Published in final edited form as:

Leukemia. 2013 September ; 27(9): 1928–1930. doi:10.1038/leu.2013.84.

Mobilization studies in C3-deficient mice unravel the involvement of a novel crosstalk between the coagulation and complement cascades in mobilization of hematopoietic stem/progenitor cells (HSPCs)

Sylwia Borkowska^{1,2}, Malwina Suszyska^{1,2}, Marcin Wysoczynski³, and Mariusz Z. Ratajczak^{1,2,†}

¹Stem Cell Institute at James Graham Brown Cancer Center, University of Louisville, Louisville, KY 40202, USA ²Department of Physiology, Pomeranian University of Medicine, 70-111 Szczecin, Poland ³Institute of Molecular Cardiology, University of Louisville, Louisville, KY 40202, USA

Hematopoietic stem/progenitor cells (HSPCs) express chemokine receptor CXCR4 and very late antigen-4 (VLA-4, also known as $\alpha_4\beta_1$ integrin) receptor on their cell surface and are retained in bone marrow (BM) niches by interaction of these receptors with their respective ligands, α -chemokine stromal-derived growth factor-1 (SDF-1) and vascular adhesion molecule-1 (VCAM-1, also known as CD106), which are expressed by cells in the BM microenvironment (e.g., osteoblasts and fibroblasts). HSPCs residing in BM are released from their niches and circulate under steady-state conditions at detectable levels in the PB, and their number increases in response to i) systemic or local inflammation, ii) strenuous exercise, iii) stress, iv) tissue/organ injury, and v) pharmacological agents [1–5]. The mobilization process has been postulated to be directed by i) a decrease in SDF-1–CXCR4 and VLA-4–VCAM-1 interactions in BM microenvironment (e.g., due to release of proteolytic enzymes or after molecular blockade by administration of small molecular CXCR4 or VLA-4 antagonists), ii) release of neurotransmitters from the synapses of the nerves that innervate the BM microenvironment (e.g., involving the dopamine and β_2 -adrenergic receptors), iii) reversal of the trans-endothelial chemotactic gradient between the BM microenvironment and plasma, iv) activation of the plasminogen system, as recently proposed, and v) activation of the complement cascade (ComC), as we have demonstrated [1–5]. Lending additional support to this latter effect, it is known that the ComC becomes activated in all mechanisms leading to mobilization of HSPCs (e.g., systemic inflammation, organ injury, strenuous exercise, as well as administration of mobilizing drugs) [2].

We previously reported that mice defective in the third complement protein (C3), which is a proximal component of the ComC, are easy mobilizers [6]. This has been explained by a

[†]Address for correspondence: Prof. Mariusz Z. Ratajczak, Endowed Stella and Henry Hoenig Chair and Director, Stem Cell Institute at James Graham Brown Cancer Center, University of Louisville, 500 S. Floyd Street, Rm. 107, Louisville, KY 40202, USA, Tel: (502) 852-1788, Fax: (502) 852-3032, mzata01@louisville.edu.

Conflict-of-Interest Statement

The authors declare no conflicts of interest.

lack of C3a formation, which normally increases retention signals for HSPCs in response to a BM SDF-1 gradient and thus prevents uncontrolled release of these cells into PB. By contrast, mice defective in the fifth complement protein (C5), which is a distal component of the ComC, display a profound defect in mobilization of HSPCs [7]. To explain this phenomenon, we demonstrated that C5 cleavage fragments (C5a and C5b) are essential for egress of HSPCs into peripheral blood (PB). Accordingly, C5a generated in the BM microenvironment above the BM–PB barrier is responsible during the mobilization process for augmenting the release of proteolytic enzymes from myeloid cells, which attenuates SDF-1–CXCR4 and VLA4–VCAM-1 retention signals in BM niches. In addition, C5a generated down from the BM–PB barrier in BM sinusoids is a potent chemoattractant for granulocytes and monocytes that, as the first cells egressing from the BM, play an important role in permeabilization of the BM–PB barrier and thus facilitate the subsequent egress of HSPCs [7]. Moreover, another C5 cleavage fragment, C5b, is involved in formation of C5b-C9, also known as the membrane attack complex (MAC), which increases the PB level of a potent chemoattractant for HSPCs, sphingosine-1-phosphate (S1P) [8]. Thus, the failure of C5-deficient mice to generate both C5a and MAC explains why they are poor mobilizers [7, 8].

Considering the fact that activation of the ComC and proper cleavage of C3 is required for formation of C5 convertase (an enzyme that cleaves C5 into C5a and C5b), one would expect that C3-deficient mice, like C5-deficient animals, would be poor mobilizers. However, as mentioned above and already reported [6], this is not the case. To address why C3^{-/-} mice, despite a defect in generation of C5 convertase, are easy mobilizers, we considered the involvement of alternative proteolytic pathways that could cleave/activate C5 in C3^{-/-} mice. For example, activation of the CoaC is a well-known side effect of G-CSF when employed in patients for stem cell mobilization [9]. Herein, based on data that C5 can be activated/cleaved by thrombin [10], which exhibits C5 convertase “like” activity, we evaluated the effect of thrombin inhibition during G-CSF-induced mobilization in C3^{-/-} and wild type (WT) mice by employing a specific, direct thrombin inhibitor, refrudan.

In our experiments, 2-month-old C3-deficient mice (C3^{-/-}) were mobilized for 6 days by G-CSF (250 µg/kg s.c.) in the presence or absence of refrudan (lepirudin), a specific thrombin inhibitor, which was administered daily for 6 days (5 mg/kg i.p.). Following mobilization, mice were sacrificed and blood was drawn from the inferior vena cava to measure the total number of white blood cells (WBC, Figure 1 A), the number of circulating clonogenic CFU-GM (Figure 1 B), and the number of Sca-1⁺c-kit⁺lineage⁻ (SKL) cells (Figure 1C). In parallel, we measured the activation of C5 by measuring the level of C5a by ELISA in the PB of C3^{-/-} mice that were non-mobilized (control), mobilized with G-CSF, and mobilized with G-CSF in the presence of refrudan.

We observed that G-CSF-induced mobilization of HSPCs was significantly reduced in C3^{-/-} mice if the mice were treated during mobilization with refrudan (Figure 1). Moreover, while as expected, we observed activation of C5 and release of C5a in C3^{-/-} mice mobilized with G-CSF, this activation was significantly inhibited in the presence of refrudan. This result confirms that C5 can be activated/cleaved in a thrombin-dependent manner [10] in the

absence of C3 and ComC-generated C5 classical convertase – and thus thrombin may substitute as C5 “like” convertase for the C3-dependent C5 classical convertase.

Based on this finding, we propose the following novel scenario for crosstalk between CoaC and ComC in the mobilization of HSPCs (Figure 2A). As reported, G-CSF administration activates both ComC and CoaC, which leads in normal WT animals to generation of C5a as the result of activation of the ComC downstream from the activated/cleaved C3 and release of C5 convertase. However, since C3^{-/-} mice cannot generate a “classical” C5 convertase, they activate/cleave C5 by activation of the CoaC and generation of thrombin, which exhibits C5 “convertase-like” activity against C5 (Figure 2B). The positive impact of thrombin on mobilization of HSPCs was subsequently confirmed in G-CSF-mobilized WT mice (data not shown). Specifically, WT mice displayed a ~20% decrease in mobilization efficiency of HSPCs in the presence of refrudan, which supports the notion that thrombin is also involved in the mobilization process in normal WT animals.

The postulated crosstalk between the CoaC and ComC involves, in addition to thrombin as reported [10], several other components of the CoaC, such as Factors XI, IX, X, and even plasmin [10]. The proteolytic compensation by other components of the CoaC explains why inhibition of thrombin by refrudan did not completely abolish the mobilization process in C3^{-/-} mice (Figure 1). On the other hand, as reported by others and confirmed by us, one must also consider that C5 can also be cleaved by several other proteolytic enzymes unrelated to the CoaC and ComC, such as cathepsin, MMP-2, and MMP-9 [10].

In conclusion, the data presented in this work demonstrate, for the first time, the existence of crosstalk between two evolutionarily ancient proteolytic cascades, the ComC and CoaC, in the mobilization of HSPCs. This has important implications, because it is known that both cascades are activated in parallel in all situations in which HSPCs are mobilized from BM into PB (e.g. infections, tissue/organ damage, strenuous exercise). Moreover, both axes show a circadian rhythm of activation [11, 12]. Due to a drop in blood pH during deep sleep at night, both cascades are activated in the early morning hours [11, 12] at a time when the peak in circulating HSPCs is observed [13]. This important fact has to be taken into consideration when studying the circadian circulation of HSPCs. It is likely that, in addition to circadian changes in the tonus of the vegetative nervous system, circadian activation of the ComC and CoaC is also involved in circadian release of HSPCs from BM into PB [11–13]. This conclusion, however, needs the support of further experimental evidence.

Acknowledgments

This work was supported by NIH 2R01 DK074720 and Maestro grant 2011/02/A/NZ4/00035 to MZR and AHA 13SDG14560005 to MW.

Reference List

1. Lévesque JP, Helwani FM, Winkler IG. The endosteal ‘osteoblastic’ niche and its role in hematopoietic stem cell homing and mobilization. *Leukemia*. 2010; 24:1979–1992. [PubMed: 20861913]

2. Ratajczak MZ, Kim CH, Wojakowski W, Janowska-Wieczorek A, Kucia M, Ratajczak J. Innate immunity as orchestrator of stem cell mobilization. *Leukemia*. 2010; 24:1667–1675. [PubMed: 20703253]
3. Lapidot T, Kollet O. The brain-bone-blood triad: traffic lights for stem-cell homing and mobilization. *Hematology Am Soc Hematol Educ Program*. 2010; 2010:1–6. [PubMed: 21239763]
4. Katayama Y, Battista M, Kao WM, Hidalgo A, Peired AJ, Thomas SA, et al. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. *Cell*. 2006; 124:407–421. [PubMed: 16439213]
5. Bonig H, Papayannopoulou T. Hematopoietic stem cell mobilization: updated conceptual renditions. *Leukemia*. 2012; doi: 10.1038/leu.2012.254.
6. Ratajczak J, Reca R, Kucia M, Majka M, Allendorf DJ, Baran JT, et al. Mobilization studies in mice deficient in either C3 or C3a receptor (C3aR) reveal a novel role for complement in retention of hematopoietic stem/progenitor cells in bone marrow. *Blood*. 2004; 103:2071–2078. [PubMed: 14604969]
7. Lee HM, Wu W, Wysoczynski M, Liu R, Zuba-Surma EK, Kucia M, et al. Impaired mobilization of hematopoietic stem/progenitor cells in C5-deficient mice supports the pivotal involvement of innate immunity in this process and reveals novel promobilization effects of granulocytes. *Leukemia*. 2009; 23:2052–2062. [PubMed: 19657368]
8. Ratajczak MZ, Lee H, Wysoczynski M, Wan W, Marlicz W, Laughlin MJ, et al. Novel insight into stem cell mobilization-plasma sphingosine-1-phosphate is a major chemoattractant that directs the egress of hematopoietic stem progenitor cells from the bone marrow and its level in peripheral blood increases during mobilization due to activation of complement cascade/membrane attack complex. *Leukemia*. 2010; 24:976–985. [PubMed: 20357827]
9. Canales MA, Arrieta R, Gomez-Rioja R, Diez J, Jimenez-Yuste V, Hernandez-Navarro F. Induction of a hypercoagulability state and endothelial cell activation by granulocyte colony-stimulating factor in peripheral blood stem cell donors. *J Hematother Stem Cell Res*. 2002; 11:675–681. [PubMed: 12201956]
10. Amara U, Flierl MA, Rittirsch D, Klos A, Chen H, Acker B, et al. Molecular intercommunication between the complement and coagulation systems. *J Immunol*. 2010; 185:5628–5636. [PubMed: 20870944]
11. Reis ES, Lange T, Köhl G, Herrmann A, Tschulakow AV, Naujoks J, et al. Sleep and circadian rhythm regulate circulating complement factors and immunoregulatory properties of C5a. *Brain Behav Immun*. 2011; 25:1416–1426. [PubMed: 21539909]
12. Wolk R, Gami AS, Garcia-Touchard A, Somers VK. Sleep and cardiovascular disease. *Curr Probl Cardiol*. 2005; 30:625–662. [PubMed: 16301095]
13. Katayama Y, Battista M, Kao WM, Hidalgo A, Peired AJ, Thomas SA, et al. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. *Cell*. 2006; 124:407–421. [PubMed: 16439213]

Figure 1. Impaired mobilization of HSPCs in C3-deficient mice in the presence of the thrombin inhibitor refludan

C3^{-/-} mice were mobilized for 6 days with G-CSF (250 μg/kg s.c. per day; n=6 mice per group) in the presence or absence of refludan (administered daily for 6 days, 5 mg/kg i.p.). The figure shows the number of circulating leukocytes (**panel A**), SKL cells (**panel B**), and CFU-GM progenitors (**panel C**) per microliter of PB. (group Ctrl – control, group R – refludan only, group G - G-CSF only, group G + R – G-CSF + Refludan). *P<0.05 (panel A), <0.005 (panel B) and <0.05 (panel C) compared with mice treated with G-CSF + Refludan. These are representative results from two independent experiments.

Figure 2. Postulated novel involvement of the complement cascade (ComC) and coagulation cascade (CoaC) in mobilization of HSPCs - thrombin substitutes for the C3-dependent C5 convertase

Panel A – G-CSF activates both the ComC and CoaC, which leads to generation of C5 convertase (via ComC) and C5 convertase-like activity by thrombin (via CoaC). C5 cleavage and release of C5a and C5b is, as reported [], crucial for egress of HSPCs from BM. **Panel B** – C5 convertase-like activity, due to activation of CoaC and generation of thrombin, compensates for the lack of C3 and thus the lack of “classical” C5 convertase in $C3^{-/-}$ mice. This substitution enables normal mobilization of HSPCs in $C3^{-/-}$ mice, despite a defect in activation of the ComC.