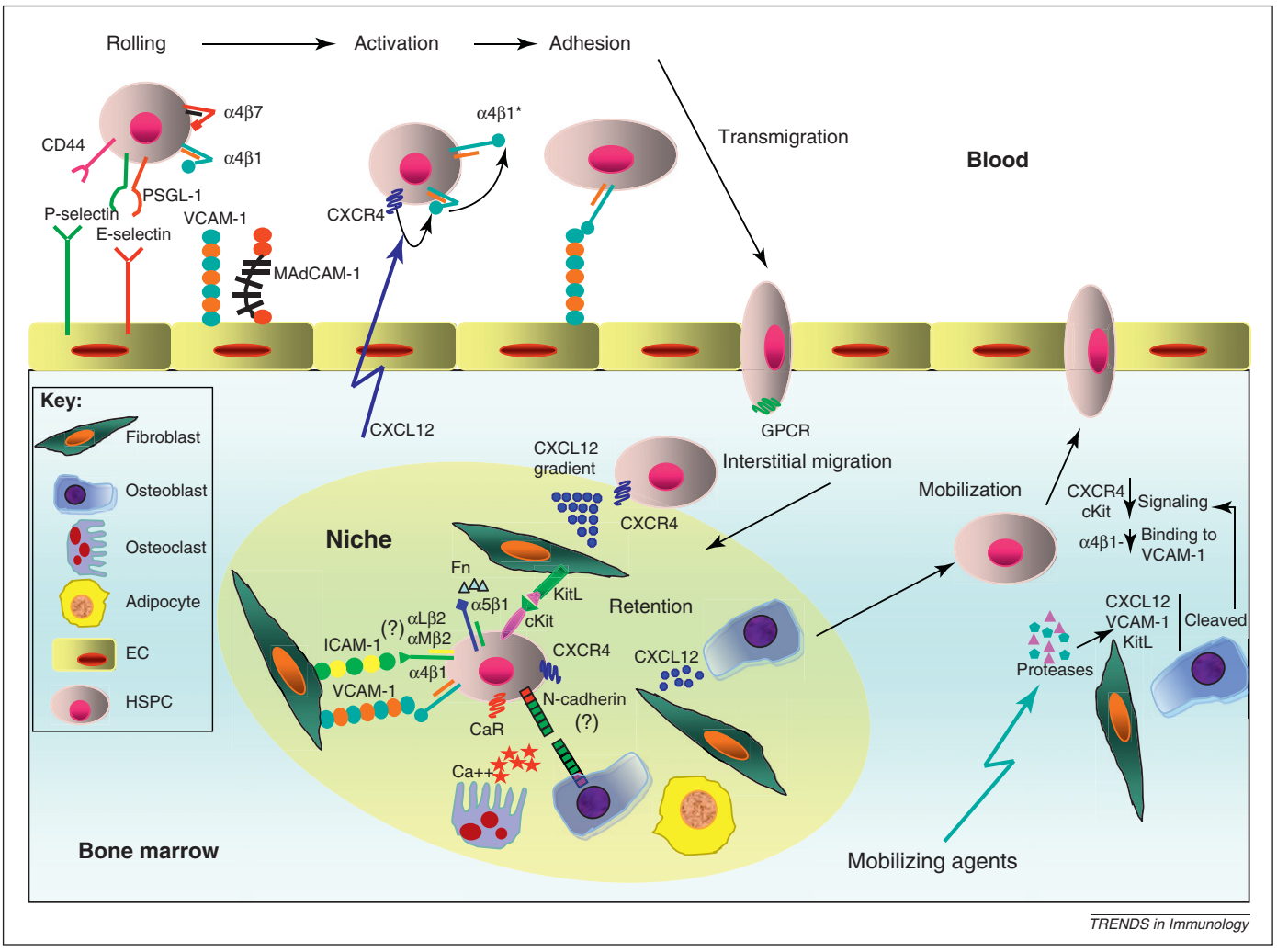

Figure 2. Migration of HSPCs within BM. HSPCs enter the BM through sinusoids, which constitutively express traffic molecules that support a unique multistep cascade for HSPC homing. First, circulating HSPCs tether to the vessel wall by engaging vascular selectins, P- and E-selectin, which bind to carbohydrate ligands that are associated with PSGL-1 and/or CD44 (HCELL) on HSPCs. Tethered cells then roll slowly, engaging both endothelial selectins and the integrin α4β1/VCAM-1 pathway. Some studies have also implicated α4β7/MAdCAM-1 in the rolling step. The rolling HSPCs are then activated by the chemokine CXCL12, which signals through CXCR4. The chemokine signal induces a conformational change in α4β1 (α4β1*), resulting in increased affinity for VCAM-1, which mediates firm arrest. Next, the adherent HSPCs transmigrate through the vessel wall following extracellular chemoattractants (possibly CXCL12) that signal through G-protein-coupled receptors. Extravascular trafficking allows HSPCs to lodge in specific niches, represented by various stromal cells (fibroblasts, osteoblasts, osteoclasts and adipocytes). Stromal cells maintain the prerequisite conditions for HSPC survival and function in the BM. HSPC retention in niches is mediated by interactions of α4β1 with VCAM-1 and fibronectin (the later also interacts with another β1 integrin – α5β1), and β2 integrins with ICAM-1, CXCR4 with CXCL12, and cKit with its ligand (KitL). Retention also involves CaR, a member of the large G-protein-coupled receptor family, and homotypic adhesion via N-cadherin. Under homeostatic and stress-induced conditions, some BM-resident HSPCs de-adhere and leave the BM (a process known as mobilization). HSPC mobilization involves upregulation of proteolytic enzymes (MMP-9, cysteine protease cathepsin K, dipeptidase CD26), which leads to cleavage of CXCL12, KitL and VCAM-1. As a result, HSPCs can detach from the BM stromal cells and enter the circulation. Fn, fibronectin; ICAM-1, intercellular adhesion molecule-1.

[46]). In addition to CD41, β1 integrins tune the migration of fetal HSPCs. The use of chimeric mice generated with β1-integrin-deficient fetal HSPCs has revealed that fetal HSPCs lacking β1 integrins form and differentiate but they cannot colonize the FL [48], suggesting an essential role of β1 integrins in fetal HSPC trafficking.

Chemokines and other chemoattractants are also important in fetal HSPC trafficking. Among these, the role of stem cell factor (SCF), also known as Kit ligand (KitL), which binds to the tyrosine kinase receptor cKit, is well established. Fetal HSPCs express high levels of cKit from early stages throughout embryonic development [49]. SCF exerts chemoattractant effects on early fetal HSPCs [17] and thus promotes their retention and controls their dwell time in the FL. Unlike adult HSPCs, early FL HSPCs do not respond to CXC chemokine ligand (CXCL)12, a major guidance signal for BM colonization [50]. However, at later stages of embryonic development, FL HSPCs acquire the ability to migrate towards CXCL12 and to colonize the BM [17]. The observation that embryos with a genetic deficiency in either CXCL12 or the major HSPC-expressed G-protein-coupled receptor for CXCL12, CXC chemokine receptor (CXCR)4, develop severe hematopoietic defects underscores the pivotal role of the CXCL12–CXCR4 axis in fetal hematopoiesis [51–53].

How HSPC migration from and into adult BM is controlled

Mechanisms for HSPC exit from BM

Anchorage of HSPCs within the BM is mediated by interactions of multiple HSPC-expressed receptors with their respective stroma-expressed ligands or soluble factors (Figure 2). Extensive studies with blocking antibodies [54], functional antagonists [55] and genetically deficient

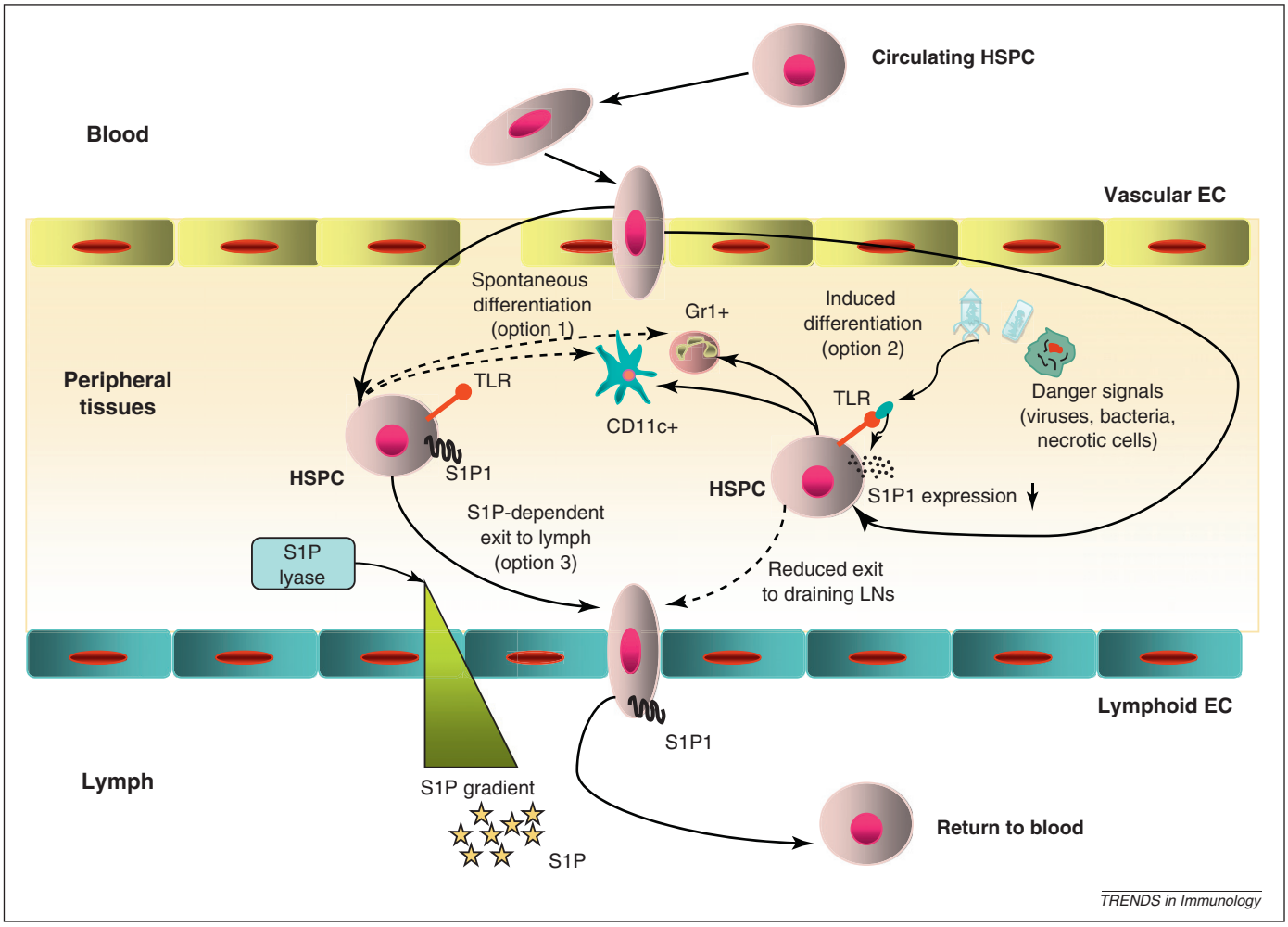

Figure 3. Migration of HSPCs within peripheral tissues. Blood-borne HSPCs have the capacity to enter peripheral non-hematopoietic tissues from the blood. The mechanisms that control this process are still poorly understood. In the organs, some HSPCs can spontaneously differentiate into immune cells (option 1). This differentiation dramatically increases under stress conditions (such as infection or tissue necrosis), because HSPCs receive signals via TLRs that promote myeloid differentiation (option 2). HSPCs that do not undergo differentiation spend ~48 h within peripheral tissues before they access local draining lymphatics (option 3); a migration step that depends on S1P and S1P1. The free S1P level is high in the lymph but low in tissues owing to constant degradation of S1P in the latter by S1P lyase. When HSPCs follow the S1P gradient and enter the lymph, they return to the circulation to access the BM or home into another peripheral tissue.

mice [56–58] have shown the particular importance of two molecular pathways for the retention of HSPCs within BM at steady-state: the adhesion molecules α4β1 integrin and vascular cell adhesion molecule (VCAM)-1, and the CXCR4–CXCL12 chemokine pathway. Additional pathways probably participate in a cooperative or synergistic fashion (reviewed in [59]). The role of the so-called leukocyte integrins that share the common β2 (CD18) chain, αLβ2 (lymphocyte function-associated antigen-1 or CD11a/CD18) and αMβ2 (macrophage-1 antigen, CD11b/CD18), is still under debate. Although some studies have reported their involvement in HSPC retention [60,61], others have indicated that the effect of β2 integrins becomes apparent only in synergy with α4β1 [62].

Signaling via the cKit–KitL pathway regulates adhesion of HSPCs to stroma by inducing activation of β1 integrins [63–65] and by promoting chemotactic and chemokinetic responses in HSPCs [66]. Moreover, several studies have demonstrated that the transmembrane form of KitL displayed on stromal cells can confer mechanically stable interactions with HSPC-expressed cKit, thus playing a direct role in cell–cell adhesion [67,68]. Another potential player in HSPC trafficking is N-cadherin, which is present on both osteoblasts and quiescent HSPCs [28]. Based on expression studies, it has been suggested that N-cadherin facilitates HSPC retention in the niche, however, the role of N-cadherin in HSPC biology has been challenged by recent observations that N-cadherin$^+$ BM cells do not possess stem cell properties [69]. Thus, there might be other molecular mechanisms used by stroma cells to retain HSPCs in the BM. Indeed, BM resident osteoblasts are a rich source of CXCL12 in humans and mice [29,70,71], thus providing an abundant local source for this potent HSPC-attracting chemokine.

Osteoblasts and osteoclasts are thought to form a functional unit tasked with physiological bone remodeling at the BM–bone interface, which is characteristically associated with a local increase in free Ca^{2+}. HSPCs can respond to variations in extracellular Ca^{2+} through the Ca^{2+} receptor (CaR), a G-protein-coupled receptor that is expressed on their surface. In the absence of CaR, HSPCs are defective in their ability to lodge in the endosteal niche, and thus do not engraft after transplantation [72]. Correspondingly, pharmacological stimulation of CaR has recently been

demonstrated to enhance CXCR4-mediated lodgment of HSPCs at the endosteal niche [73].

Despite the continuous BM retention signals that are received by HSPCs through the pathways discussed, there is always a small but significant fraction of HSPCs in the blood circulation and peripheral tissues. Experimental evidence has indicated that these extramyeloid HSPCs undergo a de-adhesion step and manage to escape from the BM by migrating into the blood; a phenomenon known as mobilization. Certain drugs can greatly enhance HSPC mobilization; an effect that has been exploited clinically to collect circulating donor HSPCs for transplantation (reviewed in [39,59]). A variety of cytokines [granulocyte colony-stimulating factor (G-CSF) is the most commonly used among them], chemokines (such as interleukin-8 and growth-regulated oncogene-β) and small molecule drugs have been used as mobilizing agents. HSPC mobilization from the niche involves upregulation of proteolytic enzymes expressed either by stromal elements [such as matrix metalloproteinase (MMP)-9 and cysteine protease cathepsin K] [34,74] or by HSPCs (e.g. the dipeptidase CD26) [75]. Activation of these enzymes leads to proteolytic cleavage of CXCL12, KitL and VCAM-1, and thus loosens the adhesive contacts between stromal cells and HSPCs.

In recent years, the role of the sympathetic nervous system (SNS) in HSPC traffic has received increasing attention [76]. The SNS innervates bone and BM stromal cells, and hematopoietic and stromal cells express neurotransmitters and neuropeptides, as well as their receptors in the BM (reviewed in [76,77]), therefore, it has been proposed that the SNS could play a role in BM homeostasis [78]. Indeed, SNS signals contribute to G-CSF-induced HSPC mobilization via β-adrenergic stimuli [79]. Under homeostatic conditions, the number of blood-borne progenitor cells in humans and mice is subject to circadian oscillations [80,81]. Studies in mice have demonstrated that circadian norepinephrine secretion leads to downregulation of CXCL12 and thus promotes HSPC egress from the BM [82].

Mechanisms for migration of HSPCs into the BM

HSPC homing, that is, migration from blood into the BM, is crucial for the clinical success of BMT. Furthermore, homing is integral to the postnatal process during which HSPCs are in constant exchange between BM and blood, to ensure homeostasis of hematopoietic activity throughout the blood-forming skeletal system [19]. To home to the BM, HSPCs must recognize endothelial cells in BM microvessels and adhere to them to resist the constant hydrodynamic shear that is exerted by the flowing blood. This recognition is achieved by interactions of HSPC-expressed adhesion molecules and chemokine receptors with endothelium-expressed binding partners, whose composition is unique to the BM microvasculature.

As a rule, the recruitment of circulating hematopoietic cells, including HSPCs, involves a sequence of at least three intravascular adhesion steps before the cells can egress into a target tissue [83,84]. The initial step, which allows the fast flowing cells to tether and slowly roll along the vascular lining, is mediated by primary adhesion molecules, which engage their respective ligands with fast binding kinetics, but a short bond lifetime. To adhere firmly, the rolling cells must receive an activation signal that is provided by soluble or surface-bound chemoattractants. In most cases, this activation signal depends on Gαi protein-coupled receptors, and can be blocked by pertussis toxin treatment. In addition, chemoattractant signals that involve Gαs have been implicated in BM homing and engraftment [27]. The activation signals trigger a conformational change in secondary receptors, namely leukocyte-expressed integrins, into a high affinity conformation, which is required for their binding to endothelial ligands of the immunoglobulin superfamily. This secondary, activation-dependent step mediates firm adhesion. Subsequently, the adherent cells transmigrate along chemoattractant gradients into and within the target tissue (reviewed in [85]).

IVM experiments have provided definitive information on the molecular pathways that mediate HSPC interactions with BM sinusoids during each consecutive step in the homing cascade (Figure 2). BM microvessels express the endothelial selectins, P- and E-selectin, as well as VCAM-1 [50]. In vascular beds outside the BM, these trafficking molecules are rarely present on endothelial cells without inflammatory stimulation. In fact, the BM is the only organ in which constitutive side-by-side expression of these three adhesion molecules has been demonstrated [9]. Interactions of endothelial selectins and VCAM-1 with their HSPC-expressed counter-receptors, P-selectin glycoprotein ligand (PSGL)-1 and low affinity α4β1 (very late antigen-4), respectively, supports HSPC tethering and rolling, whereas firm adhesion is mediated by the (chemoattractant-induced) high affinity form of α4β1 [8]. The role of another α4 integrin – α4β7 – is not completely understood. Some studies have implicated α4β7 in HSPC rolling [86] and have reported expression of the α4β7 ligand, mucosal addressin cell adhesion molecule (MAdCAM)-1, on BM endothelium [87], but others could not detect a role for this pathway in HSPC homing to BM [8]. In addition, HSPCs also express CD44, a molecule involved in rolling of hematopoietic cells [88]. A major ligand of CD44 is hyaluronate, an anionic extracellular matrix glycosaminoglycan that is widely expressed in connective, epithelial and neural tissues, including the BM [89,90]. In addition, a specialized glycoform of CD44, called hematopoietic cell E- and L-selectin ligand (HCELL), has been identified on early immature HSPCs. *In vitro* studies have indicated that HCELL binds to E- and L-selectin with higher affinity than PSGL-1; the most common selectin ligand on differentiated leukocytes [88,91,92].

Although there are numerous chemokines in the BM [93,94], only CXCL12 has been shown to be functionally relevant for HSPC recruitment [95,96]. CXCL12/CXCR4 signaling is not only responsible for HSPC retention and mobilization, but also for activation of integrins, which in turn mediate firm adhesion of HSPCs [96]. However, HSPCs are capable of homing to adult BM also via CXCL12-independent mechanisms [50], indicating that additional, yet unknown routes are involved in colonization of this organ. Accordingly, although CXCR4- and CXCL12-deficient mice die during fetal development, they do develop a BM compartment [52,53]. Nevertheless, in adult BM, primitive HSPCs ($Lin^{-}Thy1^{lo}cKit^{+}Sca1^{+}$) express a restricted reper-

toire of chemokine receptors and respond only to CXCL12 [97]. This raises the possibility that HSPCs respond to other (non-chemokine) chemoattractants. Indeed, HSPCs migrate towards gradients of the sphingolipid sphingosine 1-phosphate (S1P) to access lymph vessels from peripheral tissues (see below) [12]. However, the concentration of S1P in the BM is high in blood and low in the extravascular space [98], which makes it unlikely that this pathway contributes to HSPC homing to the BM.

How HSPC migration to and from adult peripheral tissues is controlled

The exact mechanisms that mediate retention of HSPCs in, as well as the egress from peripheral organs and the recruitment pathways responsible for HSPC trafficking from blood into extramyeloid compartments remain to be elucidated. Here, we summarize what is known so far with respect to migration into and exit from peripheral (non-BM) tissues.

Pathways for HSPC migration into non-BM tissues

Under physiological conditions, HSPCs of different maturation stages (i.e. ranging from the most primitive stem cells to lineage-committed progenitors) are found in the liver, lungs, intestines, kidneys [12,19], thymus [99], and skin [100]. Tissue damage facilitates HSPC homing into sites of injury [101–103]. The observation that HSPCs lodge in multiple organs is not surprising, because CXCL12 is produced constitutively not only by stromal cells in the BM, but also in various other tissues by different cell types, including endothelial cells and tissue DCs [70,104,105]. Moreover, CXCL12 upregulation has been noted under stress conditions [103,106]. Apart from CXCL12, E-selectin has been implicated in HSPC homing to the BM and the skin (reviewed in [100]). By contrast, stem cell homing to the spleen, a hematopoietic site in mice, does not depend on VCAM-1/α4β1 interaction, which is essential for BM colonization [107]. Thus, some but not all peripheral sites use HSPC recruitment signals similar to those that operate within the BM.

Pathways for HSPC exit from non-BM tissues

Little is known about the signals that regulate the exit of HSPCs out of tissues other than the BM. HSPCs arrive in peripheral organs via blood, but leave them predominantly via the draining lymphatics [12,39]. It has recently been shown that egress of HSPCs from peripheral tissues depends on S1P and S1P receptors (S1P receptor 1, or S1P1, in particular); a signaling pathway that has been implicated previously in lymphocyte exit from secondary lymphoid organs into the lymph [108–110]. The concentration of S1P is high in the lymph, but low in tissues due to degradation by S1P lyase, and HSPCs follow this gradient to leave peripheral organs (Figure 3). Drug-induced inhibition of S1P–S1P receptor signaling decreases the number of HSPCs in tissue-draining lymph due to impaired egress from the tissue. Stress signals that mimic an infection (induced by administration of a TLR4 agonist) reduce S1P1 expression on HSPCs, resulting in prolonged retention in peripheral organs, thus providing necessary time for HSPCs to differentiate into immune cells that are required to eliminate the danger. Tuning of HSPC retention in peripheral tissues could support innate immune responses by fostering a local and versatile supply of effector cells.

Clinical perspective and concluding remarks

A better understanding of the molecular cues that control the trafficking of HSPCs is crucial in several clinical settings; most prominently, BMT, and might lead to novel options to treat leukemia and other malignancies.

A successful outcome after BMT largely depends on the ability of intravenously injected HSPCs to find their way rapidly to specific hematopoietic niches. Several potential sources of HSPCs are routinely used in clinical protocols, including (i) HSPCs directly derived from the BM; (ii) HSPCs directly derived from cord blood; and (iii) HSPCs isolated from the peripheral blood after their mobilization from the BM by administration of drugs or cytokines. Although HSPCs from the BM reconstitute the myeloablated recipient BM rapidly [111], their use in allogeneic BMT is limited by the availability of fully HLA-matched donors [112]. In this respect, HSPCs from cord blood provide sufficient engraftment in adoptive recipients [113,114]. However, a potential caveat of this approach lies in the delayed hematopoietic reconstitution afforded by cord-blood-derived HSPCs. This in turn leads to increased incidents of early post-transplant complications. Reduced levels of surface-expressed homing-related molecules by cord blood HSPCs [115] is considered, at least in part, to contribute to this problem. As a consequence, various protocols utilizing panels of cytokines are in use to enhance the expression of adhesion molecules, MMPs and CXCR4 on transplanted cells [115,116]. Indeed, faster hematopoietic recovery directly correlates with the efficacy of migration of HSPC to the BM [117].

A better understanding of the signals that control HSPC trafficking could also be of clinical relevance beyond their obvious utility in BMT. Similar to many forms of cancer, certain leukemias are considered cell-autonomous stem cell disorders. For example, it has been demonstrated in acute myeloid leukemia (AML) that a small subset of AML cells has long-term repopulation potential and is capable of propagating and maintaining the disease [118]. These so-called leukemia stem cells (LSCs) possess similar self-renewal capacity as normal HSCs. Unlike non-hematopoietic cancer stem cells, LSCs depend on a specific microenvironment and microdomains within the BM (reviewed in [119,120]), and analogous to normal HSPCs, the trafficking of LSCs relies on expression of distinct adhesion and chemotactic molecules. In fact, recent findings have suggested that the molecular machinery used by normal HSPCs for homing and migration to supportive niches may be 'hijacked' by LSCs, and that LSCs occupy and receive crucial signals from the microenvironment that usually supports self-renewal of healthy HSCs. Correspondingly, molecular signatures involved in the trafficking of HSPCs, such as CXCL12 and CXCR4 or α4β1 integrin, have also been implicated in promoting LSC migration into the BM [121].

LSC migration and lodgment in niches is not only involved in leukemia maintenance and spreading, but also

has been implicated in primary resistance to chemotherapy, therefore, the dissection and targeting of LSC trafficking could offer new possibilities to treat leukemic diseases in the future.

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