REVIEW

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Innate immunity: a key player in the mobilization of hematopoietic stem/progenitor cells

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Abstract

The mobilization of hematopoietic stem/progenitor cells (HSPCs) from bone marrow into peripheral blood (PB) is still not fully understood. Different chemokines, cytokines, growth factors, and neurotransmitters have been described that facilitate this process. However, mounting evidence suggests that mobilization of HSPCs is a part of the immune response and is mediated by innate immunity. We discuss evidence showing that complement system cleavage fragments play a crucial role in both the retention and mobilization of HSPCs by modulating their responsiveness to stromal-derived growth factor-1 (SDF-1) gradient (by C3-derived anaphylatoxins) and by modulating the release of granulocytes into PB that subsequently facilitate the egress of HSPCs (by C5-derived anaphylatoxins).

Key words: complement, stem cell mobilization, CXCR4, SDF-1.

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INTRODUCTION

Innate or natural immunity is inborn and thus naturally present in an organism. It does not require prior sensitization to an antigen after exposure during infection or vaccination. Because it is not stimulated by specific antigens, innate immunity is generally nonspecific, in contrast to acquired immunity. In this review we present evidence that innate immunity is a key player regulating the mobilization of hematopoietic stem/progenitor cells (HSPCs) and that the mobilization of these cells could be envisioned as part of the response to infection and tissue injury.

It is well known that HSPCs circulate in peripheral blood (PB) under steady-state conditions at very low levels to maintain a pool of stem cells in balance in the bone marrow (BM) microenvironment present in bones throughout the body. Therefore, PB could be envisioned as a "highway" by which HSPCs relocate in the organism between hematopoietic endosteal and endothelial niches usually located in distant bones. It has been calculated that in mice, approximately 400 HSPCs circulate at any given moment in the PB (Goodman and Hodgson 1962; Wright et al. 2001). In addition, recent evidence suggests that while circulating in PB, HSPCs may enter tissues and return back to the PB via the lymphatic system and thoracic duct (Massberg et al. 2007). This route of "circulation/tissue patrolling" is enforced during infection, in which circulating HSPCs mobilized during infection could be recruited to the affected peripheral tissues and give rise to tissue-resident myeloid and dendritic cells. This mechanism is thus an important part of innate immunity surveillance under steady-state conditions and is enhanced in response to inflammation or organ injuries (e.g. heart infarct, or stroke).

The enforced migration of HSPCs from BM into PB is called mobilization and can be induced by some pharmacological agents (Bensinger et al. 2009; Fruehauf et al. 2005; Lapidot et al. 2005; Papayannopoulou 2004; Winkler and Levesque 2006). From a clinical point of view it is an important procedure that provides HSPCs for hematopoietic transplantation. In this review we discuss augmenting evidence that HSPC mobilization is regulated by elements of innate immunity, in particular by complement cascade (CC) proteins, cleavage frag-

ments, neutrophils, and Toll receptors (TRs), which all play a pivotal but, until recently, underappreciated role in this process.

PHARMACOLOGICAL MOBILIZATION OF HSPCs

From the historical point of view, the first hematopoietic transplants were performed with HSPCs aspirated from BM cavities. This required multiple aspirations of the iliac crests in donors, from which BM was harvested for transplantation. However, instead of being taken from BM, HSPCs are more and more frequently isolated for transplantation purposes from mobilized PB (Henon et al. 1992; To et al. 1992). The reasons for this are twofold. First, PB isolation is the easier and less stressful way to harvest such cells compared with multiple aspirations of BM cavities. Second, mobilized HSPC transplants lead to faster recovery of blood platelets and granulocytes after transplantation.

During the mobilization procedure the patient is exposed to an agent that induces HSPC egress from BM into PB. Cells that circulate in PB are subsequently harvested by leukapheresis. Generally, mobilization and HSPC harvest from PB can be performed on the patient or on a major histocompatibility complex matched related or -unrelated donor to obtain cells for autologous or allogeneic types of transplantation, respectively. Unfortunately, nearly 25% of patients, especially those pretreated with chemotherapy, do not respond efficiently to the currently recommended mobilization protocols and are deemed "poor mobilizers". Therefore, creating an understanding of the molecular mechanisms governing the process of HSPC mobilization will help to develop more efficient mobilization strategies.

The molecular mechanisms controlling the mobilization of HSPCs are still not well understood; however, attenuation of the stromal-derived growth factor 1 (SDF-1)-CXCR4 interaction between BM-secreted SDF-1 and HSPC-expressed CXCR4 plays a pivotal role in release of these cells from the BM into PB (Lapidot et al. 2005; Papayannopoulou 2004; Peled et al. 1999; Petit et al. 2002). An important role is also played by perturbation of the adhesive interaction between very late antigen-4 (VLA-4, $\alpha_1\beta_4$ integrin) expressed by HSPCs and its ligand vascular adhesion molecule-1 (VCAM-1, CD106), which is expressed in the BM microenvironment (Lévesque et al. 2001; Peled et al. 1999). Also playing some role in the retention of HSPCs in BM are other adhesion molecules as well as some growth factor-growth factor receptor axes, for example the kit ligand (KL)-c-kit receptor (c-kit) (Bellucci et al. 1999; Lévesque et al. 2003a; Lévesque et al. 2003b).

Many pharmacological agents have been described that may induce the mobilization of HSPCs. The cytokine granulocyte colony-stimulating factor (G-CSF) is currently the most frequently employed clinical drug which, after a few consecutive daily injections, may efficiently mobilize HSPCs (Lapidot and Petit 2002; Lapidot et al. 2005). Mobilization could also be induced within hours after administration of some chemokines (e.g. interleukin [IL]-8, growth-related oncogene protein-β [Gro-β], macrophage inhibitory protein-1α), multiple injection of certain growth factors (e.g. Fms-like tyrosine kinase 3, KL, vascular endothelial growth factor [VEGF]), and small molecular CXCR4 receptor antagonists (e.g. AMD3100, T140) (Andrews et al. 1994; Hattori et al. 2001; King et al. 2001; Laterveer et al. 1995; Liles et al. 2003; Mohle et al. 1998; Molendijk et al. 1986; Papayannopoulou et al. 1997; Ratajczak et al. 2004a; Reca et al. 2003). To obtain more efficient and faster mobilization, some of these compounds could be combined together (e.g. G-CSF plus AMD3100). Mobilization could be also achieved after administration of selected polysaccharides (e.g. zymosan, fucoidans) that already efficiently mobilize HSPCs in experimental animal models within one hour after a single injection (Sweeney et al. 2000; Sweeney et al. 2002; Vos and Wilschut 1979). In addition to individual patient sensitivity to a given mobilizing drug, other factors were also recently identified that may modulate mobilization. However, augmenting evidence is accumulating suggesting that innate immunity and its component CC, neutrophils, and Toll-like receptors (TLRs) play a pivotal role in the mobilization of HSPCs (Nagai et al. 2006).

To support this, work from our laboratory demonstrated that the CC is activated during mobilization and that several complement cleavage fragments (e.g. C3a, $_{desArg}$C3a, C5a, $_{desArg}$C5a anaphylatoxins) may modulate this process. As shown in Fig. 1, the CC may be activated during mobilization by the classical activation pathway that involves naturally occurring antibodies (NAbs), as seen after G-CSF administration, or by the alternative activation pathway as seen during zymosan-induced mobilization, for example. We noticed that while C3 (C3a, $_{desArg}$C3a) cleavage fragments increase the retention of HSPCs in BM, C5 (C5a, $_{desArg}$C5a) cleavage fragments enhance the egress of HSPCs into PB. This was evidenced in mobilization studies performed in C3- and C5-deficient animals which revealed that C3-deficient mice are easy mobilizers and C5-deficient mice poor mobilizers (Molendijk et al. 1986; Ratajczak et al. 2004a; Reca et al. 2003; Reca et al. 2007).

As mentioned above, NAbs play an important role during the activation of the CC via the classical pathway. These preexisting low-affinity germline-encoded Abs to biologically conserved neo-epitopes recognize antigens that become exposed in damaged tissues (e.g. in BM during G-CSF administration). After this interaction the innate immune system may direct an immune response resulting in CC activation via the classical pathway and complement deposition. Lack of NAbs, as seen, for example, in immunodeficient patients (Sekhsaria et al. 1996) or immunodeficient mice (Reca et al. 2007), accounts for these individuals being poor

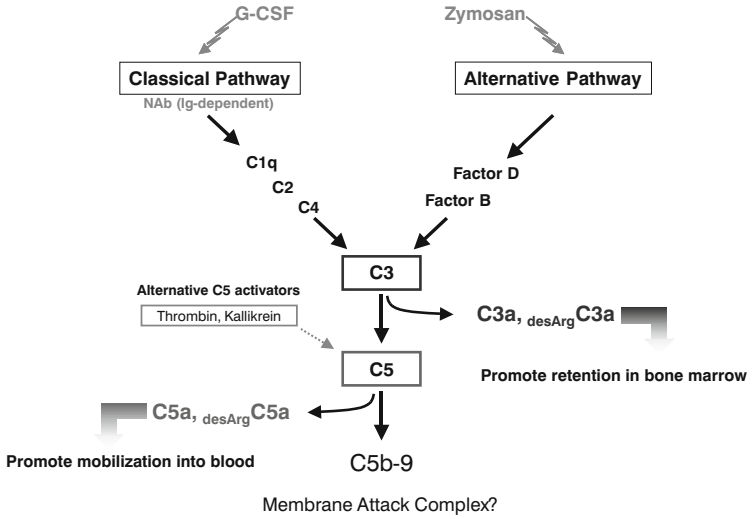

Fig. 1. The CC becomes activated in BM during mobilization and its activation products differentially modulated stem-cell trafficking. The CC becomes activated in BM after G-CSF-induced mobilization by the classical Ig (NAbs)-dependent pathway and after zymosan-induced mobilization due to activation of an alternative pathway. Some alternative mechanisms exist whereby C5 could be activated and cleaved by the proteolytic activity of thrombin and kallikrein. Upon activation of the C system, C3 and C5 cleavage products play opposite roles in the retention or mobilization of cells, respectively. While C3 cleavage fragments (C3a and $_{desArg}$C3a) enhance responsiveness of HSPCs to an SDF-1 gradient, activation of C5 promotes the mobilization of HSPCs by increasing the recruitment and degranulation of granulocytes. Thus we propose that an activated CC can affect stem-cell mobilization in a negative (C3) or a positive (C5) way. The potential contribution of other CC proteins (e.g. MAC) requires further study.

mobilizers. We reported that the poor mobilization in immunodeficient recombination activating gene 2 (RAG-2) and severe combined immunodeficient (SCID) mice (lacking B lymphocytes giving rise to immunoglobulin [Ig]-producing plasma cells) and Jh mice (selectively Ig deficient) was reversed after supplementation of wild-type Ig before mobilization (Reca et al. 2007). Whether a similar strategy could be employed in SCID patients requires further study.

Furthermore, it is well known that some cellular components of innate immunity, such as granulocytes, are required to mobilize HSPCs. To support this granulocytes are the first cells to egress from BM during mobilization (Wu et al. 2008) and that the mobilization process is severely defective in leukocyte-deficient animals (Pruijt et al. 2002). Augmenting evidence shows that granulocytes could play an important role at several steps of mobilization (Fig. 2), which is discussed later in this review.

Third, mobilization studies in a murine model revealed that it is somehow facilitated or directed by the activation of TRs on BM myeloid cells by endotoxin/lipopolysaccharide (LPS) released by intestinal anaerobic bacteria (Velders et al. 2004). This result is based on the observation that when mice are depleted of LPS-producing bacteria, they are poor mobilizers. Thus TLRs, which regulate several aspects of innate immunity and were recently found on the surface of HSPCs in addition to leukocytes, become important players in modulating the mobilization process. To support this notion, TLR2 binds zymosan, which is mentioned above as a polysaccharide employed for mobilization in mice, and TLR4 binds LPS (Sato et al. 2003; Sweeney et al. 2000; Sweeney et al. 2002; Vos and Wilschut 1979).

Below we discuss the involvement of these major components of innate immunity in more detail, focusing on activation of the CC and the role of neutrophils in the mobilization of HSPCs.

THE ROLE OF THE CC IN THE MOBILIZATION OF HSPCs

Evidence from our laboratory indicates that the CC plays a pivotal role in G-CSF- and in zymosan-induced mobilization (Ratajczak et al. 2004a; Ratajczak et al. 2004b; Ratajczak et al. 2006; Reca et al. 2003; Reca et al. 2007). Table 1 shows the summarized mobilization properties of both compounds. As shown, while G-CSF mobilizes HSPCs after a few days of daily injections, the experimental mobilizing agent zymosan may mobilize mice within one hour of injection. While G-CSF activates the CC mainly via the classical pathway, zymosan activates the CC via the alternative pathway (Fig. 1). Furthermore, it is well known that a functional barrier between blood and the BM microenvironment exists in BM at the level of the microvessel endothelium (Campbell 1986; Tavassoli 1992). We envision that while prolonged G-CSF administration may activate the CC on both sides of this barrier, zymosan activates the CC intravascularly.

Because of its clinical relevance, the G-CSF-induced HSPC mobilization model is the best studied to date. It is proposed that after G-CSF infusions, the BM gradually turns into a proteolytic microenvironment due to the release of proteolytic enzymes (e.g. elastase, cathepsin, and metalloproteinases) secreted by myeloid precursors and granulocytes in the BM (Bellucci et al. 1999; Lévesque et al. 2003a; Lévesque et al. 2003b). These enzymes degrade SDF-1 and cleave the N-terminus of the CXCR4 receptor and thus perturb the SDF-1--CXCR4 interaction, which plays a major role in the retention of HSPCs in BM. In addition, the proletolytic environment also affects the molecular integrity of the VLA-4-VCAM-1 and KL-c-kit axes. Furthermore, and at the same time, G-CSF also downregulates serpin family protease inhibitors.

In addition, as recently reported, G-CSF may directly down-regulate the expression of SDF-1 at the mRNA

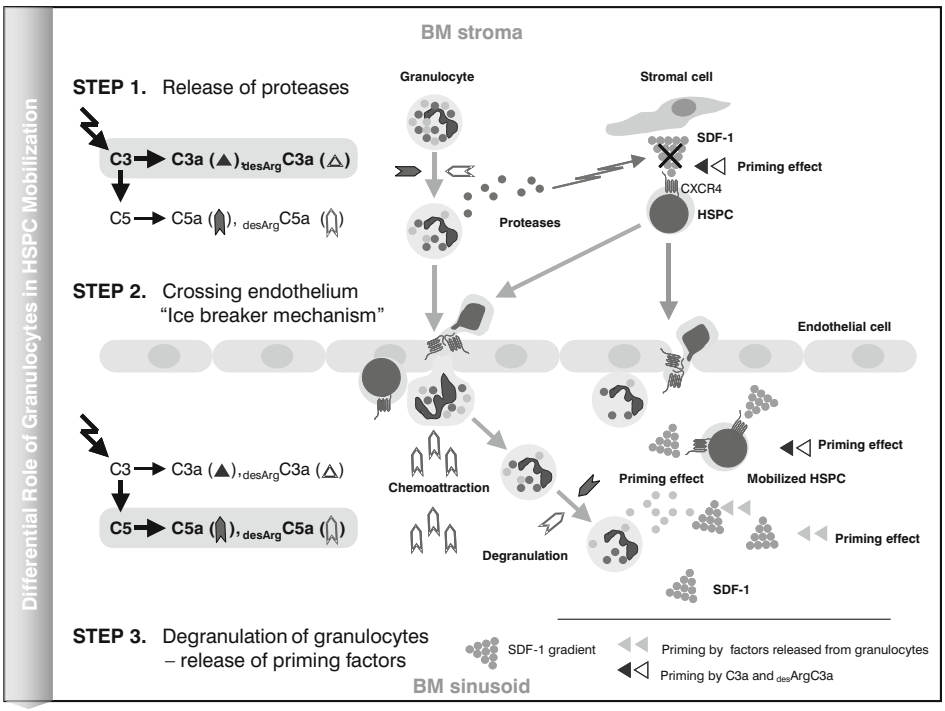

Fig. 2. Different levels of involvement of granulocytes and C5 cleavage fragments in the egress of HSPCs from BM. **Step 1:** a mobilizing agent (e.g. G-CSF) expands the number of myeloid cells in BM (granulocytes and monocytes) and activates them to secrete several proteolytic enzymes that impair the homing/retention signals of HSPCs in the BM microenvironment (e.g. SDF-1-CXCR4, VLA-1-VCAM-1, and KL-c-kitR axes). Activation of the secretion of proteolytic enzymes is potentiated by C5 cleavage fragments (C5a, $_{desArg}$C5a) that directly stimulate granulocytes. **Step 2:** Granulocytes are the first cells that egress from the BM and pave the way for HSPCs, which follow their path across the endothelial barrier ("icebreaker effect"). C5 cleavage fragments generated in the blood are strong chemoattractants for granulocytes and monocytic cells. **Step 3:** Granulocytes that egress from BM are stimulated by C5 cleavage fragments to degranulate and release several factors that prime and enhance the homing responses of HSPCs to SDF-1 gradient. In addition, this priming effect is potentiated by C3 cleavage fragments that also accumulate in PB during mobilization.

level in the BM osteoblasts that line up in the endosteal stem-cell niche (Petit et al. 2002). The end result is a decrease in responsiveness of HSPCs to an SDF-1 gradient and their proper interaction with adhesive signals in the BM microenvironment. Finally, this leads to the release of HSPCs into the circulation.

By employing several tools (e.g. fluorescence-activated cell sorting analysis, enzyme-linked immunosorbance assay, immunohistochemical staining), we demonstrated that G-CSF infusion strongly activates the CC in BM. This occurs because a neo-epitope is exposed on the surface of damaged BM cells after the induction of a proteolytic environment. This neo-epitope binds NAb and the neo-epitope-NAb complexes circulating in PB via C1q, trigger the classical Ig-dependent pathway of CC activation. Furthermore, C3 could also be cleaved/activated directly by some proteolytic proteases. Cleavage of C3 leads to the release of C3a and $_{desArg}$C3a and activation of components further downstream, including C5. In addition, because G-CSF activates the coagulation system (Bönig et al. 2001; Canales et al. 2002) and thrombin has recently been identified as a C5 activator, the CC could also be activated in G-CSF-mobilized patients as a result of activation of the coagulation cascade (Fig. 1) (Huber-Lang et al. 2006; Markiewski and Lambris 2007). In contrast, zymosan, employed as an experimental mobilizing agent, as a polysaccharide directly activates the CC by employing factors B and D, involving the Ig-independent alternative CC activation pathway (Fig. 1).

Both pathways of CC activation merge at the C3 component of the CC, which is a major CC component protein in blood. C3 cleavage releases potent anaphylatoxins, such as C3a and $_{desArg}$C3a, and creates conditions to activate components downstream from C5 to form membrane attack complex (MAC).

To evaluate a role of the CC in the mobilization of HSPCs, we employed C3- and C5-deficient mice that display deficiencies of crucial CC components. Our data revealed that C3-deficient mice show accelerated mobilization after administration of G-CSF and, at the same time, are poor zymosan mobilizers. In contrast, C5-deficient mice mobilize very poorly after both G-CSF and zymosan administration. These results provided direct evidence that innate immunity and the CC are not only activated during HSPC mobilization, but also play important roles in regulating this process. However, what became clear from these investigations was that various CC components may play different roles and

that the mobilization could be regulated differently depending on the level of CC activation.

C3-DEFICIENT MICE AS EASY G-CSF AND POOR ZYMOSAN MOBILIZERS: SEARCHING FOR A COMMON MECHANISM

Our studies revealed that C3-deficient mice show enhanced and accelerated mobilization after G-CSF administration (Ratajczak et al. 2004a). This phenomenon could be explained by the so-called priming effect of C3 cleavage fragments (C3a, $_{desArg}$C3a) on the responsiveness of HSPCs to an SDF-1 gradient, which we previously reported (Ratajczak et al. 2004a; Reca et al. 2003). Accordingly, we noticed by employing *in vitro* chemotactic assays that HSPCs respond more robustly to low threshold doses of SDF-1 when they are exposed or pre-incubated before assay with C3a or $_{desArg}$C3a. Similarly, if pre-incubated with C3a before transplantation into lethally irradiated recipients, murine HSPCs engraft faster after transplantation (Reca et al. 2003). This could also be explained by their increased responsiveness to SDF-1 homing signals after priming and exposure by C3a.

We recently provided a molecular explanation for this intriguing phenomenon based on the observation that it was lipid raft dependent (Ratajczak et al. 2004b). Lipid rafts are membrane domains rich in sphingolipids and cholesterol, which form a lateral assembly in a saturated glycerophospholipid environment (Guan 2004). The raft domains are known to serve as moving platforms on the cell surface and are more ordered and resistant to non-ionic detergents than other areas of the membrane. These domains also act as good sites for crosstalk between various cellular proteins. Lipid rafts have been shown to be important for T-cell polarization and chemotaxis (Gómez-Moutón et al. 2004; Yang et al. 2001). It was recently reported that small guanine nucleotide triphosphatases (GTPases) such as Rac-1 and Rac-2, which are crucial for engraftment of hematopoietic cells after transplantation, are associated with the lipid rafts of migrating HSPCs (Guan 2004; Yang et al. 2001). Accordingly, since CXCR4 receptor is a lipid raft-associated protein, its signaling ability is enhanced if CXCR4 is incorporated into membrane lipid rafts where it may better interact with several signaling molecules, including small GTPase Rac-1 (Guan 2004; Yang et al. 2001; Wysoczynski et al. 2005). This co-localization of CXCR4 and Rac-1 in lipid rafts facilitates GTP binding/activation of Rac-1.

We noticed that both C3a and $_{desArg}$C3a increase incorporation of CXCR4 into membrane lipid rafts and thus enhance the homing responsiveness of HSPCs to SDF-1 gradient (Ratajczak et al. 2004b; Ratajczak et al. 2006). This phenomenon also occurs during mobilization. When the proteolytic microenvironment in BM degrades the SDF-1-CXCR4 interaction, C3 fragments (C3a or $_{desArg}$C3a) activated during mobilization enhance and sensitize the responsiveness of HSPCs to a decreasing SDF-1 concentration in BM and are thus a last line of defense for keeping HSPCs from uncontrolled egress into PB. In C3-deficient mice this mechanism is missing, causing these animals to become easy mobilizers (Reca et al. 2003).

In addition to increasing responsiveness of HSPCs to SDF-1 gradient, further studies are needed to determine whether C3 cleavage fragments may also provide other pro-homing effects in BM, such as increasing the BM level of SDF-1 or the expression of selected adhesion molecules/ligands. To further support a role of C3 in BM retention, we noticed that C3a receptor (C3aR) knock-down animals are easy mobilizers as well (Ratajczak et al. 2004a). Furthermore, we observed a similar effect after C3aR blockage by employing SB 290157, which is a small molecular antagonist of this receptor (Ratajczak et al. 2004a). Thus modulation of the C3a-C3aR axis may become an important strategy for increasing the mobilization of HSPCs in poor mobilizers.

However, the issue emerges of explaining why C3--deficient mice can be easy G-CSF mobilizers while at the same time mobilizing very poorly after zymosan administration. To explain this apparent discrepancy one must remember that both these agents activate the CC in BM differently (Table 1). While prolonged administration of G-CSF activates complement efficiently in the BM microenvironment, zymosan, as a short mobilizing agent, activates the CC intravascularly (Table 1). Lack of efficient activation of the CC in the BM vessels of C3-deficient mice leads to a lack of C3a and $_{desArg}$C3a generation and thus blockage of downstream CC protein activation, particularly C5. To support this notion, during zymosan-induced mobilization in normal wild-type mice, C3 is activated intravascularly and C3 cleavage fragments prime and enhance

Table 1. Comparison of G-CSF- and zymosan-induced mobilization

G-CSF-induced mobilization	Zymosan-induced mobilization
Long-term mobilization (3–6 days) requires a few daily injections of the drug	Short-term mobilization: 1 h after a single injection of the compound
CC activated mainly by the classical pathway	CC activated by the alternative pathway
Activation of the CC on both sides of the blood-BM barrier	Activation of the CC mainly in blood, which creates an accumulation of C cleavage fragments in BM vessels

the responsiveness of BM-residing HSPCs to the SDF-1 gradient in blood. This creates a kind of "egress force" for HSPCs. This effect is missing in C3-deficient mice. Furthermore, a lack of C3 activation in these mice leads to defects in C5 activation and the release of C5a and $_{desaArg}$C5a cleavage fragments (Fig. 1). These fragments are crucial for executing the egress of HSPCs from BM, which we describe later.

C5-DEFICIENT MICE AS POOR G-CSF AND ZYMOSAN MOBILIZERS: EVIDENCE THAT MOBILIZATION IS REGULATED DIFFERENTLY AT DIFFERENT LEVELS OF CC ACTIVATION

As shown in Fig. 1, both pathways of CC activation merge on C3 and C3 activation leads to C5 cleavage, release of C5a and $_{desArg}$C5a anaphylatoxins, and the formation of MAC. Our recent mobilization studies revealed that C5-deficient mice are extremely poor mobilizers in response to both G-CSF and zymosan (Reca et al. 2007). Transmission electron microscopy (TEM) studies showed cells in the BM accumulating around vessels and displaying defective egress from the BM into PB.

This intriguing observation prompted us to study this phenomenon further. First we excluded the possibility that CC cleavage fragments may directly chemoattract HSPCs. Accordingly, in our studies neither C3 nor C5 cleavage fragments (C3a, $_{desArg}$C3a and C5a, $_{desArg}$C5a, respectively) were able to chemoattract HSPCs if employed alone. Next we looked for the ability of both cleavage fragments to enhance and prime responsiveness of HSPCs to a SDF-1 gradient. To our surprise, and in contrast to C3a and $_{desArg}$C3a, neither C5a nor $_{desArg}$C5a sensitized the responsiveness of HSPCs to SDF-1. What we noticed, however, was that $_{desArg}$C5a turned out to be strong activator and chemoattractant for granulocytes. This prompted us to explore in more detail the role of granulocytes in mobilization, which is discussed below.

However, based on C5 cleavage fragments (C5a and $_{desaArg}$C5a) being crucial for the egress of HSPCs from BM, one may ask how C3-deficient mice activate mobilized HSPCs after G-CSF administration if the CC should be blocked because of a lack of C3 cleavage that leads to C5 activation. To address this one must remember that G-CSF mobilization is performed during a few days, which is probably enough time to exert the mobilization of HSPCs by activating C5 cleavage employing alternative mechanisms. In fact, it was recently demonstrated in a very elegant paper that C5 is activated and cleaved into C5a and $_{desaArg}$C5a by the proteolytic activity of thrombin. Since the coagulation cascade (including thrombin) becomes activated during G-CSF mobilization, C5 is cleaved by an alternative thrombin-mediated activation pathway (Bönig et al. 2001; Canales et al. 2002; Huber-Lang et al. 2006; Markiewski and Lambris 2007). Alternatively, cleaved and activated C5 releases C5a and $_{desArg}$C5a that execute the egress of HSPCs from BM (Fig. 1). In the case of zymosan, which optimally mobilizes one hour after administration, there is probably not enough time for C3-deficient mice to cleave C5 by some alternative pathways in BM.

Overall, our studies in complement-deficient mice revealed that CC cleavage fragments differently regulate the mobilization of HSPCs (Fig. 1). While C3 cleavage fragments may negatively or positively regulate the egress of HSPCs in G-CSF- and zymosan-induced mobilization, respectively, C5 cleavage fragments are required for the egress of HSPCs into the circulation. Currently we are evaluating a role of other CC components performing mobilizations in C1q and C2 factor B-deficient animals. Our preliminary mobilization data in these animals support the general concept that the CC, as part of innate immunity, is required for the mobilization of HSPCs.

THE ROLE OF LEUKOCYTES IN THE MOBILIZATION OF HSPCS: OLD AND NEW ASPECTS

It is well known that granulocytes are required for HSPC mobilization; however, the mechanisms by which granulocytes induce mobilization are not completely understood. They were postulated to be a source of G--CSF-induced proteolytic enzymes in the BM that perturb SDF-1-CXCR4, VLA-4-VCAM-1, and KL-c-kit R receptor homing signals (Lévesque et al. 2001; Lévesque et al. 2003a; Lévesque et al. 2003b; Lévesque et al. 2004). On the other hand, what is intriguing in several mobilizing protocols is that granulocytes were described as the first cells to egress from BM (Glaspy et al. 1997; King et al. 2001; Pruijt et al. 1999; Sato et al. 1994; Wu et al. 2008). Similarly, PB granulocytosis is rapidly induced during infections or organ damage (e.g. heart infarct) (Kassirer et al. 1999; Kyne et al. 2000).

Because CC anaphylatoxins (C5a and $_{desArg}$C5a) are potent chemoattractants and activators for granulocytes that bind to G-protein-coupled seven-transmembrane-span C5a receptors (C5aR and C5L2) on these cells (Cain and Monk 2002; Riedemann et al. 2003), we became interested in the link between activation of the CC, release of C5a and $_{desArg}$C5a, activation and egress of granulocytes, and mobilization of HSPCs.

There are several steps at which granulocytes are involved in the egress of HSPCs from BM and we discuss them in the context of CC activation (Fig. 2). In the first step, some mobilizing agents, such as G-CSF, activate myeloid cells, including granulocytes, in BM that expand in number and secrete several proteolytic enzymes after activation that turn the BM microenvironment into a proteolytic one. Because G-CSF activates the CC, C5a and $_{desArg}$C5a become important mediators of innate immunity that directly activate the secretion of photolytic enzymes by granulocytes and monocytes. Thus the overall action of G-CSF is potentiated by C5 cleavage fragments and facilitates the

detachment of HSPCs from their niches. This "proteolytic step", described in G-CSF and cytostatics-induced mobilization (Lévesque et al. 2003a), may not be so important in the mobilization induced by other compounds (e.g. IL-8, Gro-β, AMD3100). Furthermore, because mice genetically deficient in the expression of several proteases implicated in mobilization surprisingly mobilize normally, it is implied that either activation of BM-proteases is not required or redundant protease pathways compensate during mobilization in knock--down mice (Cramer et al. 2008; Lévesque et al. 2004). Thus the overall role of proteases in HSPC mobilization remains somehow controversial.

The next step requires the egress of HSPCs from BM, where they have to cross the endothelial barrier. Since granulocytes were shown to be the first cells that egress from BM and precede the egress of HSPCs, they may "pave the way" for HSPCs that follow in their footsteps. This proposed "icebreaker" role of granulocytes is supported by our recent TEM studies. Furthermore, since $_{desArg}$C5a is a strong chemoattractant for granulocytes, activation of the CC in blood by a mobilizing agent (e.g. zymosan) and C5 cleavage may be a driving force for creating a chemotactic gradient for granulocytes to promote their egress into PB. Thus this icebreaker effect of granulocytes could be of crucial importance. Several reports postulated that HSPCs are able to egress from BM because they can secrete matrix metalloproteinase-9 (MMP-9). However, because MMP-9-deficient mice are normal mobilizers, we can assume that HSPC-derived MMPs are not so important in this process. Thus we can envision granulocytes as a population of cells that plays a crucial role in paving the way for HSPCs to leave BM. To support this role of granulocytes in mobilization, several agents employed for mobilization, such as IL-8 or GRO-β, are primarily strong activators and chemoattractants for granulocytes and induce their egress from BM.

Finally, in step three, granulocytes play other important role. Accordingly, granulocytes in the blood stream that left the BM are under the influence of C5 cleavage fragments, which force their degranulation. Our preliminary data suggest that the conditioned media harvested from granulocytes exposed to C5 or $_{deaArg}$C5a increase the responsiveness of HSPCs to the blood gradient of SDF-1 (similar to our description for C3a and $_{desArg}$C3a). Currently we are trying to identify these factors that are present in conditioned media from C5a stimulated granulocytes. Thus granulocytes together with C5 cleavage fragments accumulate in BM vessels and both become key components of innate immunity to execute the egress of HSPCs from BM into PB.

We have to consider, however, that C5a cleavage fragments may also play some other roles in HSPC mobilization, such as increasing the permeability of the BM endothelial barrier and/or enhancing the release of sphingosine 1-phosphate from endothelial cells (Massberg et al. 2007; Seitz et al. 2005). It is well known that this bioactive lipid, similar to C3a, $_{desArg}$C3a, and some products of granulocyte deregulation, may increase the responsiveness of HSPCs to SDF-1 (Fig. 2). All this may additionally potentiate the responsiveness of HSPCs to the blood level of SDF-1 and facilitate the egress of HSPCs from BM.

CONCLUSIONS AND FUTURE DIRECTIONS

We envision that the mobilization of stem cells is part of a more general immune alarm reaction in response to infection and/or tissue and organ damage that releases granulocytes, HSPCs, and other more primitive cells, such as mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs), or the so-called very small embryonic/epiblast-like stem cells (VSELs) described recently by our team, from their tissue/organ niches. We postulate that all these "circulating and patrolling" stem cells play important roles in the protection of damaged tissues.

HSPCs release stem-cell niches in BM and thus provide a place for the expansion of new stem cells, which results in an enhanced production of blood elements. In addition, HSPCs may be attracted to affected tissues where they may locally and more efficiently supply cells involved in fighting infection (e.g. granulocytes, macrophages, and dendritic cells).

Conversely, mobilized non-hematopoietic stem cells (e.g. MSCs, EPCs, or VSELs) may contribute to the regeneration of damaged organs, such as after heart infarct or stroke. It is well known that both infection and organ and tissue damage-related hypoxia are strong triggers for both CC activation (as seen in the hypoxia/reperfusion model) and upregulated expression of pivotal stem-cell chemoattractants in damaged tissues (e.g. SDF-1, hepatocyte growth factor/scatter factor [HGF/SF], VEGF). We envision that C3 cleavage fragments together with SDF-1 create a supergradient that chemoattracts stem cells to the damaged organs, for example HSPCs after transplantation to BM or VSELs mobilized from BM during heart infarct or stroke to the heart or injured brain tissue (Wysoczynski et al. 2009).

Based on these data we postulate that modulation of the CC could be an important tool to regulate the release of stem cells from their niches. We have already identified several components of innate immunity, such as C3 and C5 cleavage fragments, that directly or indirectly modulate the egress of these cells. At this point, however, we cannot exclude that other downstream C proteins (e.g. C6 or MAC) in addition to C3 and C5 may also be required for the egress of stem cells from BM. Finally, what was not addressed in this review is that innate immunity plays a role not only in mobilization, but also in engraftment of HSPCs after transplantation. Thus modulation of the CC in the BM environment may optimize the mobilization as well as homing of HSPCs after transplant. These observations should stimulate the development of new therapeutic approaches.

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