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The Role of Complement in the Trafficking of Hematopoietic Stem/Progenitor Cells

A Janowska-Wieczorek^{1,2}, LA Marquez-Curtis², N Shirvaikar², and MZ Ratajczak³

¹Department of Medicine, University of Alberta, Edmonton, AB, Canada

²Canadian Blood Services Research & Development, Edmonton, AB, Canada

³Stem Cell Institute at James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA

Introduction

Transplantation of hematopoietic stem/progenitor cells (HSPC) has become a well-established treatment for various malignant and non-malignant hematological disorders and certain solid tumors. Approximately 60,000 autologous and allogeneic HSPC transplants are performed annually worldwide. Characteristics of HSPC that make clinical transplantation feasible, apart from their regenerative potential, include their ability to be coaxed out of the bone marrow (BM), or “mobilize”, and their capacity to “home” back to the marrow space after intravenous infusion. In our investigations of HSPC mobilization and homing, the complement system has emerged as an important, yet underappreciated, modulator of this bidirectional trafficking of HSPC. Although many factors contribute to HSPC mobilization and homing, here, we focus on the role of complement cascade (CC) components C1q, C3a, C5a and C5b-C9 (membrane attack complex; MAC) and infer that their modulation in the future could have significance to improve outcomes of HSPC transplantation. After autologous and allogeneic transplantation the patient requirements for red blood cell transfusion are high (median 12; range: 8–16 units per patient),¹ and despite variations in practice, there is also substantial need for platelet transfusions (median 5; range 0–110 units per patient).² Hence, improving the strategies for HSPC collection based on better understanding of the mechanisms of mobilization and homing could also reduce the utilization of blood products.

HSPC Transplantation

Because HSPC reside primarily in the BM, HSPC for both autologous and allogeneic transplantation were traditionally collected by means of multiple aspirations from the posterior iliac crest under general anesthesia. BM transplantation was pioneered in the 1950s by a team led by E. Donnall Thomas, who showed that BM-derived stem cells infused intravenously repopulate the recipient BM and reconstitute hematopoiesis.³ In the late 1970s it was shown that during steady-state homeostasis, a small number of HSPC circulate continuously in the human peripheral blood (PB) and this number increases following treatment with chemotherapy (e.g., with cyclophosphamide) and/or growth factors and cytokines [e.g., granulocyte-colony stimulating factor (G-CSF)] that mobilize HSPC from

Corresponding Author: Anna Janowska-Wieczorek, MD, PhD, Department of Medicine, University of Alberta and Canadian Blood Services Edmonton Centre, 8249–114 Street NW, Edmonton, Alberta, Canada, T6G 2R8, Phone: (780) 431-8761, Fax: (780) 702-8622, anna.janowska@blood.ca.

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BM into the PB.^{4,5} Currently, mobilized (m)PB HSPC have almost completely replaced HSPC from BM for autologous and three quarters of allogeneic transplantations.^{6,7} Collection of mPB HSPC by leukapheresis is carried out in an outpatient setting and is therefore less invasive and without the risks associated with general anesthesia. Moreover, randomized trials have shown that neutrophil and platelet engraftment generally occurs faster after mPB transplantation than after BM transplantation, likely due to the higher number of HSPC collected in mPB and transplanted.⁸ Another possible explanation is that HSPC from mPB are exposed to CC cleavage fragments (e.g. C3a) during leukapheresis and collection, and to cationic bioactive peptides released from granulocytes (e.g. LL-37).^{9–12} More rapid engraftment reduces risk of infection, number of transfusions, and length of hospitalization. However, donor/patient responses to mobilizing agents vary; up to 5% of healthy allogeneic donors mobilize poorly and up to 60% of high-risk patients failed to mobilize at all, depending on their underlying disease, prior chemotherapy regimens, age, and other factors.⁷

An alternative to BM or mPB as source of HSPC is umbilical cord blood (CB). Since the first CB transplant in 1988, an estimated >30,000 CB transplants have been performed worldwide in both pediatric and adult patients.^{13,14,15} However, the main limitation of CB transplantation use in adults is the low HSPC (CD34⁺ cell) dose available in one CB unit, which is generally insufficient to support engraftment in adult patients. Retrospective analysis of CB transplantation outcomes in adults has shown delayed neutrophil engraftment (27 days with CB versus 18 with BM) and platelet engraftment (60 days with CB versus 29 with BM).¹⁵ Currently, efforts are being made to elucidate the mechanisms of HSPC homing and develop new strategies promoting more efficient hematopoietic reconstitution. These include use of more than one CB unit for transplantation, ex vivo expansion, and intra-bone infusion.^{16–18} In this review we focus on the complement system as a means for enhancing homing of CB HSPC.

BM niches and HSPC trafficking

Current perception of the processes of HSPC mobilization and homing derives from our understanding of the dynamic interactions between HSPC and the BM microenvironment, which comprise the stem cell niche. The concept of niches as first proposed by Schofield¹⁹ describes three-dimensional spatially organized anatomical compartments in the BM where stem cells reside and are maintained. Mounting evidence later revealed that the BM niche provides not only a simple static structural support but also topographical information and the appropriate physiological cues to control the dynamic balance of stem cell quiescence, self-renewal, differentiation and apoptosis, as well as HSPC localization and migration.^{20,21} The existence of the endosteal/osteoblastic and the vascular niches has been suggested. The endosteal/osteoblastic niche close to the bone, a site of relative hypoxia where immature osteoblasts are in close contact with HSPC, plays a major role in the maintenance of hematopoietic stem cell (HSC) quiescence.^{22–24} The vascular niche consisting of sinusoidal vessels provides a microenvironment rich in nutrients, growth factors, and oxygen, and plays a role in HSC proliferation and differentiation, and ultimately the egress of mature progenitors into the circulation.^{22,23,25}

HSPC mobilization is primarily mediated by alterations in the cellular components of the BM niche.²⁶ Perivascular mesenchymal stem cells (MSC), macrophages, sinusoidal endothelial cells, osteoblasts, and sympathetic nerve fibers form the niches that harbor HSPC during homeostasis and mediate their egress in response to mobilizing agents.^{21,27,28} For example, suppression of resident monocytes/macrophages leads to decreased expression of factors required for HSPC retention and results in HSPC mobilization.²⁹ Furthermore, depletion of endosteal macrophages (osteomacs) that form a canopy over mature osteoblasts

at sites of bone formation and support osteoblast function elicited robust mobilization of HSPC, suggesting that BM macrophages play a critical role in the maintenance of endosteal HSPC niches.³⁰ On the other hand, it was recently demonstrated that nestin-expressing perivascular MSC are either in direct contact with HSPC or in clusters around them. These nestin⁺ MSC express HSPC maintenance genes and, upon their deletion, significant reduction in BM HSPC is observed, owing at least in part to their mobilization towards extramedullary sites.³¹ The BM is highly innervated, with nerve fibers running along blood vessels, and increasing evidence indicates a major role for signals coming from the sympathetic nervous system in the regulation of HSPC retention, homing and mobilization.^{27,31}

The cellular components of the BM microenvironment transmit and receive signals through soluble factors (e.g., growth factors, cytokines and chemokines, hormones), bioactive lipids, cell adhesion molecules, extracellular matrix (ECM), neural inputs, and the vascular network. Stromal cell-derived factor (SDF)-1 (also known as CXCL12) is a chemokine that strongly attracts HSPC, which express its receptor CXCR4.^{32,33} SDF-1 is constitutively expressed at high levels by osteoblasts and endothelial cells and represents a potent retention signal for HSPC. HSPC are also retained in the niche by adhesion molecules acting through cell-to-cell contact (e.g., via the very late antigen (VLA)-4/vascular cell adhesion molecule (VCAM)-1 axis) and attachment to ECM components. With the HSPC firmly entrenched in the BM niches, it can be envisioned that their mobilization to PB would require the breaking down of adhesive interactions, alterations in chemotactic gradients (e.g., an increase in the sphingosine-1 phosphate (S1P) gradient in BM sinusoids) and proteolysis of ECM and other molecules that promote anchorage of HSPC to their niches (e.g., those released from granulocytes and monocytes stimulated by G-CSF or the C5a complement cascade cleavage fragment).^{10,34}

During steady-state hematopoiesis, the continuous traffic of HSPC between the BM and PB, albeit at a very slow rate, contributes to maintaining normal hematopoiesis. However, stress conditions such as inflammation or injury greatly amplify the egress of HSPC from the BM. These processes are mimicked in clinical mobilization in which HSPC are recruited from the BM to PB by means of pharmacological agents and collected for use in transplantation.^{5–7} Several cytokines, growth factors, and chemokines [G-CSF, granulocyte macrophage (GM)-CSF, Flt-3 ligand, interleukin (IL)-8, stem cell factor (SCF), hepatocyte growth factor (HGF), SDF-1 and GRO β] can trigger mobilization in varying degrees.^{35–37} Recent studies have demonstrated that thrombolytic agents, such as microplasmin, tenecteplase, and recombinant tissue plasminogen activator, enhance G-CSF-induced mobilization in murine models,³⁸ and the proteolytic enzyme membrane type 1-matrix metalloproteinase (MT1-MMP) is upregulated by G-CSF and contributes to ECM degradation, thus enhancing the migration of HSPC.^{39,40} HSPC can also be easily mobilized by CXCR4 receptor antagonists (AMD3100 also known as plerixafor, T140) or agonists (CTCE-0021, ATI-2341) and after blockage of VLA-4 integrin on HSPC by BIO 4860.^{10,11,41,42}

Conversely, homing occurs when transplanted HSPC travel from the blood circulation and establish residence within the BM niche. Early on, the homing process was described as the “rolling, crawling and nesting” of HSPC into the marrow stromal space.⁴³ Even then, seminal studies had already identified the significant contributions of a wide variety of adhesion molecules and their receptors in mediating cell-to-cell and cell-to-matrix interactions, and the role of the SDF-1/CXCR4 axis in the retention of HSPC in the BM niche was also realized.^{44–47} It soon became evident that proteolytic enzymes capable of degrading ECM components could play a role in the migration of HSPC.⁴⁸ It is now recognized that homing is a complex multi-step process that involves signaling through adhesion molecules, chemotactic molecules, and their receptors, proteases and other factors.

In addition, GM-CSF, IL-3 and SCF, which activate VLA-4 and VLA-5 and increase the adhesiveness of HSPC, as well as Flt3-ligand, SCF, IL-3, IL-6 and HGF which, in addition to prostaglandin E₂(PGE₂), upregulate the expression of CXCR4 on HSPC, also affect the homing of HSPC.³³ A flexible hierarchy of cooperating homing pathways with the dominant players characterized by significant functional overlap and constant repositioning within changing cytokine milieu has been postulated.⁴⁹

Thus, existing empirical evidence demonstrates the interplay of cellular components and various signaling molecules in modulating HSPC trafficking. Our recent research has shown that activation of the CC takes place in both the mobilization and homing of HSPC, and we discuss it below in detail.

Activation of the CC

The human CC has been traditionally recognized as a supportive first line of host defense against infections. It is now known that its functions extend far beyond the elimination of foreign bodies by acting as a rapid and efficient immune surveillance system that discriminates between healthy host tissue, cellular debris, apoptotic cells, and pathogenic microbes and responds accordingly.^{50,51} The complement proteins are important elements of the innate immune response that act in a cascade to induce their physiological effects. Three main pathways for complement activation are recognized namely the classical, alternative, and lectin pathways, as recently reviewed.^{50,51}

The classical pathway, often referred to as the antibody (IgG)-dependent pathway, is initiated by non-covalent binding of the 3-subunit component 1, composed of C1q, the recognition subunit of the complex, and two chains each of C1r and C1s, proteases which are activated upon surface binding of C1q. Activated C1 cleaves C4 and C2 releasing smaller fragments (C4a and C2b) and larger fragments (C4b and C2a). C2a coordinates with C4b to form an enzymatic complex termed C3 convertase, with the ability to cleave C3 into larger C3b and smaller C3a, an anaphylatoxin with pro-inflammatory properties (Fig. 1).⁵⁰ Deposition of C3b on the target surface induces the formation of C5a convertase, which cleaves C5 into C5b and C5a, another anaphylatoxin. C6, C7, C8 and C9 bind serially to C5b to form the membrane attack complex (MAC), which initiates cell lysis when inserted into the cell membrane of an invading cell. The alternative (IgG-independent) pathway is triggered by spontaneous C3 hydrolysis which exposes its internal thioester group to form C3a and C3b. Upon covalent binding to a pathogenic membrane C3b is bound by Factor B to form a complex, which, in the presence of Factor D, is cleaved into Ba and Bb. Bb remains covalently bonded to C3b to form C3bBb, which has proteolytic activity, catalyzing the hydrolysis of C3 in the blood into C3a and C3b. Deposition of C3b molecules leads to the formation of a C5 convertase which cleaves C5 into C5a and C5b, as in the classical pathway (Fig. 1).⁵⁰ The lectin pathway is homologous to the classical pathway, but with the opsonin, mannose-binding lectin (MBL), and ficolins, instead of C1q. This pathway is activated by binding MBL to mannose residues on the pathogen surface, which activates the MBL-associated serine proteases, MASP-1, and MASP-2 (very similar to C1r and C1s, respectively), which can then split C4 into C4a, and C4b and C2 into C2a and C2b. C4b and C2b then bind together to form the C3-convertase, as in the classical pathway.⁵⁰ Because all three complement pathways merge with C3b deposition on a target and C3b is the initiating factor of the alternative pathway, complement activation can be initiated by the classical or lectin pathway and amplified by the alternative pathway.

The anaphylatoxins C3a and C5a are constantly released and trigger signals through their corresponding G-protein-coupled receptors, C3aR and C5aR (also called CD88). They may also engage an alternative C5L2 receptor.⁵² Human C3aR is expressed on monocytes/

macrophages, neutrophils, eosinophils and basophils and mediates the chemotaxis of eosinophils and mast cells. C5aR is expressed on neutrophils, eosinophils, basophils and monocytes, as well as epithelial and endothelial cells. Activities initiated in granulocytes and monocytes via C5aR include cytoskeletal remodeling, shedding of L-selectin, and upregulating adhesion molecules, chemotaxis, granule release and synthesis of cytotoxic reactive oxygen metabolites. Activation of granulocytes and monocytes by C5a and its derivative ^{desArg}C5a play an important role in the release of proteolytic enzymes that cause detachment of HSPC from their niches.^{10,53–55} Furthermore, three cell-associated receptors for C1q have been described: cC1qR which closely resembles calreticulin; C1qRp, a phagocytosis-promoting receptor; and gC1qR, which recognizes the globular head region of C1q.⁵⁰ The activities triggered by C1q include the induction of chemotaxis and chemokinesis in mast cells and neutrophils, increased expression of adhesion molecules in endothelial cells and promotion of phagocytosis. It is apparent that the complement receptors are involved in the signaling pathways modulated by the complement component proteins.

Because circulating HSPC could be recruited to the affected peripheral tissues during infection or injury as an important part of innate immunity surveillance,⁵⁶ we examined whether HSPC mobilization is regulated by elements of innate immunity, in particular by CC proteins.

Mobilization and the Role of Complement

Many reviews on the biology of HSPC mobilization have been published recently^{11,26,57,58} together with investigations of strategies to improve HSPC collection.^{7,42,59–61} Based on a review of clinical trials using chemotherapy, cytokines or combination regimens for mobilization of HSPC, it was concluded that the optimal mobilization strategy is still unknown.⁶¹ Key interactions between HSPC and the BM stroma have been identified and have become potential targets to enhance mobilization. It is important to recognize that different mobilization regimens could have different effects on the graft (e.g., expression of genes involved in cell cycle, apoptosis, cell adhesion and chemotaxis), which may affect the outcome of HSPC transplantation.⁶²

The complement system is an essential component of innate immunity that we recently postulated to be one of the major players in HSPC mobilization.^{10,53,55} As it has been observed that G-CSF-induced mobilization is impaired in patients suffering from severe combined immunodeficiency disease (SCID), i.e., who lack functional B and T lymphocytes,⁶³ we proposed that the egress of HSPC from the BM occurs as part of the immune response. Since the classical pathway of CC is activated by a subclass of immunoglobulins (known as naturally occurring antibodies) poor mobilization in these patients could be explained by lack of CC-activating immunoglobulins that recognize the neoepitope antigen on BM cells expressed during mobilization. Like these SCID patients, immunodeficient mice are also poor mobilizers due to their lack of CC-activating immunoglobulins.⁶⁴

To explain the roles of naturally occurring antibodies, expression of neoepitope on BM cells during mobilization, and activation of CC, it was suggested that mobilizing agents (e.g., G-CSF) stimulate granulocytes and monocytes in BM to release proteolytic enzymes (Fig. 2) that turn the BM microenvironment into a proteolytic one.^{65,66} This can be perceived as a cause of “BM injury” leading to exposure of neoepitope on BM damaged cells. Such an event triggers local activation of the CC. The neoepitope binds naturally occurring antibodies (NA-Ig) that circulate in the PB and, via C1q triggers CC activation through the

classical pathway generating C3 and C5 cleavage fragments (Fig. 1).^{53,54} This mechanism of CC activation is typical for G-CSF-induced mobilization.

In contrast, polysaccharides (e.g., zymosan) activate the CC by the alternative pathway, which in turn triggers various reactions that likewise generate C3 and C5 cleavage fragments (Fig. 1).⁵³ C3 could also be cleaved directly by proteases released from granulocytes after administration of mobilizing agents. In fact, the concentration of C3 cleavage fragments (C3a, ^{desArg}C3a, iC3b) in the BM increases during mobilization.⁶⁷ In addition, the proteolytic activities of thrombin and kallikrein also activate C5 (Fig. 1).

As shown in Fig. 1, CC cleavage fragments play opposite roles in the mobilization of HSPC. While C3 cleavage fragments inhibit mobilization by promoting BM retention, C5 cleavage fragments and the activation of C5b-C9 (MAC) promote mobilization. This is supported by the observation that while C3-deficient mice (lacking C3 cleavage fragments) are easy mobilizers,⁶⁸ C5-deficient mice (lacking C5 cleavage fragments and not generating MAC) mobilize very poorly.^{53,54,69}

We demonstrated that C3 cleavage fragments enhance/prime the responsiveness of HSPC to an SDF-1 gradient, thus promoting retention of these cells in the BM.⁷⁰ In fact, as mentioned above, studies using C3-deficient (C3^{-/-}) mice, as well as studies using C3a receptor-deficient (C3aR^{-/-}) mice, revealed that the C3a-C3aR axis protects HSPC against their uncontrolled egress from the BM and that the blockade of this axis with the C3aR antagonist SB 290157 increases HSPC mobilization.⁶⁸ On the other hand, C5-deficient mice respond poorly to G-CSF-induced mobilization (in contrast to their wild type counterparts),⁵⁴ suggesting an important role of the distal part of the CC in promoting egress of HSPC from the BM into PB.

To further elucidate the role of C5 cleavage fragments in the mobilization of human HSPC, we evaluated the plasma levels of the cleavage fragment ^{desArg}C5a. We found that ^{desArg}C5a levels are significantly higher and correlate with CD34⁺ cell and WBC counts in patients who are good mobilizers.⁵⁵ Although C5 cleavage fragments did not chemoattract myeloid progenitors (CFU-GM), ^{desArg}C5a strongly chemoattracted both granulocytes and monocytes (Fig. 2). Consistently, the C5a receptor CD88 was not detected on CD34⁺ cells but appeared on more mature myeloid precursors, monocytes and granulocytes. Moreover, we found that G-CSF-mobilized mononuclear and polymorphonuclear cells from PB had a significantly higher percentage of cells expressing CD88 than those from their non-mobilized PB counterparts. Furthermore, C5a stimulation of granulocytes and monocytes decreased CXCR4 expression and chemotaxis towards an SDF-1 gradient, and increased secretion of MMP-9 and expression of MT1-MMP and carboxypeptidase M by these cells (Fig. 2).⁵⁵ These observations support the notion that C5 cleavage fragments facilitate the egress of HSPC into mPB by increasing the secretion of proteolytic enzymes from granulocytes, thereby attenuating the function of the SDF-1/CXCR4 and VCAM-1/VLA-4 axes (Fig. 2). C5 cleavage fragments also directly chemoattract granulocytes that “pave the way” for subsequent egress of HSPC (Fig. 2).⁵⁴

Activation of the distal part of the CC is also crucial in generating C5b-C9 (MAC), the activation of which leads to the release of S1P into BM sinusoids from erythrocytes, the major reservoir of S1P in the PB (Fig. 2). We observed that, at physiologically relevant concentrations, S1P is several magnitudes more potent in chemoattracting BM-residing HSPC than SDF-1. In fact, we observed that the plasma SDF-1 levels do not correlate with the mobilization efficiency of CD34⁺ HSPC in patients and that normal and mobilized plasma chemoattracts HSPC independently of SDF-1.^{34,58,71,72} S1P has been previously shown to chemoattract HSPC and to participate in the egress of HSPC from extramedullary

tissues to lymph.^{56,73} Consistent with the activation of CC during mobilization, we observed an elevation of the S1P level in PB after G-CSF induced mobilization which explains HSPC egress into the circulation in a S1P chemotactic gradient-dependent manner.^{10,34} Furthermore, since PB under steady-state conditions contains a significant amount of free S1P that creates a strong chemotactic gradient for HSPC,³⁴ we postulate that retention of HSPC in the BM is an active process that counterbalances the S1P chemoattracting gradient continuously present in the PB (Fig. 2).

Taken together, our data support the concept that the mobilization process is part of a more general immune response to infection or tissue injury that causes release of granulocytes, monocytes and HSPC from hematopoietic organs. We propose that modulation of the CC could be an important tool to regulate the release of HSPC from their niches. We also have evidence that, similar to G-CSF, mobilization induced by the CXCR4 antagonist AMD3100 (plerixafor) depends on activation of the CC.⁶⁹ AMD3100 restores mobilization in mice that do not activate the proximal steps of CC (i.e., are C3-deficient), but is not as effective in mobilizing mice that are unable to generate the distal components of CC activation (i.e., are C5-deficient). In contrast to G-CSF, however, AMD3100 activates the complement system directly at the C5 level. Our cell egress kinetic data showed that granulocytes and monocytes constitute the first wave of cells mobilized into the PB by AMD3100, followed by HSPC. AMD3100 upregulates the expression of MMP-9, cathepsin G, and neutrophil elastase that cleave/activate C5 in this BM-derived granulocyte and monocyte population.⁶⁹ Overall, these data support the notion that C5 cleavage fragments and the distal steps of CC activation are required for optimal mobilization of HSPC (Fig. 2). Interestingly, eculizumab, a humanized monoclonal antibody against C5 that inhibits a terminal complement activation, is employed to inhibit MAC-dependent lysis of erythrocytes in patients with paroxysmal nocturnal hemoglobinuria,⁷⁴ and we can expect that this antibody would inhibit mobilization of HSPC; however, to our knowledge no such studies have been performed.

Homing and the Role of Complement

In recent years there have been a number of comprehensive reviews on HSPC homing.^{11,25,33,75} Homing is defined as the early event that lodges and firmly retains HSPC in the BM prior to their proliferation and expansion. Clearly, the success of clinical HSPC transplantation relies on the inherent ability of transplanted HSPC to home efficiently to the appropriate BM niche and to engraft. Migratory events that lead to HSPC homing are fairly rapid, taking place within 5 to 15 hours and not later than 2 days post-transplantation.⁷⁵ Homing occurs when intravenously administered HSPC recognize and interact with the microvascular endothelial cells of the BM, adhere to the vessel wall with sufficient strength to overcome the considerable shear stress exerted by the flowing blood, and then extravasate across the vascular wall along an SDF-1 gradient generated by osteoblasts in the endosteal niche (Fig. 3). This homing gradient of SDF-1 is significantly potentiated by several small molecules, such as C3a, cathelicidine (LL-37), uridine triphosphate (UTP) or PGE₂, released in the BM microenvironment after pre-conditioning for transplantation by radio-chemotherapy. These small molecules increase the responsiveness of HSPC to the SDF-1 gradient by increasing incorporation of CXCR4 into membrane lipid rafts (C3a, LL-37), by upregulating CXCR4 levels on HSPC (PGE₂), or by other unknown mechanisms (UTP).^{9,12,70,76,77}

As it does during mobilization, pre-conditioning for transplantation (by lethal irradiation in murine models) activates the CC in BM as confirmed by ELISA assay, which detects C3a and C5a cleavage fragments in plasma^{54,55} and by histochemical detection of MAC in BM tissue (Fig. 3).^{12,78} The importance of the CC in stem cell homing/engraftment is supported

by the fact that C1q-, C3- and C5-deficient mice show delayed hematopoietic recovery after transplantation of HSPC.^{58,79,80}

When investigating the role of C1q in HSPC trafficking, we found that its receptor (C1qRp; also known as human CD93) is present on HSPC from human BM, mPB and CB CD34⁺ cells and on myeloid, megakaryocytic and erythroid progenitors (Fig. 3). Moreover, C1q alone is not a chemoattractant for CD34⁺ cells, but it primes SDF-1-dependent chemotaxis as well as SDF-1-dependent trans-Matrigel migration of CD34⁺ cells.⁷⁹ The presence of functional C1qRp on human CD34⁺ cells allows these cells to respond better to the SDF-1 gradient and be retained within BM niches (Fig. 3). Having shown that C1q enhances the responsiveness of CD34⁺ cells to an SDF-1 gradient in vitro, using C1q-deficient mice for in vivo studies we subsequently found that they are more sensitive to mobilization induced by G-CSF than wild type mice, suggesting that C1q is involved in the retention of HSPC in the BM niches and that perturbation of this axis facilitates egress of HSPC into the blood.⁷⁹

Moreover, to explain why C3-deficient mice engraft poorly, we showed that C3a and ^{desArg}C3a increase/prime responsiveness of HSPC to the SDF-1 gradient,^{9,80,81} which decreases in BM after pre-conditioning for transplantation.⁷⁸ A molecular explanation for this intriguing phenomenon is based on the observation that the actively signaling CXCR4 receptor is associated with lipid rafts (Fig. 3).^{9,80} These membrane domains are rich in sphingolipids and cholesterol, which form a lateral assembly in a saturated glycerophospholipid environment. 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 are also good sites for crosstalk between various cell signaling proteins. For example, it has been 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 lipid rafts on migrating HSPC.^{82–84} Since the CXCR4 receptor is a lipid raft-associated protein, its signaling ability is enhanced when CXCR4 is incorporated into membrane lipid rafts where it may interact better with several signaling molecules, including Rac-1.^{83,84} This co-localization of CXCR4 and Rac-1 in lipid rafts facilitates GTP binding/activation of Rac-1.

Thus, generation of C3 cleavage fragments in the BM microenvironment may act in some way as a mechanism to increase the responsiveness of HSPC to a SDF-1 gradient. In C3-deficient mice this phenomenon is attenuated, explaining why these animals show delayed engraftment. In support of this, increases in the level of C3a and ^{desArg}C3a in BM after myeloablative conditioning promotes homing of HSPC.⁸⁰ In addition, the C3 cleavage solid phase iC3b fragment that is deposited in the BM microenvironment tethers HSPC and increases their adhesion to BM.⁷⁰

Furthermore, evidence is accumulating to indicate that the C3a binding receptor (C3aR) is expressed by HSPC and may be involved in their homing (Fig. 3). Accordingly, transplantation of C3aR^{-/-} HSPC into lethally irradiated mice resulted in a delay in neutrophil and platelet recovery and a decrease in the number of CFU-GM progenitors in the BM after transplantation. Consistent with these murine data, blockage of C3aR on human CB CD34⁺ cells by the C3aR antagonist SB290157 impairs their engraftment in non-obese diabetic/SCID mice.⁸¹ Overall, these results show that the C3a/C3aR axis plays a role in the homing of HSPC to the BM, first by enhancing responsiveness to the SDF-1 gradient and second, by modulating several functions pertinent to trans-endothelial migration. We have been proposing that one of the strategies to be developed to improve engraftment is the ex vivo priming of HSPC before transplantation with small molecules such as C3a.¹⁸ This strategy is currently being tested in transplantation centers (Charlottesville, VA and

Minneapolis, MN, USA) where umbilical CB-derived HSPC are ex vivo primed with recombinant C3a for 30 minutes before infusion into the patients.

In addition to C3 cleavage fragments, we found that the antimicrobial cationic peptide LL-37, which is secreted from granulocytes and BM stroma, is able to prime/enhance the responsiveness of murine and human HSPC to an SDF-1 gradient.¹² This indicates that the responsiveness of HSPC to the BM SDF-1 gradient could be positively modulated by several small molecules, just as we observed with valproic acid, hyaluronic acid and thrombin.^{85,86} Since the CC becomes activated during mobilization of HSPC and their subsequent harvest via leukapheresis,⁸⁷ we envision that an mPB graft contains HSPC that are primed by C1q, C3 cleavage fragments, and LL-37 released from C5a-activated granulocytes present in the leukapheresis product. This may explain why mPB engrafts faster than BM or CB cells.¹²

Finally, our recent observation that C5-deficient mice also engraft poorly with HSPC indicates that the MAC could be involved in the homing of HSPC (Fig. 3).⁷⁸ MAC released in the final steps of CC activation is present in PB in two forms (Fig. 1). The first form, lytic MAC, forms transmembrane channels that disrupt the phospholipid bilayer of target cells, leading to cell lysis and death. However, another form, sublytic MAC, may bind to cell membranes independent of any receptor, and does not lyse cells, but activates multiple signaling pathways and has wide-ranging effects on many cell types leading to cellular responses, such as secretion, adherence, aggregation, chemotaxis and even cell division. Recently we reported and proposed that MAC increases the homing responses of HSPC to S1P and ceramide 1-phosphate (Fig. 3).^{58,78}

Conclusion

A new paradigm is emerging which shows that complement cascade proteins play an important role in both the mobilization and homing of HSPC. These findings show promise for clinical applications both ex vivo and in vivo that could improve the outcome of HSPC transplantation. Ex vivo priming of HSPC with C3a shows the most promise and is currently being tested in clinical trials.

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Figure 1.

Complement cascade (CC) activation during HSPC mobilization. The CC may become activated through the classical pathway (which is dependent on naturally-occurring antibodies (NA-Ig) and is triggered by complement protein C1q), the lectin pathway, and the alternative pathway (which is triggered by Factors B and D). All three pathways merge at complement protein C3, whose activation leads to the release of C3 anaphylatoxin and complement protein C5. C5 is enzymatically cleaved to release anaphylatoxin C5a. Some alternative mechanisms exist, whereby C5 could be activated by the proteases thrombin and kallikrein. The arginine terminal residue of both C3a and C5a is immediately cleaved to yield $_{\text{desArg}}\text{C3a}$ and $_{\text{desArg}}\text{C5a}$, which have different biological functions. While C3a and $_{\text{desArg}}\text{C3a}$ cleavage fragments enhance the responsiveness of HSPC to the bone marrow to a stromal-cell derived factor (SDF)-1 gradient, thus promoting their retention in the bone marrow, C5a and $_{\text{desArg}}\text{C5a}$ facilitate the egress of granulocytes and pave the way for the mobilization of HSPC. The final step of CC activation is the generation of the cytolytic end-product C5b-C9 membrane attack complex (MAC).

Figure 2.

Schematic representation of some of the interactions in response to G-CSF-induced mobilization. HSPC are retained in the bone marrow niche by adhesive interactions, such as between vascular adhesion molecule (VCAM)-1 and very late antigen (VLA)-4, and chemotactic interactions, such as between SDF-1 (green triangles) and CXCR4 receptor. Mobilizing signals (e.g., G-CSF) expand the number of granulocytes and activate the complement cascade. C5 cleavage fragments activate myeloid cells via the C5a receptor (C5aR) to release proteases (red and purple circles) which disrupt VCAM-1/VLA-4 and SDF-1/CXCR4 interactions. Membrane type-1 matrix metalloproteinase (MT1-MMP, black diamond) accumulates on the surface of granulocytes and HSPC and further facilitates their egress. In addition, C5 cleavage fragments chemoattract granulocytes and pave the way for the mobilization of HSPC across the endothelium. MAC generated in the final step of CC activation enhances the release of sphingosine-1 phosphate (S1P) from erythrocytes. S1P is also a potent chemoattractant for HSPC which express the S1P receptor.

Figure 3.

Schematic representation of some of the interactions occurring during HSPC homing. Conditioning for transplantation induces a proteolytic microenvironment (e.g., release of matrix metalloproteinase (MMP)-9) that activates the complement cascade and leads to the generation of the MAC. In addition, the concentrations of the bioactive lipids S1P and ceramide-1 phosphate (C1P) increase in the bone marrow after conditioning for transplantation. Both S1P and C1P are potent chemoattractants for HSPC. Moreover, agents priming responses to SDF-1 (e.g., C3a, which acts through its receptor C3aR) enhance the incorporation of CXCR4 into the lipid rafts of the cell membrane of HSPC. All these interactions lead to chemoattraction and lodgement of HSPC in the bone marrow niche.