Hematopoietic stem cell mobilization

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Recruitment of hematopoietic stem/progenitor cells (HSC) from the bone marrow into peripheral blood following treatment with chemotherapy and/or cytokines is termed mobilization. The release of HSC from the bone marrow is a physiological phenomenon for the protection of HSC from toxic injury, as circulating cells can re-engraft bone marrow, or to maintain a fixed number of HSC in the bone marrow (homeostatic mechanism). In fact, trafficking to blood is an important death pathway to regulate the steady-state number of HSC.[1] Bone marrow cells also enter peripheral blood in response to stress signals during injury and inflammation of hematopoietic and non-hematopoietic tissues.[2-4]

Several randomized trials have demonstrated the clinical advantages of the reinfusion of autologous mobilized peripheral blood stem cells over bone marrow HSC.[5,6] These advantages include shorter duration of granulocytopenia, enhanced immune reconstitution, reduced morbidity and mortality, shorter hospital stay and saving of financial resources. However, several factors affect HSC mobilization: age, type and dose of cytokines used and, in the autologous setting, the patient's diagnosis, mobilizing chemotherapy regimen, number and type of previous chemotherapy cycles or radiation, and interval from last chemotherapy cycle. Indeed, depending on these characteristics, a significant proportion of patients receiving standard mobilization for the purpose of autologous transplantation or allogeneic donors still fail to mobilize bone marrow cells into the periphery.[7,8] Although an extensive description of factors influencing HSC mobilization[9] and current clinical trials involving transplantation of peripheral blood stem cells is beyond the scope of this perspective article, we report here some of the bio-molecular mechanisms leading to the release of HSC from the bone marrow to help the understanding of novel strategies under development to make stem cell collection more effective.

Mechanisms of stem cell mobilization

HSC in the bone marrow are spatially distributed in highly organized three-dimensional microenvironmental niches. The endosteal stem cell niche is located at the endosteum of the bone marrow, where osteoblasts are the main regulators of HSC functions such as proliferation and quiescence. Furthermore, a significant proportion of HSC are closely associated with sinusoidal endothelial cells in the endothelial niche where they are ready to enter peripheral blood and start differentiation.[10,11] However, bone marrow niches are not stably occupied by quiescent stem cells; rather, the release of these cells into the circulation, and their migration, homing and re-engraftment to the bone marrow are physiological processes that are part of their development. Emerging evidence suggests that pharmacologically enhanced mobilization of HSC alters their interplay with stromal and hematopoietic cells regulating bone remodeling by osteoclast/ osteoblast interactions.[12] Stress-induced signals activate neutrophils and osteoclasts which, in turn, cause shedding and release of membrane-bound stem cell factor (SCF), proliferation of HSC, activation and/or degradation of adhesion molecules such as VLA-4 and P/E selectins. Moreover, a major role in stem cell mobilization is played by the inactivation of the chemokine stromal cell derived factor-1 (SDF-1)CXCL12, interleukin-8 (IL-8)/CXCL8 along with the proteolytic activity of elastase, cathepsin G, proteinase 3, CD26 and metalloproteinase (MMP)-2 and 9 which disrupt the SDF-1/CXCR4 axis resulting in HSC release. Granulocyte colony-stimulating factor (G-CSF) treatment increases the number of bone-resorbing osteoclasts which secrete high levels of the mobilizing chemokine IL-8, the proteolytic enzymes MMP-9 and cathepsins that cleave SDF-1. SDF-1 expressed by endosteal osteoblasts recruits osteoclast precursors (i.e. monocytes) from the peripheral blood to the bone marrow.[9] Interestingly, G-CSF suppresses the expression of SDF-1 on osteoblasts, thus inducing stem cell release in the circulation by sympathetic stimulation of the peripheral nervous system.[13] The bone remodeling process, osteoclast/osteoblast balance, niche alterations, HSC activation and mobilization are, therefore, partially driven by the same pathways and molecules.

Increased leukocyte release (including HSC) from the bone marrow is part of the host's immune system defense response during inflammation. Similar mechanisms are involved in the homing of HSC and cells of the immune system to injured tissues, including the bone marrow. For instance, many non-peptide molecules, such as leukotrienes and other lipid mediators,[14] are involved in the modulation of both inflammation and HSC homing to the bone marrow. Of note, inflammation and homing phenomena occur simultaneously, *in vivo*, in the bone marrow microenvironment after exposure to DNA-damaging agents,[15,16] when the secretion of survival and migration factors, such as SDF-1, peaks. In turn, the increased secretion of SDF-1 is able to enhance the homing and survival potential of donor HSC. Thus, increased production of SDF-1 during alarm situations is part of the host's defense mechanisms capable of counteracting the effects of DNA-damaging

agents leading to cell death.

Extracellular nucleotide triphosphates (e.g. ATP and UTP) are mainly released by activated lymphocytes, macrophages and platelets, as well as necrotic and apoptotic cells, and are known to increase dramatically during inflammation *in vivo*, behaving as danger warning molecules and actively participating in the inflammatory response. We have recently demonstrated[17] that these inflammatory mediators may induce paracrine or autocrine signaling pathways, in cooperation with other chemokines such as SDF-1, to activate and recruit HSC to the bone marrow. Inflammatory molecules may also participate in stem cell trafficking to different organs and tissues. Indeed, SDF-1 expression is increased not only in the bone marrow microenvironment, but also in several other organs upon tissue injury in order to recruit HSC participating in tissue regeneration.[2,18] In this view, recent data document the mobilization of HSC after myocardial infarction[3] or ischemia/reperfusion liver injury following liver transplantation.[4] The mechanisms leading to human HSC recruitment to the damaged liver of immunodeficient mice were recently investigated by Kollet *et al*.[2]

Taken together, these results underline the concept that bone marrow mobilized stem cells show a much broader differentiating potential than that exploited by conventional stem cell transplantation to reconstitute bone marrow function in cancer patients.[18]

Novel molecules to improve stem cell mobilization

The better understanding of the molecular pathways leading to the egress of HSC from the bone marrow or homing of these cells to the marrow has prompted the development of novel strategies and investigational agents to improve stem cell collection prior to autologous or allogeneic transplantation. Specifically, several chemokines and small chemoattractant molecules regulating HSC migration are under study. Most of these molecules, directly affecting HSC/niche interaction, exert their activity within minutes or hours after administration as compared to the 4-6 days required by conventional cytokines such as G-CSF or granulocyte-macrophage colony-stimulating factor. AMD3100 is currently the most promising mobilizing agent under investigation. AMD3100 is a bicyclam molecule that specifically and reversibly blocks SDF-1 binding to CXCR4. Initial phase I studies demonstrated the safety of AMD3100 and its efficacy at mobilizing peripheral blood stem cells in healthy volunteers and cancer patients.[19,20] Subsequent clinical trials showed that a single dose of 240 μg/Kg of AMD3100 was equally effective as standard G-CSF treatment for HSC mobilization in healthy individuals, and also that AMD3100 and G-CSF[21] had synergistic activity. Recently, Flomemberg *et al*.[22] demonstrated, in a randomized trial, that the combination of AMD3100 and G-CSF is superior to G-CSF alone and that the addition of AMD3100 to G-CSF enables the collection of HSC in patients who had been poor mobilizers in response to G-CSF alone. Concurrently, animal studies have shown the long-term engraftment capacity of AMD3100-mobilized HSC;[23] and these preclinical data support the use of AMD3100 to mobilize HSC in allogeneic stem cell donors.[24]

The CXCR4/SDF-1 interaction is also the target of the novel cyclized CXCR4 agonist peptide CTCE-0021[25] and the short modified peptide T-140.[26] The latter agent induces mobilization of HSC within a few hours, synergizes with G-CSF and appears to be more potent than its analog, AMD3100, in a murine model.

GRO-β (CXCL2) is a member of the CXC chemokine family, which includes IL-8, binding CXCR2 receptor. GRO-β and IL-8 share the same mechanism of action, which is neutrophil-dependent upregulation of MMP-9 activity. GRO-β and its N-terminal 4-amino acid truncated form, SB-251353, induce rapid mobilization of HSC in mice and non-human primates with enhanced long-term repopulating capacity as compared to murine HSC mobilized with G-CSF. Of note, GRO-β-mobilized HSC showed increased homing and engraftment efficiency after transplantation and these processes were less dependent on the SDF-1/CXCR4 axis.[27]

Further attempts to modify the HSC/bone marrow niche interaction to improve stem cell mobilization and/or homing and engraftment include the use of parathyroid hormone. This hormone, and the locally produced parathyroid hormone-related protein expand and activate osteoblasts through a specific receptor which, in turn, increases the number of HSC and marrow cellularity.[28] Although preliminary results from a phase I clinical trial[29] demonstrated the capacity of parathyroid hormone, combined with G-CSF, to rescue a significant proportion of patients in whom one or two previous mobilization attempts had failed, its mechanism of action seems more suitable to improving the engraftment of patients who receive a limited number of HSC.

Additional strategies to make stem cell collection more efficient include the expansion of HSC in the bone marrow which are then available for release into peripheral blood upon the concomitant use of a mobilizing agent such as G-CSF. This strategy was successfully employed, using a combination of recombinant human growth hormone and G-CSF, in heavily pretreated patients with hematologic malignancies.[30] Moreover, the expansion and kinetic activation of bone marrow myeloid cells, including committed progenitors and $CD34^+$ cells, following G-CSF administration,[31] enabled G-CSF-primed bone marrow HSC to be harvested from poor mobilizers. Transplantation of autologous G-CSF-primed bone marrow cells resulted in rapid and sustained hematologic recovery after transplantation in myeloablated cancer patients.[32]

Pegfilgrastim is a pegylated form of filgrastim with an extended terminal elimination half-life and self-regulat-

ing serum levels as a function of neutrophil count. Recent data demonstrate that pegfilgrastim, at doses ranging from 6 to 12 mg alone or together with chemotherapy, is capable of efficiently mobilizing peripheral blood stem cells, thus resulting in rapid reconstitution of hematopoiesis when transplanted into patients with multiple myeloma or lymphomas.[33,34] Moreover, a single injection of 12 mg of pegfilgrastim has been used to mobilize peripheral blood stem cells in allogeneic stem cell donors.[35] In this issue of the journal, Russell *et al*. report on lymphoma patients randomized to receive either conventional filgrastim or two different doses of pegfilgrastim (a single injection of 6 or 12 mg) to mobilize peripheral blood stem cells.[36] Although the results indicate that pegfilgrastim is roughly equivalent to filgrastim for stem cell mobilization and collection, a few issues should be carefully considered. First, the study was conducted on a small number of patients (n=90) and this precludes definitive conclusions. Perhaps more importantly, there was a major drop-out rate so that too few patients completed the trial. Of note, a significant proportion of patients (28%) did not mobilize peripheral blood stem cells at all with no difference between the three treatment groups despite the relatively low burden of previous therapy (see Table 2 and exclusion criteria in *Design and Methods*). Thus, the issue of poor mobilizers is not solved by the use of the pegylated form of filgrastim. Interestingly, pegfilgrastim seems to mobilize peripheral blood stem cells to levels suitable for apheresis earlier than filgrastim, inducing a shift to the left in the kinetics of release of progenitors.

In addition to the different kinetics of mobilization of $CD34^+$ HSC, treatment with pegfilgrastim results in a different graft composition compared to that following treatment with filgrastim. As for allogeneic stem cell donors, it is interesting to note that monocytes represent around 40% of leukapheresis products with a 21-fold increase compared to baseline values.[35] Previous investigations have shown that monocytes mobilized along with peripheral blood stem cells secrete large amounts of IL-10 which may affect T-cell function *in vivo*. Moreover, in an animal model, pegfilgrastim proved to be markedly superior to standard filgrastim for the prevention of graft-versus-host disease following allogeneic stem cell transplantation, due to the generation of IL-10-producing regulatory T cells.[37]

On this background, in this issue of the journal, Bruns *et al*. report the results of their comparison of molecular and functional characteristics of stem/progenitor cell subsets mobilized by pegfilgrastim and filgrastim.[38] Of note, gene expression profiling and functional studies demonstrated that pegfilgrastim induces the mobilization of higher percentages of $CD34^+Lin^-CD38^-$ primitive stem/progenitor cells and fewer megakaryocyte-erythrocyte progenitors. Moreover, the gene expression pattern of pegfilgrastim-mobilized $CD34^+$ cells indicated a developmentally earlier cell type, given the increased expression of the *HOX* family of homeobox genes, *MLL* and *MEIS1* genes and reduced expression of erythroid differentiation genes and genes related to the late stage of myeloid maturation. However, one note of caution in the interpretation of the data concerns the low threshold of a 1.2-fold change to define genes differentially expressed between filgrastim- and pegfilgrastim-mobilized $CD34^+$ cells.

Moreover, these findings appear to be in contrast with the increased cycling activity of total $CD34^+$ cells and more primitive $CD34^+Lin^-CD38^-$ cells mobilized by pegfilgrastim as compared to stem cells mobilized by filgrastim. In particular, it is impressive to see that 30% of early $CD34^+Lin^-CD38^-$ cells were in the S-, G2M-phase of the cell cycle after only 3 hours of incubation with early-acting cytokines. Whereas the kinetic features of pegfilgrastim-mobilized cells may suggest that their engraftment capacity is impaired, the results of the clinical trials[36] did not demonstrate any difference in the recovery of hematopoiesis after myeloablative conditioning therapy and transplantation of filgrastim- or pegfilgrastim-mobilized cells.

Taken together, these results indicate that stimulation with pegfilgrastim may result in altered gene expression and different functional properties of mobilized HSC, mature myeloid and selected lymphoid subsets. However, larger randomized clinical trials should investigate whether these intriguing differences will translate into clinical benefit for cancer patients.

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