Published in final edited form as:
Methods Mol Biol. 2012 ; 904: 1–14. doi:10.1007/978-1-61779-943-3_1.

Mobilization of Hematopoietic Stem/Progenitor Cells: General Principles and Molecular Mechanisms

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Abstract

Hematopoietic stem/progenitor cell mobilization can be achieved by a variety of bone marrow niche modifications, although efficient mobilization requires simultaneous expansion of the stem/progenitor cell pool and niche modification. Many of the mechanisms involved in G-CSF-induced mobilization have been described. With regard to mobilization of hematopoietic stem/progenitor cells, challenges for the future include the analysis of genetic factors responsible for the great variability in mobilization responses, and the identification of predictors of mobilization efficiency, as well as the development of mobilizing schemes for poor mobilizers. Moreover, improved regimens for enhanced or even preferential mobilization of nonhematopoietic stem/progenitor cell types, and their therapeutic potential for endogenous tissue repair will be questions to be vigorously pursued in the near future.

Keywords

G-CSF; Mobilization; Hematopoietic stem/progenitor cell

1. Introduction

Although mature hematopoietic cells are physiologically released from bone marrow to peripheral blood, their immature counterparts are found in circulation in very low frequencies. An enforced egress, referred to as “mobilization,” of a modest proportion of the latter cells from bone marrow to peripheral blood can be enacted by a variety of systemic “stressors.” Stem cell mobilization was uncovered mostly through empiric observations rather than rationally designed treatments. Why and how stem/progenitor cells physiologically escape the BM environment is not entirely clear, but it is very likely that the process of mobilization makes use of physiological molecular pathways leading to mobilization.

The considerable scientific interest in mobilization of immature cells is fuelled by its clinical relevance. Its importance in autologous repair mechanisms was demonstrated when after partial irradiation radiation-depleted marrow is repopulated from noncontiguous nonirradiated marrow sites, presumably by itinerant stem cells (1). Quantitatively, however, of greater clinical relevance at the current time, is the collection of mobilized cells by apheresis, enabling allogeneic transfer or temporary cryopreservation of autologous stem/progenitor cells for hematopoietic “stem cell” transplantation (2, 3).

Protocols for several mobilization approaches are reported in this book and several recent comprehensive reviews have been published on clinical aspects or the cellular and molecular mechanisms of mobilization (4–8). This mini review focuses on issues relevant to G-CSF mobilization, because of its unique clinical importance and the plethora of studies on G-CSF mobilized cells. Mobilization by some other modalities is touched upon only because of their mechanistic insight and because they may display a synergistic or additive activity with G-CSF.

2. General Mobilization Principles

Under steady-state conditions, stem/progenitor cell location is almost exclusively restricted to the marrow, where these cells apparently reside in specific, supportive microenvironments (9–11). Environmental cues from stromal cells or matrix could influence cell fate, and are, under resting conditions, also responsible for their firm retention in the marrow. Active egress of stem/progenitor cells from bone marrow could be the default response when their restraining mechanisms are released, i.e., the HSPC could be inherently nomadic unless restrained. While this may appear to be a philosophical issue, the answer to this question could allow for a rational development of mobilizing agents. Currently available data on stem cell mobilization suggest that indeed the breakdown of retention mechanisms is sufficient for mobilization.

Several common properties of mobilized hematopoietic cells have been emphasized irrespective of the mobilizing agent. Thus, mobilized immature cells are predominantly noncycling, in contrast to the cells left behind in the marrow (12–14), they express little VCAM-1, and low levels of many integrins (14–16). Specifically data generated with fast-acting mobilizing agents suggest that these phenotypic changes precede egress of cells from marrow, suggesting in turn that these properties are prerequisites for mobilization, rather than changes induced by the milieu in the peripheral blood (15). Likewise, gene expression patterns of mobilized immature subsets have been described; they differ markedly from their counterparts residing in unstimulated marrow (17, 18). Thus, in CD34+ cells from G-CSF mobilized blood, myeloid genes and cell cycle-associated genes were relatively up-regulated. These changes likely indicate differences in the heterogeneous mix of cells contained in the CD34+ fraction, entirely compatible with known effects of G-CSF, rather than necessarily pointing to molecular events involved in mobilization. In agreement with that, an extensive body of evidence has accumulated on differences in the ratio between primitive and more mature hematopoietic subsets, depending on the mobilizing agent. Thus, several publications have commented that AMD3100-alone mobilized immature cells are, on average, more functionally and phenotypically primitive than G-CSF- or G-CSF + AMD3100-mobilized ones (19, 20), resembling more closely the distribution in a steady-state marrow. This observation may be explained by the relative skewing of a G-CSF stimulated marrow towards less primitive (more mature) cells, i.e., the mobilized fractions are representative of marrow contents at the time of mobilization. As was reported many years ago, a G-CSF mobilized marrow is relatively depleted of immature hematopoietic subsets, and the marrow does not assume its normal cellular composition for several weeks after discontinuation of G-CSF (21).

The precise locations from which mobilized immature cells originate, or the exact site of their egress, are not clear. A reasonable proposition is that egress into blood would require apposition to medullary blood vessels, most likely to medullary venous sinusoids. Mobilization by G-CSF is associated with a relative depletion of periosteal niches of hematopoietic stem cells, migration of stem cells to vascular niches where much of the proliferation occurs (5), followed by egress of both mature and immature subsets. With chemokine-induced mobilization the rapid kinetics likely do not allow for migration across significant distances, which may explain the relatively lower potency, and the synergism between G-CSF and AMD3100 (15). Of interest, data generated with the Gi protein inhibitor Pertussis toxin, which renders hematopoietic cells completely incapable of migration (22), clearly show that the ability to migrate is not a critical capacity of a mobilizable cell, i.e., that stem/progenitor cell pools might reside on the luminal side of medullary blood vessels. In aggregate, these data may indicate that although mobilized cells would at some time cross perivascular pools before they exit the bone marrow, they could initially originate from

other bone marrow locations further away and that such movement to the perivascular space increases the number of mobilizable cells.

How large is the fraction of stem cells that can be induced to leave the marrow? At first glance, extrapolation from the mouse model indicates that the efficiency of mobilization with G-CSF might be modest. After a similar mobilization regime as in humans (nine doses q12h) several thousand CFU-C per mL of blood, i.e., no more than 10,000 CFU-C in total, will be in circulation. The number of circulating CFU-C after G-CSF in the mouse (10,000 in a C57Bl/6 mouse, more in some other strains) must be compared against a CFU-C content of approximately 60,000 per steady-state (unmobilized) femur (15), which is estimated to represent 1/16 of the total marrow mass (23). Thus, the total number of CFU-C of the mouse is one million, 100 times the number that is found in circulation after G-CSF. However, this may not necessarily mean that only 1% of “stem cells” are mobilized by G-CSF, since other relevant variables in the equation are completely unfathomable. The transit time of mobilized cells is elusive (minutes to a few hours have been suggested) (24, 25), and their fate has not been completely elucidated. In other words, once the cells are in circulation, how long do they remain there, and when they leave the circulation, how many home back to marrow or are lost to other organs is unclear. Conceivably, many circulating cells could interact with and be siphoned off by nontarget organs. In that case the true number of mobilized cells would be much higher than the number in circulation suggests. What is the evidence for such “steal” effects? Experimental evidence has been provided that the spleen of a G-CSF mobilized mouse accumulates significant numbers of immature cells, so that mobilization of splenectomized mice is more pronounced (26). Trafficking of mobilized immature cells through the intestinal lymphoid system and to adipose tissue has also been shown (27, 28). The possibility that this likewise pertains to other organs must be entertained. Since unlike the spleen, other organs do not support immature hematopoietic cells, it is difficult to experimentally address this issue with currently available technology, but again, tracking experiments in transplanted animals demonstrate accumulation of progenitor cells in nonhematopoietic tissues (29). Thus, reliable estimates of the potency of G-CSF mediated mobilization cannot be given.

How is mobilization quantified? Ultimately, the cell of interest in the context of hematopoietic cell mobilization is the stem cell. Yet the stem cell is defined functionally, as a cell capable of self-renewal and long-term multilineage reconstitution in an appropriately conditioned host. It must be remembered that any other “stem cell” enumeration assay than the long-term engraftment assay (30) is measuring some surrogate parameter, so many caveats must be considered when interpreting the results of such assays. In addition to its tediousness, even a stem cell assay (limiting dilution transplantation and readout of long-term engraftment) has its limitations, since it tests at the same time stemness and transplant related properties like homing, niche-integration, retention, etc. Thus, if cells which would be capable of self-renewal and long-term repopulation in terms of their epigenetic status, i.e., are bona fide stem cells, are impaired in their ability to interact with the niche or to proliferate, the number of stem cells might be underestimated.

In vitro colony assays have been used by most to quantify mobilization, or to compare mobilization efficiency (30–33). Since cells giving rise to colonies in colony assays are progenitor cells, i.e., more mature specimen, CFU-assays are not a true measure of stem cell mobilization. However, cumulative evidence indicates that during progenitor cell mobilization stem cells are always comobilized, but the relative frequency among the immature cells may vary, depending on the mobilizing agent. Thus, the CFU-C assay may be the most practicable assay for assessment of mobilization, but its shortcomings must be born in mind.

Phenotypic analyses of “stem cells” using more or less complex surface marker panels have also been used. These assays are most problematic, because mobilizing agents can induce changes in surface phenotype (e.g., c-kit expression on immature cells is all but suppressed on G-CSF mobilized cells); thus, the stem cell phenotype of a stem cell in a steady-state marrow is likely different from that in mobilized peripheral blood (34, 35). With less complex surface marker panels (e.g., CD45/CD34), the relative mix between primitive and more mature subsets contained in this phenotypically defined, yet functionally heterogeneous population is not considered and can lead to misinterpretations of stem cell mobilization efficiency.

3. Mobilization by G-CSF

The clinically most relevant mobilizing agent, G-CSF, expands the number of stem cells at the same time that it induces proliferation/ maturation towards the granulocytic lineage, and it causes marked alterations in the hematopoietic stroma in the marrow. Together these changes result in the release, or mobilization, of hematopoietic stem/progenitor cells. It seems clear that the summation of expansion and mobilization is responsible for the rather potent mobilization efficiency of G-CSF compared to other mobilizing agents. In humans, after a conventional course of G-CSF (5 μg/kg every 12 h, nine total doses) the number of circulating progenitor cells is increased approximately 60-fold, to 60–100 CD34+ cells/ μL.

Preliminary data indicate that other types of immature cells are comobilized alongside hematopoietic stem/progenitor cells, including endothelial and mesenchymal stroma cells (36–38). It is not unreasonable to hypothesize that the same changes which cause hematopoietic stem/progenitor cell mobilization are also involved in mobilization of these other stem cell specimen, but definitive data are lacking.

Recently, significant advances have been made with respect to the molecular and cellular events involved in G-CSF mediated hematopoietic stem/progenitor cell mobilization. Informative data document that mobilization is not a direct effect of G-CSF on the stem cell proper. The receptor for G-CSF, G-CSFR, is conspicuously absent from hematopoietic stem cells (39). Indirect cues must therefore be responsible for numeric and spatial changes in the stem cell population. Recent data by the Link and Levesque laboratories suggest a chain of events starting with G-CSF mediated stimulation of certain marrow-resident macrophages which appear to relay signals to osteoblasts (also G-CSFR negative), which then down regulate SDF-1 gene transcripts (40–42). Proteolytic cleavage of SDF-1 off of stromal binding sites has also been demonstrated and functionally implicated, as truncation of SDF-1, resulting in nonfunctional SDF-1 molecules can compete with full-length SDF-1 for CXCR4 binding sites. The cellular and molecular architecture of a G-CSF treated bone marrow is significantly changed compared to a steady-state marrow. For instance, cleavage of a number of surface-bound chemokines, cytokines, receptors, etc. has been demonstrated. Some evidence has been provided that these changes are the work of proteases, which are elaborated during G-CSF mobilization, together with down regulation of protease-inhibitors during mobilization. However, the critical role of MMP9 emphasized in some studies (43) has not been con firmed, and even mice deficient in a whole panel of proteases responded to G-CSF with the expected efficiency (44). Further, deficiency in CD26 is associated with impaired mobilization by G-CSF (45). CD26 is a broad dipeptidase that (among many other putative target molecules) cleaves SDF-1 into a nonfunctional variant, which competes with SDF-1 for CXCR4 binding. It was proposed that the inability to cleave SDF-1 was responsible for the attenuated G-CSF responsiveness of the CD26-deficient mice (45). At this point in time, a definitive contributory role of other proteases to mobilization cannot be pinpointed.

As the marrow is exposed to G-CSF and the described profound changes in marrow architecture are happening, HSPC expand in regions located more centrally and closer to the blood vessel. Data from the Levesque laboratory suggest that this is at least in part a reflection of (a) greater oxygen needs of proliferating cells and (b) greater oxygen consumption in a proliferating marrow, i.e., during G-CSF stimulation, HSPC move towards higher oxygen concentrations (46). The potent mobilizing activity of certain chemotherapy drugs like cyclophosphamide has been solely attributed to endogenous G-CSF, since G-CSFR deficient mice treated with cyclophosphamide show the expected rebound proliferation in marrow, but egress of immature cells from marrow is virtually absent (47). The mechanisms involved in mobilization by cyclophosphamide would then likely be the same as during mobilization with exogenous G-CSF.

4. G-CSF-Enhancing and Alternative Activities in Mobilization

Several cytokines (including GM-CSF, FLT3 ligand and SCF, the c-kit ligand) mobilize HSPC and synergize with G-CSF in stem cell mobilization (47–50). These modalities have in common similarly slow kinetics as G-CSF mobilization, suggesting a combined effect of proliferation and mobilization, as with G-CSF. Some clinical data with GM-CSF and SCF, the latter predominantly in combination with G-CSF, have been reported, but their clinical relevance is modest. The mechanisms of mobilization with these cytokines have not been studied in any detail. Considering the role of the coagulation/complement cascade in hematopoietic cell trafficking (51–53) and the strong activation of this system by GM-CSF (54), a contribution of this pathway is conceivable.

A different group of mobilizing agents has gained a lot of attention in the last few years, namely, CXCR4 antagonists of various chemistries. The effectiveness of this intervention has been demonstrated in mice, monkeys, dogs and humans. One CXCR4 antagonist, the bicyclam AMD3100 (Mozobil, Plerixa for) is licensed for clinical mobilization in combination with G-CSF + chemotherapy for patients failing to adequately mobilize with G-CSF + chemotherapy alone (15, 31, 55–60).

Preliminary data from the Di Persio laboratory, reported at the 2010 ISBT meeting, indicate that when given alone, as with G-CSF, other species of immature cells are also comobilized by CXCR4 antagonists, although their nature has not been definitively elucidated. The mechanism of action of CXCR4 antagonists appears to be interference between stromal SDF-1 and CXCR4 on the HSPC surface. The kinetics is rapid, quite unlike those of G-CSF, and no conclusive evidence has been provided that these CXCR4 inhibitors elicit changes in the hematopoietic niche. Proliferation is not a feature of mobilization with CXCR4 antagonists, which was thought to explain the relatively low potency of these inhibitors. Preliminary data from our group indicates, however, that novel, more potent CXCR4 inhibitors can exceed the mobilization achieved with a 5-day course of twice-daily G-CSF, at least in mice (unpublished data). The frequency of stem cells in CXCR4-antagonist mobilized grafts among the cells with an immature phenotype is greater than after G-CSF. It appears that this reflects the frequencies within a steady-state marrow as opposed to a G-CSF-treated marrow, so this observation should not be surprising. Clearly mobilization with CXCR4 antagonists argues against a hypothesis put forth about mechanisms of G-CSF mobilization, i.e., inversion of an SDF-1 gradient, where mobilization of HSPC would be in response to greater concentrations outside the marrow than inside (supposedly because SDF-1 is cleaved from the stroma, to circulate in blood and bone marrow fluid). Because of the very short half-life of SDF-1 in plasma, this seemed unlikely, but definitive evidence against this hypothesis comes from the following observation: since CXCR4 antagonists effectively block SDF-1 directed migration, yet potently synergize with (rather than antagonize) G-CSF mobilization (15), it is clear that in mobilization cells do not respond to

SDF-1, but are on the contrary made temporarily unresponsive. Data about mobilization with CXCR4 agonists are in line with this hypothesis (61), since they lead to down regulation of CXCR4 surface expression on HSPC. Thus, the SDF-1-CXCR4 axis acts as a retention pathway, which is disturbed by various means in mobilization with G-CSF and CXCR4 antagonists or agonists. Data generated in mice transplanted with CXCR4 deficient hematopoietic cells are in agreement with these observations (62).

Considering that several authors have proposed interference of the CXCR4/SDF-1 pathway as the mechanism of action of G-CSF mediated mobilization, the well-documented synergism between G-CSF and CXCR4 antagonists is surprising. The simplest explanation may be (1) that after G-CSF, this pathway is only partially obstructed and (2) that CXCR4 antagonists may find a larger population to mobilize in a G-CSF treated marrow than in an untreated one, because of expansion of the pool and of relocation to perivascular regions.

The inhibitor of Gi protein signals, including of SDF-1/ CXCR4 signals, Pertussis toxin was reported to elicit potent and protracted HSPC mobilization. Specifically, Pertussis toxin also synergized with G-CSF induced mobilization (22). Why Gi protein blockade leads to mobilization is not clear. While irrelevant from a clinical perspective, the implications for mobilization mechanics are of interest. Pertussis toxin mobilized HSPC are incapable of migration. This suggests that activation of migratory signals may not be required for mobilization. Similarly, GRO-β induced mobilization is seen despite the fact that it inhibits migration in vitro (32). These data could be interpreted to indicate that mobilizable pools of HSPC reside not in the marrow immediately adjacent to bone surfaces, CAR cells and other stromal elements, but in regions adjacent to the venous sinuses in marrow. Such a location would be equally compatible with the rapid kinetics of IL-8, GRO-β and CXCR4 antagonists—in either case distant transmarrow migration might not be feasible within the relevant time frame. Fenestrae in the septum segregating the spaces between the marrow space and the venous sinuses have been described as the site of passage of mature cells into blood. Conceivably, these fenestrae could also be used by immature hematopoietic cells during mobilization. This hypothesis would also be compatible with the observation that HSPC in G-CSF treated marrow are preferentially located close to blood vessels (46).

VLA4 is an adhesion molecule expressed on HSPC. It is normally present in its low-affinity conformation, but affinity can be induced by a number of cytokines, and it changes during cell cycle transit (14). Like CXCR4 and SDF-1, VLA4 finds cognate ligands in the marrow stroma, including, but potentially not limited to, VCAM-1, fibronectin, and osteopontin, thus serving as a stem cell retention pathway. Studies in mice, nonhuman primates or humans, using genetic, small-molecule- or antibody-mediated ablation of VLA4 or of several of its ligands in marrow have demonstrated mobilization of HSPC into peripheral blood (31, 63–67). The kinetics follow an intermediate time course. Although reduced expression of VLA4 is also a feature of G-CSF mobilized HSPC (14–16), suggesting VLA4 down regulation as another mechanism of G-CSF mobilization, VLA4 inhibition or genetic deletion was synergistic or at least super additive with G-CSF. Similarly to what we postulated for synergism between CXCR4 antagonists and G-CSF, interference of G-CSF induced events with VLA4-mediated adhesion is likely incomplete, while direct targeting of the molecule is complete. As we have shown, VLA4 blockade is effective at mobilizing HSPC in mice, monkeys, and humans, albeit with low potency (31, 64, 68). As an indication that VLA4-inhibition and CXCR4 blockade are mobilizing HSPC by independent mechanisms, we and others have demonstrated synergism of the two modalities in monkeys and mice (31, 67). Lower VLA4 expression was also observed on HSPC mobilized with CXCR4 antagonists and with a variety of other mobilizing agents (15). This could either indicate down regulation of VLA4 under the influence of mobilizing agents and preferential mobilization

of these VLA4-dim cell populations, or assumption of a VLA4-dim phenotype during the transition from marrow to blood.

Two other chemokines, GRO-β and IL-8, mobilize HSPC with very rapid kinetics. With respect to GRO-β, it was shown that this mobilization was dependent on MMP9, suggesting that the target cell may be a mature neutrophil (32, 69). For IL-8, contradictory results about the role of MMP9 have been reported, yet a dependence on G-CSFR likewise suggests a role for mediators released from mature neutrophils (47, 70, 71). The proposed chain of events leading to mobilization for these two molecules is granulocyte degranulation, release of proteases, severing of retention factors, resulting in stem cell release.

Several other examples of stroma or niche modification have also been reported which resulted in stem cell mobilization. Several of these involved modification of stromal ligands for established retention factors. Examples include very different substances, such as Fucoidan, which displays competitive displacement of chemokines, including SDF-1 (which is present in the stem cell niche as a surface-bound molecule) (72, 73), anti-VCAM-1 antibodies (74) and VCAM-1-deleted (75) or osteopontin-deleted (33) mice, which blocked/ablated relevant VLA4 ligands in the stroma. Other mobilizing agents likely exerted their effects indirectly, through induction of endogenous G-CSF (e.g., parathyroid hormone) (76). Moreover, a variety of mediators have been associated with mobilization that seemingly have very little in common, and where the molecular mechanics are sometimes poorly defined. These include sympathomimetics (77, 78), cannabinoid receptor agonists (79), complement (52, 53), elevated lipoprotein levels (80), defibrotide (81), glycosaminoglycans (82), and endotoxin (83). None of these have gained any clinical relevance, but the abundance of mobilizing agents indicates the precariousness of the equilibrium between marrow retention and egress, and may in the future support the rational development of mobilizing strategies for poorly mobilizing patients, or for individuals who are intolerant to G-CSF.

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