The doctor prescribed a fat-free diet for stem cell mobilization

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Mobilized hematopoietic stem and progenitor cells (HSPC) are widely utilized for clinical stem cell transplantation. HSPC mobilization by the cytokine granulocyte colony-stimulating factor (G-CSF) had been used clinically for several decades. Nevertheless, the underlying mechanisms and the factors leading to large variations in mobilization yields in healthy donors are poorly understood. In this issue of *Haematologica*, Suzuki and colleagues[1] shed light on the effect of dietary fat content on G-CSF-stimulated mobilization, deciphering the regulatory role of ω3-polyunsaturated fatty acids (PUFA) processed by bone marrow (BM) neutrophils (and to a lesser extent by other cell types) as part of the mobilization process in mice. The authors show that G-CSF-mediated activation of peroxisome proliferator-activated receptor (PPAR)δ signaling first requires cues from the sympathetic nervous system via β1/2-adrenergic receptors in BM neutrophils, which in turn increases PPARδ expression and activity. However, PPARδ is a negative regulator of HSPC mobilization. A shrewd approach to bypass this negative regulation was to feed mice for a brief period with a fat-free diet. As a result of the low ω3-PUFA content in this diet, the lack of ω3-PUFA/PPARδ activation decreased transcription of the negative regulator angiopoietin-like protein 4 (Angptl4), which in turn increased BM vascular permeability and facilitated enhanced HSPC mobilization. This simple, albeit novel approach could be easily assessed in order to address the problem of poor clinical HSPC mobilization in some healthy donors. However, BM neutrophils are not the only players in the complex multifaceted process of HSPC mobilization. Hence, the intriguing study by Suzuki *et al.*, in addition to its novelty regarding the machinery activated in BM neutrophils during HSPC mobilization, opens new research directions regarding HSPC cell-intrinsic signaling.

Signals driving HSPC retention in the BM *versus* their egress to the blood are tightly balanced during steady-state homeostasis in order to facilitate blood and immune-cell production on demand along with preservation of the undifferentiated HSPC BM reservoir. Thus, physiological HSPC egress to the blood is dynamically modulated by homeostatic light/dark cycles and circadian rhythms involving β1/2-adrenergic receptor signaling[2] as well as BM blood vessel permeability and hormone/cytokine secretion.[3] These signals in mice balance the daily rhythms of BM HSPC differentiation[3] and egress during daylight to replenish the blood.[2] Melatonin secretion at night reduces BM vessel-permeability and egress, exerting anti-inflammatory effects, which reprogram stem cell self-renewal.[3] Pro-inflammatory signals enforced by bacteria-mimicking lipopolysaccharide challenge and by G-CSF treatment in mice modulate this balance, skewing it towards differentiation and mobilization, to address the urgent need for immune-competent cells. The negative-regulatory function of Angptl4 seems to be part of the balance machinery addressing the need to preserve BM HSPC from exhaustion and hematopoietic failure. G-CSF is known to evoke pro-inflammatory stimuli in the BM, involving signals from the nervous system, which exert dramatic changes in myeloid cells, osteolineage cells,[4] bone metabolism,[5] and blood vessel permeability.[6] HSPC also respond to G-CSF-induced signals, showing robust metabolic changes,[7,8] which prepare them for the dynamic state, essential for making the active journey to the blood.

An interesting question is whether HSPC "sense" changes in BM lipid mediators during daily light/dark cycles an following

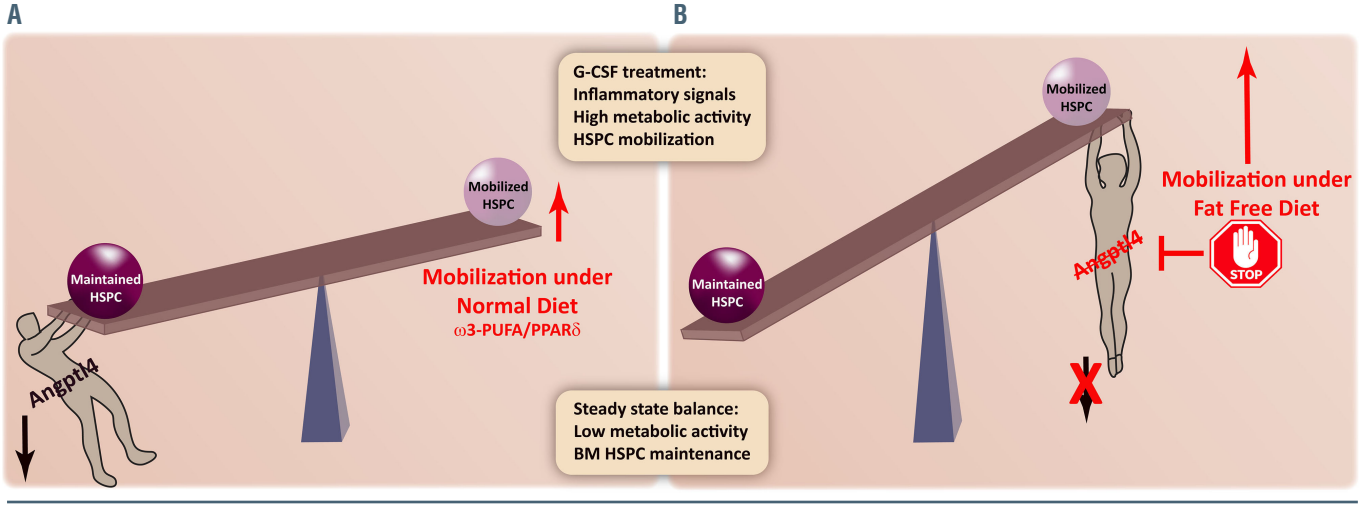

Figure 1. Fatty acid content of the diet affects granulocyte colony-stimulating factor-induced mobilization of hematopoietic stem and progenitor cells. In addition to signaling in bone marrow neutrophils, as reported by Suzuki *et al.*,[1] the fatty acid content of the diet may also affect granulocyte colony-stimulating factor (G-CSF)-induced mobilization via cell-intrinsic signaling in hematopoietic stem and progenitor cells (HSPC). (A) G-CSF-induced mobilization under normal diet provides fatty acids including the key ω3-PUFA that activates PPARδ/Angptl4 signaling in order to balance and maintain bone marrow HSPC despite the pro-inflammatory cues. (B) G-CSF-induced mobilization under a fat-free diet deprived of the key ω3-PUFA, prevents the activation of PPARδ/Angptl4 inhibitory signaling that yields higher rates of HSPC mobilization.

G-CSF stimuli. Furthermore, what signaling do these mediators induce in HSPC and particularly, do they involve changes in reactive oxygen species levels, as well as in Angptl4 expression and activity? Some hints come from several reports. HSPC bear the lipolytic machinery (phospholipase C-β2) to control pharmacological (G-CSF and AMD3100) HSPC mobilization.[9] Angiopoietin-like proteins 1-7 play multiple roles in the regulation of hematopoietic stem cell activity including quiescence, expansion, self-renewal, and homing.[10] A major candidate for such future studies could be Angptl4. In humans, ANGPTL4 maintains the *in vivo* repopulating capacity of CD34$^+$ cord blood HSPC.[11] In mice, the PML-PPARδ-FAO pathway influences reactive oxygen species generation and stem cell division. Depletion of PPARδ, which serves as a fatty acid nutrient sensor, reduced stem cell quiescence, and their repopulating potential since it controls asymmetric divisions that are essential for HSC maintenance.[12] Interestingly, Angptl4 is upregulated in the BM under inflammatory conditions induced by bacterial lipopolysaccharide challenges, leading to increased secretion of G-CSF and Angptl4 from BM stromal cells, which also expand BM myeloid progenitors.[13] Thus, Angptl4 in HSPC balances the cells' response to pro-inflammatory effects in order to preserve their BM maintenance and long-term function. Suzuki *et al.* suggest that temporal attenuation of Angptl4 upregulation may further increase the efficiency of G-CSF-induced mobilization (Figure 1).

Another physiological life condition is aging, which is associated with stress and pro-inflammatory cues, an increase in marrow vascular permeability, adipocytes, and a decrease in hematopoietic cellularity. Adipocytes accumulate in the BM during obesity and aging, and notably also following a high-fat diet in mice. This change in the ratio of adipocytes/hematopoietic cells reprograms mesenchymal stem cells towards adipogenic rather than osteogenic differentiation, which reduces the rates of bone regeneration and hematopoiesis recovery.[14] In addition to pro-inflammatory signals, in humans G-CSF also induces a pro-coagulant state and increased thrombin activity.[15] The efficiency of G-CSF-induced mobilization in healthy donors for clinical HSPC transplantation can be predicted by the surface expression levels of the major coagulation- and inflammation-related thrombin receptor, protease activated receptor 1 (PAR1) on mature peripheral blood leukocytes and CD34$^+$ HSPC before mobilization is conducted.[16] Importantly, this surface PAR1 expression also predicts HSPC repopulating potential in transplanted patients and PAR1 signaling in mice is essential for steady-state egress and for directional *in vitro* migration of HSPC to a gradient of the major stem cell chemokine CXCL12.[16,17] It would be of great interest in future studies to elucidate a potential cross-talk between these two axes, the coagulation and inflammation-related thrombin/PAR1/nitric oxide axis and ω3-PUFA/PPARδ/Angptl4 signaling, with the purpose of improving G-CSF-induced mobilization.

The manuscript by Suzuki *et al.* provides important insights into the signaling pathways activated in BM neutrophils by G-CSF stimuli, and the cross-talk with the lipid content in the BM as a major driving force for the intensity of HSPC mobilization from the BM into the blood.

Disclosures

No conflicts of interest to disclose.

Contributions

OK, EKM and TL wrote the commentary together.

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