

THE ROLE OF THIRD COMPLEMENT COMPONENT (C3) IN HOMING OF HEMATOPOIETIC STEM/PROGENITOR CELLS INTO BONE MARROW

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1. INTRODUCTION

Transplantation of hematopoietic stem/progenitor cells (HSPC) is routinely employed in the clinic to treat patients with hematological malignancies, inborn or acquired defects of hematopoiesis and immunodeficiencies. Prior to transplantation in order to destroy old pathological hematopoiesis, the patient receives a myeloablative dose of radiochemotherapy, and subsequently the patient's own autologous- or harvested from histocompatible donor allogeneic-HSPC are infused intravenously in an attempt to establish new hematopoiesis. It is well established that the α -chemokine stromal derived factor-1 (SDF-1) and the G-protein-coupled seven-span transmembrane receptor, CXCR4, axis plays a pivotal role in directing the homing of HSPC from the blood to the bone marrow (BM)^{1,2}.

Chemokines, small proinflammatory chemoattractant cytokines that bind to specific G-protein-coupled seven-span transmembrane receptors present on the plasma membranes of target cells, are the major regulators of cell trafficking. More than 50 different chemokines and 20 different chemokine receptors have been cloned³. Chemokines usually bind to multiple receptors, and the same receptor may bind to more than one chemokine. However, there is one exception to this rule: the α -chemokine SDF-1, which binds exclusively to CXCR4, and

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has CXCR4 as its only receptor. This fact alone suggests that the SDF-1–CXCR4 axis plays a uniquely important biological role. In fact from all the chemokines tested so far, SDF-1 is the only chemoattractant for HSPC¹.

While SDF-1 is secreted by BM stroma^{4,5}; CXCR4 is present on the surface of HSPC⁶. SDF-1 secreted by stromal cells in the developing BM is critical for its colonization by fetal liver-derived HSPC during embryogenesis⁷⁻¹². In support of this, murine embryos with SDF-1 or CXCR4 knockout have a profound decrease in HSPC in their BM cavities^{9,13}. Furthermore, during adult life the SDF-1–CXCR4 axis plays a pivotal role in retention of HSPC in the BM microenvironment^{1-2,7-9,11-14}. Thus it is not surprising that proper functioning of the SDF-1–CXCR4 axis is crucial in directing homing/engraftment of HSPC into BM after transplantation^{1,15}. On the other hand, perturbation of the SDF-1–CXCR4 axis, for example, by mobilizing factors (e.g., G-CSF) is essential for egress of hematopoietic stem/progenitor cells from the BM into peripheral blood (PB)¹⁶⁻¹⁸.

Data from our laboratories demonstrate that complement (C) emerges as a new regulator of SDF-1-mediated homing of HSPC into BM as well as a regulator of HSPC egress/mobilization from BM into peripheral blood¹⁹⁻²¹. In this review we will present our data that the cleavage fragments of the third component (C3) of the complement system, C3a and C3a_{desArg}⁷ play an important role in modulating responsiveness of HSPC to an SDF-1 gradient. Thus a new concept emerges that trafficking of HSPC is tightly connected with activation of C and innate immunity. We will discuss these data in our review.

2. THE FUNCTION OF CXCR4 RECEPTOR DEPENDS ON LIPID RAFT FORMATION

The function of CXCR4 on HSPC is regulated at multiple levels (Figure 1). First, expression of CXCR4 is regulated at the transcriptional level by several factors (e.g., hypoxia)²²⁻³⁰. Second, CXCR4 as well as its ligand SDF-1, are subject to proteolytic degradation by some proteases (e.g., metalloproteinases (MMPs), dipetidylpeptidase CD26, and carboxypeptidase) that are expressed in the hematopoietic microenvironment and serum^{31,32}. Third, CXCR4 after interaction with SDF-1 is internalized from the surface and subsequently may be recirculated from the endosomal compartment again to the cell surface^{15,33,34}. Fourth, CXCR4 is the subject of negative regulation by regulators of G-protein signaling (RGS) proteins³⁵. Finally, it has been recently demonstrated that CXCR4 requires association with membrane lipid rafts for optimal signaling (Figure 1)³⁶⁻³⁹.

Lipid rafts are membrane domains 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 nonionic detergents than other areas of a membrane⁴⁰. These domains also act as good sites for crosstalk between various cellular proteins^{39,41}. Lipid rafts have been shown to be important for T-cell

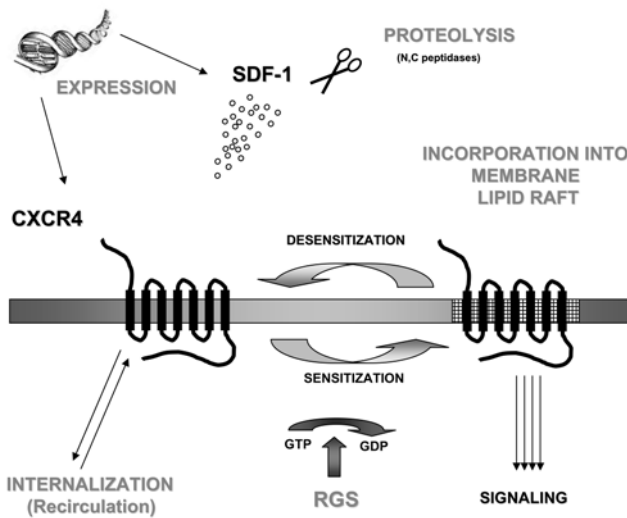


Figure 1. Different levels of regulation of CXCR4 function on hematopoietic cells. First, expression of CXCR4 is regulated at the transcriptional level by several factors (e.g., hypoxia). Second, CXCR4 as well as its ligand SDF-1 are subject to proteolytic degradation by several proteases that are expressed in the hematopoietic microenvironment and serum. Third, CXCR4 after interaction with SDF-1 is internalized from the surface and is recirculated from the endosomal compartment at different rates. Fourth, CXCR4 is the subject of negative regulation by RGS proteins. Finally, functionality of the CXCR4 receptor depends of its incorporation into membrane lipid rafts, and several signals from other membrane receptors or integrins may increase the incorporation of CXCR4 into membrane lipid rafts, increasing its signaling.

polarization and chemotaxis^{39,42}. It has been recently reported that small GTPases such as Rac-1 and Rac-2, which are crucial for engraftment of hematopoietic cells after transplantation, are present in lipid rafts of migrating HSPC^{43,44}.

Thus, incorporation of CXCR4 into membrane lipid rafts results in better responsiveness of CXCR4⁺ cells to an SDF-1 chemotactic gradient. We noticed that several small inflammation-related molecules, such as fibrinogen and fibronectin cleavage fragments and hyaluronic acid, increase incorporation of CXCR4 into membrane lipid rafts and thus modulate the responsiveness of CXCR4⁺ HSPC to an SDF-1 gradient³⁶.

3. COMPLEMENT IS ACTIVATED IN BM DURING MEYLOABLATIVE CONDITIONING FOR HEMATOPOIETIC TRANSPLANATION

The protein components of complement (C) are activated through proteolysis in a cascade-like fashion (by pathways known as classical, alternative, or lectin),

leading to generation of activated/cleaved proteins that bind to the C-activating surface and small liquid-phase activation peptides with potent proinflammatory properties that have been termed anaphylatoxins. Furthermore, all pathways of C activation merge at the level of C3, which is present at relatively high levels in serum (1 mg/ml). Cleavage of C3 is essential for activation of downstream C components^{45,46}.

Generally two groups of C3 cleavage fragments are distinguished — fluid phase (C3a, C3a_{des-Arg}) and cell- or extracellular matrix-bound (C3b, iC3b, C3dg, C3d) fragments (Figure 2). C3a and C3b are the first cleavage products of C3, and each has a short half-life in plasma. C3a is processed by serum carboxypeptidase N to C3a_{des-Arg} (long half-life cleavage product), and C3b is cleaved into iC3b (long half-life cleavage product) by factor I. The C3dg and C3d fragments (very long half-life) are generated from substrate-bound iC3b by proteolysis with various proteolytic enzymes including factor I, plasmin, and leukocyte elastase⁴⁵⁻⁴⁷.

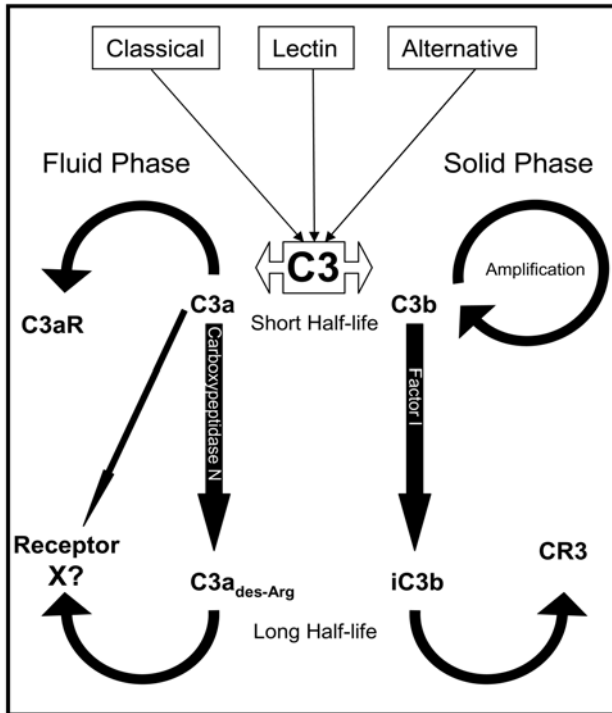


Figure 2. Activation/cleavage of C3 and generation of bioactive peptides. Cleavage/activation of C3 secreted by BM stromal cells is initiated by a C3-convertase to generate fluid-phase C3a and stromal cell-bound C3b. Both C3a and C3b have a short half-life. Fluid C3a is rapidly degraded to C3a_{des-Arg} and bound C3b is proteolyzed to iC3b. While C3a activates the C3aR and another non-identified receptor (Receptor X?), C3a_{des-Arg} binds to this yet-unidentified Receptor X only. iC3b tethers HSPC by interacting with CR3 (Mac-1).

We noticed that during myeloablative conditioning for transplantation of HSPC (e.g., after lethal γ -irradiation or conditioning by cyclophosphamide) C3 is cleaved/activated in PB and BM²¹. In support of this notion we have found that, during myeloablative conditioning for transplantation while C3 is cleaved and C3a_{des-Arg} becomes detectable by ELISA, iC3b is deposited on BM-derived fibroblasts and endothelial cells as determined by FACS analysis of these cells stained with anti-iC3b antibodies conjugated with Oregon green¹⁹. Since BM tissue responds to myeloablative conditioning by activating C, we hypothesized that C3 activated in BM may play an important role in promoting regeneration of BM after myeloablative treatment¹⁹.

C3 cleavage fragments bind to several specific C3 receptors (Figure 2). While C3a binds to the G-protein coupled, seven transmembrane-spanning C3a-receptor (C3aR), the receptor for C3a_{des-Arg} has not been identified yet (Receptor X). The C3aR is predominantly expressed on the surface of human mast cells, eosinophils, monocytes, and activated T-lymphocytes. Its major functions include chemotaxis of eosinophils and recruitment and degranulation of mast cells⁴⁸. Recently, we showed that a functional C3aR is also expressed by normal human HSPC and lineage-expanded hematopoietic precursors from myeloid, erythroid, and megakaryocytic lineages²¹.

The receptor for extracellular matrix-bound C3 cleavage fragment iC3b, CR3, is the $\alpha_M\beta_2$ -integrin also known as CD11b/CD18 or Mac-1. CR3 is expressed on granulocytes, monocytes/macrophages, and NK cells, and more importantly has been shown to be expressed on a significant proportion of HSPC. Thus, HSPC and their differentiated progeny express functional receptors for both fluid-phase and surface-bound C3 cleavage fragments⁴⁹⁻⁵¹.

4. THE ROLE OF COMPLEMENT IN REGULATING THE BIOLOGY OF HSPC

Since HSPC expresses C3a²¹ and iC3b⁴⁹ binding receptors, C3aR, and CR3, respectively, we became interested in whether C3 cleavage fragments play a role in regulating the biology of these cells. First, we tested if addition of C3 cleavage fragments to in-vitro growing hematopoietic clonogenic progenitor cells would increase their proliferation. However, neither C3a nor C3a_{desArg} affected the clonogenic growth of human CD34⁺ or murine Sca-1⁺ HSPC. Both the number and size of myeloid, erythroid, and megakaryocytic colonies for human and murine BM-derived cells growing in standard methylcellulose media were not affected regardless if C3a or C3a_{desArg} were added (0–1 μ g/ml) to the cultures. Thus C3 cleavage fragments do not directly affect the proliferation of human or murine HSPC²¹.

C3a and C3a_{desArg} do not chemoattract HSPC alone²¹, but both compounds, as mentioned above, sensitized the responses of human CD34⁺ and murine Sca-1⁺ cells to an SDF-1 gradient. We observed that C3a-C3aR axis signaling influ-

enced the SDF-1-dependent homing of HSPC to BM by promoting their (i) chemotactic response to SDF-1, (ii) adhesion to VCAM-1, (iii) expression/secretion of metalloproteinase-9 (MMP-9), and (iv) migration across subendothelial basement membranes. Interestingly, a short exposure (priming) of Sca-1⁺ cells to C3a before transplantation accelerated their engraftment in lethally irradiated mice. Hence, we concluded that the C3a–C3aR axis modulated responses dependent upon the SDF-1–CXCR4 axis, thereby regulating the homing of HSPC into BM. Based on these data, a new regulatory link between the C system and the SDF-1–CXCR4 axis has been established²¹. However, the question of whether C may also modulate in addition to the SDF-1–CXCR4 axis the chemokine–chemokine receptor axes as well requires further studies.

On the other hand, as expected, we noticed that the receptor for extracellular matrix-bound iC3b, CR3, plays a role as reported in adhesion of CR3⁺ HSPC in BM. However, while CR3 is mostly expressed on more differentiated hematopoietic progenitor cells (HPC)⁴⁹, recent data suggest that CR3 is also present on a substantial number of hematopoietic stem cells (HSC). In support of this, infusion of antibodies against CR3 into mice releases (mobilizes) both HSC and HPC from BM into PB. Based on this the CR3–iC3b interaction is crucial for retention of HSPC in BM⁵².

To evaluate the effect of iC3b in tethering HSPC in BM we employed a different strategy. Since iC3b is deposited onto C-activating surfaces and our previous studies had shown that iC3b was deposited onto BM stroma damaged by gamma radiation¹⁹, to determine if such surface-bound iC3b deposited onto BM stroma also played a critical role in BM recovery, experiments were carried out to explore the role of CR3 in tethering HSPC to sites of BM damage marked by deposited iC3b.

In these experiments Sca-1⁺ cells from wild-type (wt) and CR3^{-/-} mice were tested for adhesion to iC3b deposited *in vitro* onto cultured BM stroma cells. BM stroma cells from C3^{-/-} mice (to exclude the potential contribution of C3 produced by stromal cells) were γ -irradiated (to express a neoepitope recognized by a natural IgM antibody present in normal serum) and subsequently exposed to serum (source of IgM and C3) from wt or C3^{-/-} mice. The deposition of iC3b was confirmed on the surface of stroma cells exposed to wt serum only by FACS using an affinity-purified goat anti-mouse C3 Ab-Oregon green^{20,21}. For adhesion assays, Sca-1⁺ cells were employed from wt or CR3^{-/-} mice. After 1 and 6 hr of adhesion, non-adherent cells were discarded from the adhesion cultures and the cells in the wells were trypsinized and subsequently plated in methylcellulose cultures stimulated to grow CFU-GM colonies¹⁹.

We noticed that after 1 hr of adhesion CFU-GM was tethered to stroma cells in a CR3–iC3b-dependent manner. In prolonged adhesion (6 hr) this effect was not visible, suggesting that this CR3–iC3b-mediated interaction is important in early adhesion and may be compensated later on by other adhesion molecules. Thus, the CR3–iC3b axis may play an important role in the tethering, adhesion, and retention of HSPC in BM and thus also contribute to marrow reconstitution

from myeloablative treatment (e.g., irradiation). We hypothesize that by increasing the adhesiveness of HSPC within the hematopoietic microenvironment the C system promotes their better interaction and exposure to BM stroma-expressed growth factors, adhesion molecules, and cytokines. These interactions are crucial for self-renewal and expansion of HSPC¹⁹.

5. HEMATOPOIESIS IN C3-DEFICIENT MICE UNDER NORMAL STEADY-STATE AND STRESS SITUATIONS

Since no obvious hematological deficiencies were described in mice deficient in complement C3 (C3^{-/-}), these mice are considered to be hematologically normal⁵³. To address this issue better we studied several hematological parameters in these mice and compared them to control C3^{+/+} wt littermates. Table 1 shows that C3^{-/-} mice display (i) normal peripheral blood cell counts as compared to control C3^{+/+} wt littermates, (ii) similar numbers of clonogenic progenitors from the myeloid, erythroid, and megakaryocytic lineages in the bone marrow cavities as well as (iii) a similar total number of mononuclear cells present in their bone marrow cavities, and (iv) a number of transplantable colony forming unit-spleen (CFU-S) cells.

Table I. Hematological Parameters in C3^{-/-} and Normal Wild-Type C3^{+/+} Littermates

	Peripheral Blood			BM Progenitors			BM Cellularity	Spleen
	Leukocytes (/μL)	Platelets (/μL)	Hematocrit (%)	CFU-GM (/400,000 cells)	CFU-Meg (/400,000 cells)	BFU-E (/400,000 cells)	BM MNC /femur	CFU-S / 10 ⁵ cells
C3^{+/+}	6,145 +/- 1,355	634,000 +/- 75,473	46.2 +/- 2.7	256 +/- 18.2	43 +/- 7.9	182 +/- 29.8	11.65 X 10 ⁶	9 +/- 1.9
C3^{-/-}	5,604 +/- 524	557,999 +/- 70,804	45.5 +/- 2.8	277 +/- 25.7	37 +/- 6.2	158 +/- 8.0	10.60 X 10 ⁶	10 +/- 3.0

However, normal hematopoiesis as observed in steady-state conditions does not preclude the possibility that hematological abnormalities may become visible under stressed situations when the demand for blood supply increases. Thus, we have sublethally irradiated C3^{-/-} and control C3^{+/+} animals (500 cGy) and evaluated the recovery of peripheral blood counts. Interestingly, we noticed that C3^{-/-} mice had a delayed recovery of platelet and leukocyte counts by about 5–7 days¹⁹.

Similarly, $C3^{-/-}$ mice displayed a significant delay in hematopoietic recovery after transplantation of wt HSPC¹⁹. Transplantation of histocompatible wt Sca-1⁺ cells into $C3^{-/-}$ mice resulted in (i) a decrease in day 12 CFU-S, (ii) a 5–7 day delay in platelet and leukocyte recovery, and (iii) a reduced number of BM CFU-GM progenitors at day 16 after transplantation. Since HSPC from $C3^{-/-}$ mice engrafted normally into irradiated wt mice, the observed delay in recovery was likely related to a defect in the hematopoietic environment of $C3^{-/-}$ mice. Thus, our data strongly suggest that C3, even if dispensable for hematopoiesis under steady-state conditions, is needed for optimal regeneration of BM from sublethal irradiation and for proper engraftment of HSPC after transplantation¹⁹.

6. MOLECULAR EXPLANATION OF THE DEFECT IN HOMING/ ENGRAFTMENT OF HSPC IN C3-DEFICIENT MICE

The early stages of BM seeding by HSPC, which precedes their proliferation/differentiation, are collectively termed “homing.” Homing can be divided into several overlapping steps. CXCR4⁺ HSPC infused into PB have to respond to a chemotactic SDF-1 gradient in BM, attach to BM endothelium, transmigrate through the basal membrane in a metalloproteinase-dependent manner, and finally home to a niche where they have to subsequently survive, expand, and proliferate^{1,54}. Thus, homing of HSPC to BM niches is the first step in the engraftment process, during which HSPC self-renew and differentiate into precursor cells for all the hematopoietic lineages. The expansion of transplanted HSPC and proper colonization of BM tissue is directly responsible for final reconstitution of the BM tissue.

Since $C3^{-/-}$ mice do not have C3, the C3 fragments C3a, C3a_{des-Arg}, and iC3b were examined for a role in HSPC engraftment. Since C3a binds to the G-protein-coupled seven transmembrane spanning C3aR and iC3b binds to the integrin-type CR3 receptor⁴⁸, we assumed that the molecular action of C3 cleavage fragments will be different for fluid phase C3a/C3a_{des-Arg} and for surface bound iC3b. As mentioned above, several small molecules (e.g., fibronectin and fibrinogen fragments, hyaluronic acid) that are present in supernatants from leukopheresis products may modulate the responsiveness of HSPC to an SDF-1 gradient by enhancing, as we assumed, the incorporation of CXCR4 into membrane lipid rafts. Our recent research on human hematopoietic cells primed by fibrinogen and fibronectin confirmed that sensitization/priming of cell chemotaxis to an SDF-1 gradient is dependent on the cholesterol content in the cell membrane and incorporation of the SDF-1 binding receptor, CXCR4, and the small GTP-ase Rac-1 into membrane lipid rafts. This co-localization of CXCR4 and Rac-1 in lipid rafts facilitated GTP binding/activation of Rac-1³⁶.

Based on these observations, we asked if C3a and C3a_{des-Arg} may also prime (increase) the responsiveness of HSPC to an SDF-1 gradient, similarly as fibronectin and fibrinogen fragments, by increasing incorporation of CXCR4 into

membrane lipid rafts. Thus, the hypothesis was investigated that the priming effect of C3a and C3a_{des-Arg} is dependent on lipid raft formation¹⁹. Accordingly, chemotaxis assays were performed with low doses of SDF-1, either alone or with a priming concentration of C3a or C3a_{des-Arg}. To determine the contribution of lipid rafts, cells were preincubated for 30 min before chemotaxis in 2.5-mM hydroxypropyl- β -cyclodextrin to extract cholesterol from the membranes and to perturb lipid raft formation. These studies with hematopoietic cells suggested that the priming effect of C3a and C3a_{des-Arg} on SDF-1 chemotaxis was blocked by this raft-disrupting agent. To provide more evidence that the priming effect of these small C3 fragments is dependent on lipid raft formation, confocal-like and Western blot analysis were carried out to assess CXCR4 incorporation into membrane lipid rafts in hematopoietic cells primed by C3a and C3a_{des-Arg}. As expected, CXCR4 in hematopoietic cells stimulated by C3a and C3a_{des-Arg} became incorporated into cell membrane lipid rafts¹⁹. Figure 3 depicts the role of C3 cleavage fragments in lipid raft formation. However, the type of C3 receptors involved in this process needs further clarification.

Generally, it is widely accepted that, while the seven transmembrane-spanning G-protein coupled C3aR binds anapylatoxin C3a, its degradation product, C3a_{desArg}, binds to another unidentified receptor and the seven-transmembrane span C5L2 orphan receptor was postulated to be a possible candidate for this latter interaction⁵⁵. However, based on our observations that blockade of C3aR by C3aR antagonist SB290157 only partially inhibited the priming effect, and not only C3a but also C3a_{desArg} primes the responsiveness of HSPC to an SDF-1 gradient, we envisioned that another C3a_{desArg}/C3a binding receptor may exist that could be responsible for the observed priming effect¹⁹. This was additionally supported by our data that cells from mice with C3aR knocked-out showed a priming effect to both C3a and C3a_{desArg}¹⁹.

To clarify this issue, another group of investigators recently took advantage of C5L2 knockout mice and demonstrated that in these animals, which do not have the C5L2 receptor, C3a_{desArg} still is able to increase the responsiveness of HSPC to an SDF-1 gradient⁵⁶. This strongly suggests that another C3a and C3a_{desArg} binding receptor exists on HSPC. It is not clear at this point if this receptor would be another member of the seven-transmembrane span receptor family, similar to C3aR and C5L2, or a different type of molecule⁵⁷.

Next, we became interested in the molecular mechanisms of surface-bound C3 cleavage fragment, iC3b, in homing of HSPC to the BM. We noticed that fluid-phase iC3b may also prime the responsiveness of HSPC to an SDF-1 gradient and increase incorporation of CXCR4 into membrane lipid rafts (not published). More importantly, however, iC3b as discussed above seems to play a role as a tethering molecule in BM. The retention of HSPC in BM is controlled by an adhesive interaction between adhesion receptors on HSPC and stroma. These receptors include integrins (VLA-4, VLA-5, Mac-1), selectins (L-selectin), CD44, members of the immunoglobulin superfamily, tyrosine kinase receptor-c-kit, and chemokine receptor CXCR4^{2,7,8,10,18,58-60}. Recent studies showing

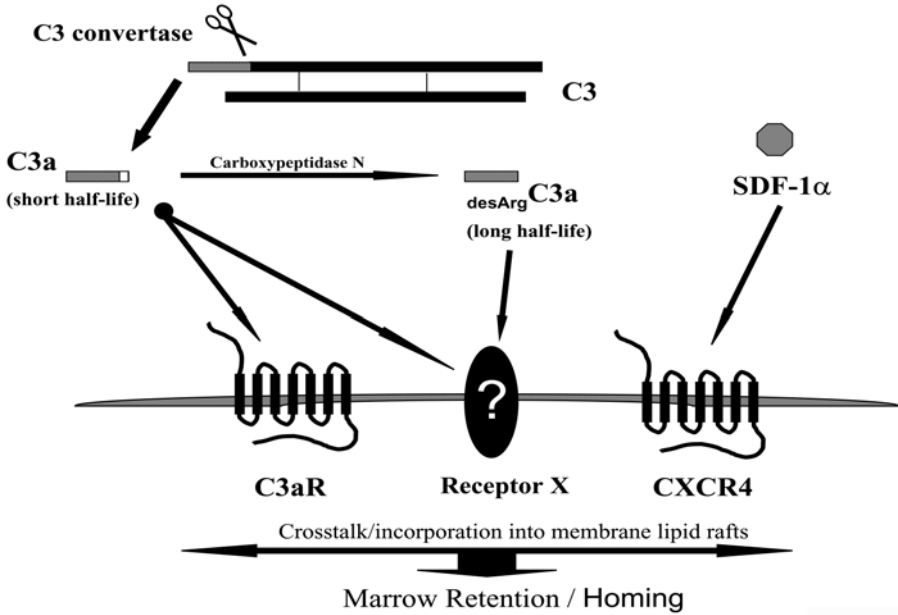


Figure 3. Schematic diagram of the hypothetical role of C3 cleavage fragments (C3a and C3a_{desArg}) in sensitizing the responsiveness of HSPC to an SDF-1 gradient. C3 is activated in BM in response to marrow damage (e.g., after conditioning for transplantation by TBI or chemotherapy). Cleavage/activation of C3 in the BM microenvironment is initiated by a C3-convertase to generate C3a anaphylatoxin. Subsequently C3a is quickly cleaved by carboxypeptidases, which removes the terminal arginine residue from the 78-aminoacid C3a molecule. Although C3a has a short half-life, the C3a inactivation product, C3a_{desArg}, has a long half-life. We postulate that C3a and C3a_{desArg} fragments increase the responsiveness of HSPC to SDF-1 gradients by enhancing the incorporation of CXCR4 into membrane lipid rafts. A recent observation supports the concept that, while the seven transmembrane-span G-protein coupled C3aR binds C3a only, another non-identified receptor (Receptor X?) binds both C3a and C3a_{desArg}. Activation of both of these receptors enhances incorporation of CXCR4 into membrane lipid rafts and thus its better association with downstream signaling proteins.

expression of CR3 on HPC and significant enhancement of G-CSF-induced mobilization by administration of blocking antibodies against β_2 -integrins indicated the involvement of CR3 in the homing/retention of HPC in BM⁶¹. Supporting this, our recent studies in CR3^{-/-} mice revealed that these mice are more sensitive to mobilization by suboptimal doses of G-CSF as compared to their wt littermates²⁰. Taken together, these data suggest that CR3 participates in retention of HSPC in the BM.

Based on our investigations, the following model is proposed for the role of C3 cleavage fragments in hematopoiesis (see Figure 4). Cleavage/activation of C3 secreted by BM stromal cells and macrophages is initiated by a C3-

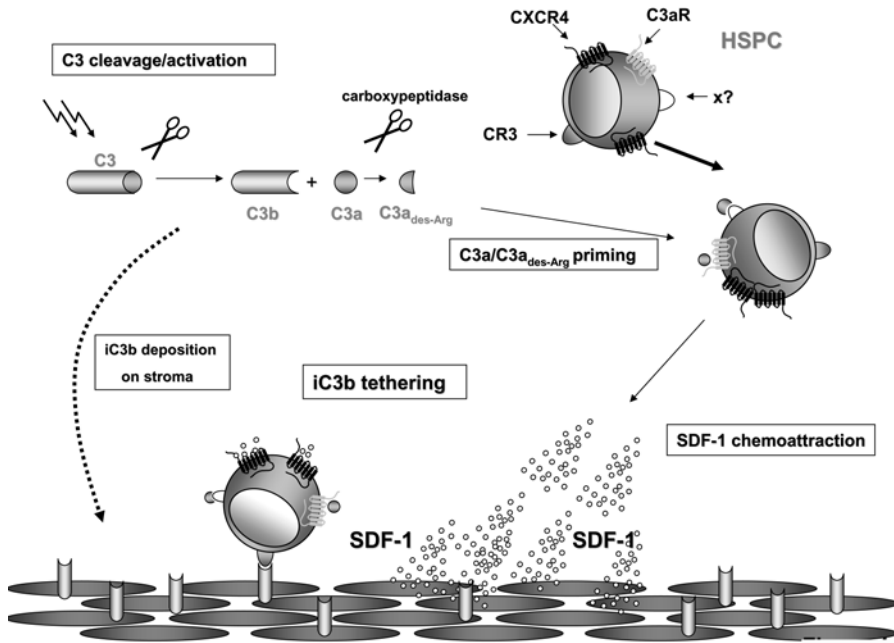


Figure 4. Schematic diagram of the hypothetical role of C3 fragments in retention of HSPC in BM. C3 is activated in BM in response to marrow damage (e.g., after conditioning for transplantation by TBI). Cleavage/activation of C3 secreted by BM stromal cells and macrophages is initiated by a C3-convertase to generate fluid-phase C3a and stromal cell-bound C3b. Both C3a and C3b have a short half-life. Fluid C3a is rapidly degraded to C3a_{des-Arg} and bound C3b is proteolyzed to iC3b. We postulate that while C3a and C3a_{des-Arg} fragments increase the responsiveness of HSPC to SDF-1 gradients, iC3b tethers HSPC onto BM stroma. The HSPC tethered by the iC3b–CR3 axis are effectively targeted to the site of injury and may interact better with stroma cells because of both CR3 signaling resulting from ligation to iC3b and/or enhanced recognition of lower-affinity adhesion molecules.

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7. CONCLUSIONS

We presented evidence that a crosstalk between C and the SDF-1—CXCR4 axis plays an important and underappreciated role in engraftment of HSPC. Accordingly, a novel mechanism for HSPC engraftment was identified that involves (i) complement activation in BM after myeloablative conditioning for transplantation^{19,21} and (ii) release of specific CXCR4 incorporation into membrane lipid rafts (thus potentiating HSPC responses to SDF-1 gradients), whereas iC3b deposited onto irradiated BM cells functions to tether CR3(CD11b/CD18)⁺HSPC to damaged stroma¹⁹.

On the other hand, our data on BM regeneration strongly suggest that activation/cleavage of complement becomes important in stressed situations as seen in tissue/organ damage. In support of this notion, activation of C3 and the presence of biologically active cleavage fragments was reported in several other models of tissue injury (e.g., liver damage)^{62,63}. These data support the concept that innate immunity plays an important role in regeneration of damaged organs. We postulate that it is likely that, similarly as in BM, activation of C that results in generation of C3 cleavage fragments plays an important role in regulating chemoattraction and homing of CXCR4⁺ non-hematopoietic tissue committed stem cells (TCSC) for organ regeneration^{64,66}. We recently reported that these TCSC reside in the BM and are released into circulation during organ injury^{65,66}. Trafficking of these cells, similarly as HSPC is directed by SDF-1, and C3 cleavage fragments may play an important role here as well. Thus, C emerges as an important co-regulator of trafficking not only of HSC but also of TCSC as well. Modulation of the C system along with the SDF-1—CXCR4 axis may result in the development of more efficient regeneration therapies.

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