Comment on Juarez et al, page 707

**PBSCs Sphingolinking along S1P/S1P₁ axis**

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In this issue of *Blood*, Juarez and colleagues reveal a need for the Sphingosine-1-Phosphate/S1P receptor 1 (S1P/S1P₁) axis in the egress of hematopoietic stem cells/hematopoietic progenitor cells (HSCs/HPCs) from the bone marrow after CXCR4 antagonist mediated mobilization in mice. The authors also demonstrate an additional peripheral blood stem cell (PBSC) mobilization benefit for S1P₁ agonist co-treatment in combination with a CXCR4 antagonist (see figure) but not human granulocyte colony-stimulating factor (G-CSF).

The observation that HPC numbers are elevated in the blood of patients after chemotherapy eventually led to the realization that HSCs/HPCs could be induced to exit the bone marrow and enter the circulation, a process referred to as mobilization. In the setting of hematopoietic stem cell transplantation (HSCT), patients are usually given chemotherapy, immunotherapy, and/or radiation therapy to treat their malignancy and then receive either their own autologous cells or allogeneic donor cells collected from a healthy individual. Currently, mobilized peripheral blood is the primary source of hematopoietic cells used for hematopoietic transplantation. This is particularly true in the adult transplantation setting where bone marrow (BM) harvest procedures have fallen out of favor, and where single umbilical cord blood (CB) collections are often too small in volume and/or cell number to be adequate for an adult recipient. While there is no universal consensus on optimal CD34⁺ cell yield after PBSC mobilization, infusions of greater than 2.0 to 2.5 x 10⁶ cells/kg are generally associated with fast and sustained hematopoietic recovery. Larger doses of CD34⁺ cells may result in faster recovery after HSCT, and possibly higher overall recipient survival. Therefore, strategies to improve HSC/HPC yield during mobilization would have therapeutic benefit.

The ideal mobilization agent or regimen has yet to be identified, and numerous different mobilization approaches exist. Recombinant G-CSF is the most commonly used mobilizing agent in the clinical setting of allogeneic HSCT. Filgrastim (Neupogen; Amgen Inc), or methionyl human G-CSF (r-metHuG-CSF) is a G-CSF analog produced by recombinant DNA technology. It was initially used to treat chemotherapy-induced neutropenia, and is now known to increase the number of circulating HSCs. Mobilization with G-CSF alone is successful in approximately 85% of healthy volunteers. Additional mobilizing agents that have also been studied include recombinant human...
The role for the SDF-1/CXC4 axis in retention of HSCs/HPCs in the BM under steady-state conditions is widely accepted. However, its role in stem cell trafficking, the processes of homing or mobilization, has needed additional clarification. At its most basic level, the question that persisted was; “If the SDF-1 gradient across the BM-PB barrier drives the homing of HSCs/HPCs into the BM during HSCT, what drives the release of cells from the BM microenvironment into the PB when the SDF-1/CXC4 axis is disrupted?” Mechanistically, it appears that the homing of HSCs/HPCs from the PB into the BM is facilitated by the SDF-1/CXC4 axis and that disruption of this axis results in the release of HSCs/HPCs. However, the model by which the SDF-1/CXC4 axis changes during mobilization, thereby facilitating the release of HSCs/HPCs is unclear. It is in this context that the SLP active phospholipid has entered the discussion as a novel chemoattractant for HSCs/HPCs and a possible explanation for the mechanism behind release of HSCs/HPCs into PB. SLP, a product of 2 sphingosine kinases (SK1 and SK2), interacts with at least 5 G-protein–coupled seven-transmembrane–spanning receptors, S1P1, on the surface of target cells to induce cell migration. HSCL have been shown to express SLP receptors and migrate in response to SLP.5

Here, Juarez et al studied the role of the SLP/S1P axis in PBSC mobilization and determined that (1) pharmacologic inhibition of the SLP/S1P axis (using FTY720; Novartis) or use of S1P1, mice inhibits CXCR4 antagonist (AMD3100)–mediated mobilization but not G-CSF–induced mobilization, (2) use of SK1−/− mice inhibits AMD3100 mobilization, (3) SLP plasma levels increase after AMD3100 treatment in the PB of mice but not humans, and (4) treatment of mice with a SLP1 agonist (SEW2871) results in a dose-dependent augmentation of mobilization when co-administered with AMD3100. These data are consistent with other reports that suggest differing mechanisms of action between AMD3100 and G-CSF–induced mobilization of PBSCs. An unresolved question in the field is whether an increase in plasma SLP concentrations in the PB in response to stimulus is indeed a component of the mechanism by which PBSC mobilization occurs. Others have suggested that a SLP chemotactic gradient is continuously present in the PB, retention of HSCs/HPCs in BM is an active process that counters SLP gradient, and increased plasma SLP levels occur during mobilization.9 The data presented here confirm that that is true in mice but will have to be examined in humans. Additional data in humans as well as mice using highly sensitive assays that are able to detect subtle changes in relatively low concentrations of SLP may help to resolve this question. Even with this remaining controversy it is clear that a combination strategy to disrupt the endogenous HSC/HPC retention mechanisms, such as the SDF-1/CXC4 axis, while augmenting the SLP/S1P axis is likely to enhance PBSC mobilization and yield clinical benefit within the field of stem cell therapeutics/regenerative medicine.

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REFERENCES

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Functional transfer of microRNA by exosomes

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Cells can communicate directly with each other through cell-cell contact or at a distance using secreted soluble mediators. A third mode of intercellular communication may be mediated by exosomes, an emerging novel pathway with unprecedented potential. In this issue of Blood, Montecalvo and colleagues demonstrate that dendritic cells (DCs) secrete exosomes that are loaded with distinct sets of microRNA, dependent on the status of DC activation.1 Moreover, they show that DC exosomes can fuse with target cells, thereby delivering their membranous and cytosolic contents. Finally, using a clever setup, they provide proof of principle that, after being transferred by exosomes, microRNA can repress mRNAs in target cells.
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