Novel Evidence That the Mannan-Binding Lectin Pathway of Complement Activation Plays a Pivotal Role in Triggering Mobilization of Hematopoietic Stem/Progenitor Cells by Activation of Both the Complement and Coagulation Cascades

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Novel evidence that the mannan-binding lectin pathway of complement activation plays a pivotal role in triggering mobilization of hematopoietic stem/progenitor cells by activation of both the complement and coagulation cascades

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Hematopoietic stem progenitor cells (HSPCs) circulate at low levels in peripheral blood (PB) and follow changes in circadian rhythm. Evidence has accumulated that their egress from stem cell niches is significantly augmented in a complement cascade (ComC)-dependent manner. The number of HSPCs circulating in PB increases during infection, tissue or organ injuries and particularly after administration of pharmacological drugs, such as granulocyte-colony stimulating factor (G-CSF) or the CXCR4 receptor antagonist AMD3100, and pharmacological mobilization is a means to obtaining HSPCs for hematopoietic reconstitution.

ComC and CoaC activation pathways are involved in triggering the mobilization of HSPCs after administration of G-CSF or AMD3100. MBL is a soluble receptor-like molecule that activates the ComC by engaging the so-called MBL-associated serine proteases (MASP-1 and -2). The MBL pathway of ComC activation in triggering the mobilization of HSPCs has been previously demonstrated, plays a role in the mobilization process. On the basis of these findings, we hypothesized that the MBL-initiated ComC and CoaC activation pathways are involved in triggering mobilization of HSPCs and that MBL–MASP deficiency results in poor mobilization efficiency.

In our experiments, we employed 2-month-old, MBL-deficient (MBL/−) and MASP-1-deficient (MASP-1/−) mice as well as their normal wild type (WT) littermates, and animals were mobilized with G-CSF (100 μg/kg daily for 3 or 6 days) or AMD3100 (5 mg/kg). Following mobilization, we measured the total number of white blood cells, the number of circulating clonogenic colony-forming unit granulocyte/macrophage (CFU-GM) progenitors and the number of Sca-1+ c-kit+ lineage− (SKL) cells in PB. In parallel, we evaluated activation of the CoaC after administration of G-CSF or AMD3100 in experimental animals by employing C5a ELISA. We found that MBL-deficient and MASP-1-deficient mice did show impairment in mobilization of HSPCs.

Therefore, we became interested in the potential role of the MBL pathway of ComC activation in triggering the mobilization of HSPCs after administration of G-CSF or AMD3100. MBL is a soluble pattern-recognition receptor circulating in PB that is involved in the first line of defense of innate immunity and, as mentioned above, activates the ComC by engaging the so-called MBL-associated serine proteases (MASP-1 and -2). The MBL–MASP pathway also activates the CoaC, which, as also recently demonstrated, plays a role in the mobilization process.

On the basis of these findings, we hypothesized that the MBL-initiated ComC and CoaC activation pathways are involved in triggering mobilization of HSPCs and that MBL–MASP deficiency results in poor mobilization efficiency.
in some of the experiments with an inhibitor of the CoaC (refudan).

We found that MBL-KO (Figure 1a) and MASP-1-KO (Figure 1b) mice are poor mobilizers in response to mobilizing agents compared with WT littermates. Moreover, to exclude defects in hematopoiesis in animals employed in this study that could be responsible for the observed mobilization defects, we found that under steady-state conditions MBL-deficient (Supplementary Figure 1) and MASP-1-deficient (Supplementary Figure 2) mice have normal PB cell counts (Panels A), red blood cell parameters (Panels B), numbers of bone marrow-residing HSPCs (Panels C) and numbers of clonogenic progenitors (Panels D) compared with WT animals.

Since, as mentioned above, the MBL–MASP-1 complex has been reported to also activate the CoaC, and thrombin provides C5-like convertase activity to activate/cleave C5a, 6,8 which is pivotal for egress of HSPCs from BM into PB, we performed mobilization studies in MBL−/− and WT mice in the presence or absence of the CoaC inhibitor refudan. Figure 1c shows that, as expected, control mice exposed to refudan have impaired G-CSF-induced mobilization. However, administration of refudan did not augment the mobilization defect in MBL−/− mice, which indicates that the MBL–MASP pathway is most likely the crucial pathway in activation of the CoaC following G-CSF administration.

Overall, the salient observation of our work is that MBL and its downstream effector MASP-1 play a pivotal role in activation of the CoaC during G-CSF- and AMD3100-mediated mobilization of HSPCs. For example, Figure 2a demonstrates defective generation of C5a in MBL−/− and MASP-1−/− animals, which explains our previous results in which mice that have a defect in activation of the classical pathway (C1q−) mobilize HSPCs into PB normally, because distal ComC pathway and C5 in C1q− mice is properly activated in MBM–MASP-dependent manner. We also demonstrate that, in addition to the ComC, the CoaC, which augments mobilization of HSPCs by providing thrombin-mediated C5-like convertase activity is also activated during mobilization in an MBL–MASP-dependent manner. On the basis of these and other published results, we propose the mechanistic scenario depicted in Figure 2b, which portrays mobilization of HSPCs in response to pharmacological agents (G-CSF or AMD3100).

Specifically, the first step during mobilization is activation of Gr−1 granulocytes and monocytes in the BM microenvironment, which are a source of several proteolytic 9,10 and, as recently demonstrated, also lipolytic enzymes 11 that together cooperate to

![Figure 1](image-url)
impair retention signals for HSPCs in BM niches as well as disturb membrane lipid raft integrity. The fact that experiments with mouse mutants for several proteolytic enzymes that are released from activated Gr-1+ cells in BM have failed so far to identify a crucial enzyme\(^3,^{12}\) suggests redundancy among enzymes and the involvement of several other proteases, such as cathepsin K. Moreover, it is widely acknowledged that proteolytic enzymes digest proteins involved in retention of HSPCs in BM niches, such as stromal-derived factor 1 and vascular cell adhesion molecule 1, expressed in the BM microenvironment, with the corresponding digestion proteins involved in retention of HSPCs in BM niches, such as stromal-derived factor 1 and vascular cell adhesion molecule 1, expressed in the BM microenvironment, with the corresponding proteolytic and lipolytic BM microenvironment, Gr-1+ cells also mobilize HSPCs. Mobilizing agents, G-CSF or AMD3100, activate Gr-1+ neutrophils and monocytes and enhance secretion of ROS by these cells. In the BM microenvironment, ROS expose neoepitopes. Moreover, during mobilization, several types of DAMP molecules are released. Neoepitope–IgM complexes as well as DAMPs are recognized by MBL, which activates the ComC and the CoaC in a MASP-dependent manner. CS convertases (classical and ‘CS-like’) generated in the next step cleave C5 to release cleavage fragments crucial to executing egress of HSPCs from BM.

Figure 2. (a) Defective cleavage of C5 in MBL- and MASP-1-deficient mice during mobilization. Plasma collected from PB of mice mobilized with G-CSF (short or long) or AMD3100 show lower levels of C5a compared with WT mice. Results shown as a percentage of mobilized WT mice, \(P \leq 0.05\). (b) Interplay of components of innate immunity (the ComC, Gr-1+ cells, naturally occurring IgM antibodies), and the CoaC in the mobilization of HSPCs. Mobilizing agents, G-CSF or AMD3100, activate Gr-1+ neutrophils and monocytes and enhance secretion of ROS by these cells. In the BM microenvironment, ROS expose neoepitopes. Moreover, during mobilization, several types of DAMP molecules are released. Neoepitope–IgM complexes as well as DAMPs are recognized by MBL, which activates the ComC and the CoaC in a MASP-dependent manner. CS convertases (classical and ‘CS-like’) generated in the next step cleave C5 to release cleavage fragments crucial to executing egress of HSPCs from BM.
In conclusion, we have identified a previously unrecognized role for the MBL–MASP-1 pathway in triggering both ComC and CoaC activation during the HSPC mobilization process. This finding explains the pivotal role of the MBL pathway in triggering activation of the proximal part of the ComC and explains why, with a deficiency in activation of classical pathway components (C1q), mobilization of HSPCs proceeds normally as long as the MBL pathway remains intact.15 Taking into consideration that ~10% of normal people are poor activators of the MBL pathway15 and that this percentage may correspond with the ~10% of the normal healthy population that are poor mobilizers, we are currently investigating whether MBL deficiency correlates with poor mobilization status in patients. If our hypothesis is correct, the MBL level could become an important predictive parameter for identifying poor mobilizers. Finally, our results again confirm a pivotal role of the ComC and other elements of innate immunity as well as involvement of the CoaC in the mobilization process.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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Letters to the Editor

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