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HEMATOPOIESIS AND STEM CELLS

Comment on Mende et al, page 3387

Extramedullary hematopoietic stem cells

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In this issue of *Blood*, Mende et al[1] apply single cell sequencing and functional studies to characterize human hematopoietic stem and progenitor cells (HSPCs) isolated from extramedullary sites. Use of single cell technologies has provided many insights into our understanding of hematopoiesis, particularly when coupled with functional analyses. These studies have demonstrated unrecognized levels of cellular complexity; identified new, functionally distinct populations of cells; detected novel transcriptional regulators; and revealed the existence of multiple hematopoietic hierarchies, each with distinct regulatory features.[2] Models of hematopoiesis have evolved from specific cell states with well-defined points of transition to one of a continuous model of cell state during hematopoietic differentiation.[3]

Single cell studies of HSPCs have added to our understanding of their diversity and their changes in hematopoiesis,[4-8] with most focusing on HSPCs that reside in the bone marrow. What about the rare HSPCs that are found outside the marrow? What is their function? Small numbers of HSPCs are detectable in peripheral blood at steady state, with numbers influenced by circadian rhythm. HSPCs have also been found in lung, spleen, liver, gut, and gingiva, with ill-defined residence times.[9] In states of hematopoietic stress, such as anemia, inflammation, infection, cardiovascular disease, and autoimmune disease, or after pharmacologic mobilization, HSPCs migrate to extramedullary sites, primarily the spleen and liver, where they differentiate into effector cells, beginning the process of extramedullary hematopoiesis.

Mende and colleagues from the University of Cambridge begin to address this knowledge gap, applying single cell sequencing and functional studies to study of human HSPCs isolated from spleen, peripheral blood, and mobilized peripheral blood (extramedullary HSPCs) and comparing them to bone marrow (medullary) HSPCs. HSPC compositions in medullary and extramedullary sites were distinct, with bone marrow containing more myeloid and megakaryocyte progenitors, whereas spleen and peripheral blood harbored significantly fewer late progenitors of the myeloid, megakaryocyte erythroid mast cell basophil lineage. Primed multipotent progenitors (MPPs), a subset of HSCPs with marked myeloid or erythroid megakaryocyte lineage priming, were significantly enriched at extramedullary sites. Paralleling the relative lack of late extramedullary progenitors, most progenitors in spleen and peripheral blood had significantly decreased proportions of cycling cells. Modeling indicated that extramedullary HSPCs had significantly limited progenitor expansion incompatible with sustained ongoing hematopoiesis at these sites.

Comparative transcriptome analyses demonstrated that extramedullary hematopoietic stem cells/multipotent progenitors (HSC/MPPs) isolated from peripheral blood, mobilized peripheral blood, and spleen share a common transcriptional signature that is distinct from bone marrow HSC/MPPs. Extramedullary HSC/MPPs were more lineage-primed compared with those from bone marrow. They also had higher expression of genes associated with short-term HSCs and lineage committed progenitors, the master regulator of quiescence exit *CDK6*, of surface proteins associated with HSC differentiation and activation, and not unexpectedly, of surface proteins associated with altered cytoskeleton organization and cell migration and adhesion. In contrast, medullary HSC/MPPs had significant enrichment of gene signatures and cell surface markers of long-term HSCs.

Unmobilized peripheral blood HSPCs from healthy individuals displayed a bias toward erythroid-megakaryocytic differentiation. Functionally, this bias was conveyed by a subgroup of phenotypic $CD71^+$ HSC/MPPs that were highly abundant in peripheral blood but rare in other adult tissues that exclusively produced erythrocytes and megakaryocytes. In transplantation studies, unmobilized peripheral blood from normal individuals at steady state demonstrated the presence of HSC/MPPs with long-term repopulating ability. However, they were extremely rare and at much lower levels than that found in cord blood or mobilized peripheral blood. $CD71^+$ HSC/MPPs had no repopulating ability.

With aging, the HSC/MPP pool from unmobilized peripheral blood demonstrated markedly higher bone marrow- to spleen-type score ratios than younger donors, and it produced significantly smaller erythroid colonies than HSC/MPPs from young donors, indicating decreased proliferation potential with age. Erythroid progenitors from older donors expressed significantly lower levels of master regulators of the erythroid lineage such as GATA-1, KLF1, and MYC.

Is the unique bias toward erythroid-megakaryocytic differentiation exhibited by peripheral blood HSPCs altered in hematologic disease? HSC/MPPs from patients with essential thrombocythemia and, surprisingly, β-thalassemia demonstrated increased myeloid production at the expense of the erythroid lineage. The authors suggest that disease-driven changes in the microenvironment and/or hematopoietic dysfunction in the marrow leads to a shift in the differentiation balance of peripheral blood HSC/MPPs.

To further place these studies in a clinical context, extramedullary HSPC composition and function were examined in spleens from 2 patients with hereditary spherocytosis, an example of chronic hematopoietic cell stress. HSC/MPPs from HS spleens displayed increased erythroid transcriptional priming and produced more erythroid colonies in vitro than those from control spleens. HS splenic HSPCs also had a much higher ratio of early erythroid to myeloid progenitors than control. Although there was no change in myeloid expansion of splenic HSPCs, there was increased differentiation along the erythroid line, although not to the level modeled in bone marrow. Taken together, the authors conclude that splenic HSPCs contribute to erythropoiesis in response to anemia in humans.

This study provides an atlas of HSPC transcriptome data for investigators to query and use for comparative studies. It also demonstrates that circulating HSPCs hold promise for use in clinical applications, particularly in disease states because the composition and function of circulating HSPCs may serve as markers for bone marrow dysfunction. They may provide insights into disorders such as cardiovascular disease and stroke, select malignancies, myelofibrosis, and some autoimmune diseases, in which increased numbers of circulating peripheral blood HSPCs have been observed. Could peripheral blood HSPCs provide additional mechanistic insights to our understanding of disease, allowing better diagnosis, monitoring, and treatment? The studies by Mende and colleagues provide a tantalizing "yes" while at the same time providing a beautiful blueprint for future work.

Conflict of interest disclosure: The author declares no competing financial interest. ■

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IMMUNOBIOLOGY AND IMMUNOTHERAPY

Comment on Ma et al, page 3402

Hyperuricemia reduces neutrophil function

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In this issue of *Blood*, Ma et al[1] report that high levels of soluble uric acid (sUA) reduce neutrophil function in patients with chronic renal failure. It is well appreciated that individuals suffering from various forms of chronic renal failure are immunosuppressed.[2] Indeed, infection is the second most common cause of death in patients with kidney disease.

There are many factors that may contribute to the immunodeficiency of renal failure, but the molecular mechanisms that cause these immune defects remain poorly investigated. Clearly, both innate and adaptative immune responses can be affected. In the innate compartment, impairment of both neutrophil and monocyte/macrophage function have been reported. Monocytes from uremic patients manifest reduced cytokine production when stimulated, whereas neutrophils display reduced migratory capacity. In the mononuclear system, one of the molecular mediators of reduced cellular function was found to be high levels of sUA.[3] Hyperuricemia at a level of 7 to 12 mg/dL (found in patients with more severe renal dysfunction) leads to inhibition of monocyte Toll-like receptor (TLR) signaling, which impairs cytokine production and $CD14^+$ monocyte migration in vitro. The authors of the Ma et al article found that UA impairs β2 integrin activation and signaling, which leads to reduced neutrophil migration into inflammatory sites in vivo.

UA is taken up into cells via glucose transporter 9 (Glut9 or SLC2A9). Mice lacking SLC2A9, specifically in hepatocytes, develop moderate hyperuricemia; when the animals are fed an inosine-rich diet, they develop serum UA levels in the range of 7 to 12 mg/dL seen in humans with end-stage renal failure.[4] By using this model, Ma et al found reduced neutrophil recruitment into air pouches (formed on the back of the mouse) in response to 2 different inflammatory stimuli. Most directly, this group used intravital microscopy to directly measure neutrophil rolling and adhesion in the vasculature of the hyperuricemic mice. In response to