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HEMATOPOIESIS AND STEM CELLS

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STAT1 and MHCII: guardians of stressed HSCs

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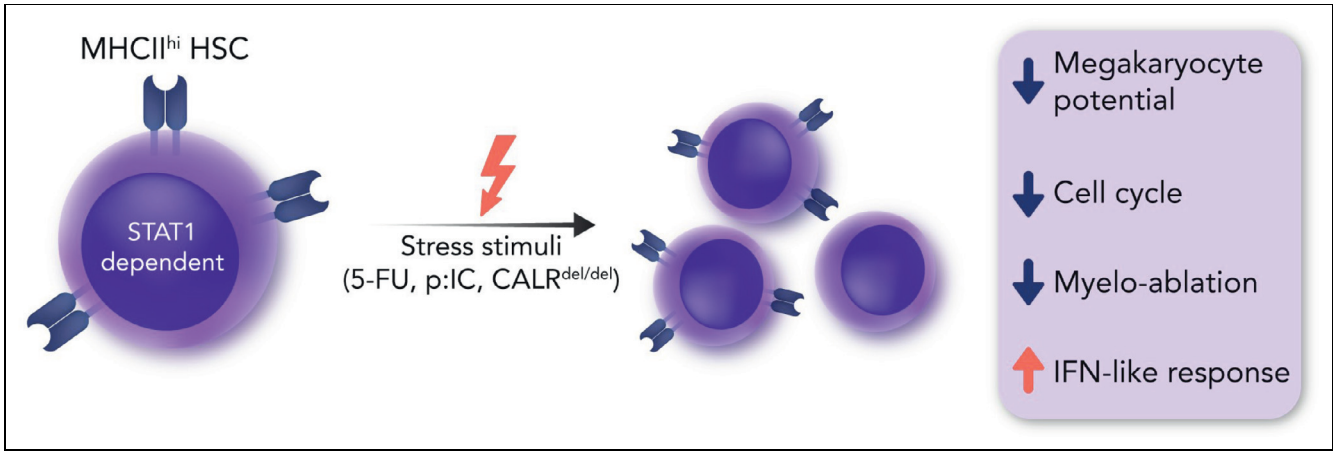
In this issue of *Blood*, Li et al[1] add a fresh perspective to our understanding of hematopoietic stem cell (HSC) functions. The authors explain that signal transducer and activator of transcription 1 (STAT1) is a critical steady-state regulator of HSC biology. Specifically, STAT1 is a key regulator of a subset of HSCs characterized by elevated levels of major histocompatibility complex II (MHCII^{hi}). This MHCII^{hi} HSC subset resists stress-induced myeloablation and is not expanded in calreticulin mutant (CALR$^{del/del}$) mice (see figure).

In addition to activating antiviral protection, interferon (IFN) signaling drives HSCs out of their quiescent state, promotes differentiation, and induces apoptosis. STAT1 as key downstream player in IFN signaling mediates these effects.[2] Our knowledge of the role of STAT1 in HSCs under homeostatic conditions has been sparse. In the study by Li et al, the authors conducted an in-depth characterization of STAT1-deficient mice, which showed increased numbers of immunophenotypic dormant HSCs but indicated a reduced functional HSC pool based on single-cell RNA sequencing. This reduced function was reflected by altered competitive transplantation behavior and an altered response upon challenge with 5-fluoruracil (5-FU), in line with a recent report by Marié et al.[3] In steady-state conditions, STAT1 deficiency is associated with reduced expression of MHC molecules, interferon-stimulated genes (ISGs), viral defense genes, and viral sensing or tumor immune surveillance. The impact of STAT1 on ISG expression is expected, yet extraordinary in this context, because ISGs are observed in the absence of any stimuli. This may be the result of previous inflammatory events or it may represent a condition of preparedness in HSCs that enables them to react to any potential insult. An alternative explanation could be that tonic IFN signaling (the production of vanishingly low quantities of IFN) affects HSCs, which has been observed in diverse immune cell types.[4] In addition to the effects of phosphorylated STAT1 (pSTAT1) in the canonical JAK/STAT signaling cascade, which is triggered upon activation, effects may also be linked to nonactivated unphosphorylated STAT1 (u-STAT) functions. An example of a u-STAT function is provided by u-STAT5, which enters the nucleus and binds DNA (thereby regulating gene expression) and influences cellular functionality in hematopoietic progenitor cells.[5] A similar concept can be postulated for STAT1 in HSCs. In this case, STAT1 has a dual role in HSCs; u-STAT1 regulates quiescence whereas pSTAT1 forces HSCs out of quiescence into proliferation upon stimulation by IFN.

The HSC pool is heterogeneous; distinct HSC subsets give rise to distinct lineage outputs upon transplantation.[6] Li et al expand our knowledge and add a new facet to this subject by defining a functional HSC subpopulation characterized by high MHCII levels, low apoptotic rates, and deep quiescence. MHCII^{hi} HSCs are protected from stress stimuli such as 5-FU or polyinosinic:polycytidylic acid. The existence of MHCII^{hi} HSCs requires the presence of STAT1, as STAT1-deficient mice lack this subpopulation. In differentiated cells, STAT1 contributes to MHCII expression via the regulation of *Ciita*, the master regulator of constitutive and IFN-γ–induced MHCII expression.[7] In STAT1-deficient HSCs under homeostatic conditions, *Ciita* levels are unaltered, which suggests a mechanism independent of *Ciita*. It remains unknown whether STAT1 directly regulates MHCII or whether MHCII^{hi} HSCs are lost because of an enhanced differentiation into MHCII^{lo} HSCs in STAT1 knockout mice. High ISG expression in the absence of stress stimuli has previously been observed in stem cells and linked to protection from viral infection.[8] It is attractive to speculate that MHCII^{hi} HSCs are particularly protected and represent a reservoir for hematopoiesis in case of severe viral infections.

The fact that the absence of STAT1 does not affect *Ciita* expression is surprising and indicates an unconventional role for STAT1 in HSCs. We recently investigated 2 other STAT family members, STAT5A and STAT5B, in HSCs and found a role for STAT5B as a key driver of self-renewal and quiescence. In this regard, STAT5B and STAT1 have overlapping or common functions because STAT5B-deficient HSCs phenocopy STAT1-deficient HSCs in transplantation studies or after 5-FU challenge. The absence of STAT5B in HSCs also reduces ISG levels based on single-cell RNA sequencing data. The specific effects of STAT5B, but not STAT5A, in HSCs were unexpected because STAT5A and STAT5B were considered to have largely redundant functions.[9] Both STAT1 and STAT5B modify stem cell quiescence with unusual functions. Activated STAT1 hinders proliferation in differentiated cell types, but it induces proliferation in HSCs. And vice versa, activated STAT5



STAT1-dependent MHCII^{hi} HSCs are less responsive to diverse stress stimuli. High levels of MHCII define a novel HSC subset dependent on STAT1. This subset is less responsive to stress-induced (5-FU or polyinosinic:polycytidylic acid [p:IC]) proliferation and apoptosis. In mutant CALR mice, MHCII^{hi} HSCs have a low capacity to expand and differentiate into the megakaryocytic lineage. Professional illustration by Somersault18:24.

induces proliferation in differentiated cell types, but it maintains quiescence in HSCs.[2,9,10]

The authors also investigated the roles of MHCII^{lo} and MHCII^{hi} HSCs in CALR$^{del/del}$ mice. In the murine model, MHCII^{lo} HSCs are expanded and likely drive thrombocytosis, whereas MHCII^{hi} HSCs show reduced megakaryocytic potential and expansion capacity. The enhanced megakaryocytic bias of MHCII^{lo} HSCs is in line with the observation that these cells gave rise to a higher degree of myeloid repopulation in transplantation settings. The discrepancy in CALR$^{del/del}$–induced expansion between MHCII^{lo} and MHCII^{hi} HSCs provides a potential therapeutic window to specifically target MHCII^{lo} disease-driving HSCs while leaving MHCII^{hi} dormant HSCs unaffected. It remains to be determined whether and how MHCII^{lo} and MHCII^{hi} HSCs differ at a molecular level, and whether the expansion of MHCII^{lo} HSCs also dominates other stem cell–driven hematopoietic malignancies.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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https://doi.org/10.1182/blood.2022016850

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TLR1/2-stimulated DCs "prime" HSCs via IL-1β

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Bone marrow dendritic cells (BMDCs) are increasingly recognized as an important cellular component of the hematopoietic stem cell (HSC) perivascular niche. In this issue of *Blood*, Li et al[1] report that BMDCs respond to Toll-like receptor 1/2 (TLR1/2) agonist stimulation by producing interleukin 1β (IL-1β), which is sensed by HSCs, leading to their expansion.

Mature hematopoietic cell production during both homeostasis and stress relies on a fine-tuned process of environmental sensing and intracellular signaling by HSCs within their microenvironment, also referred to as the "HSC niche." Integration of cellular extrinsic signals such as inflammatory or pathogen-associated molecular pattern molecules (PAMPs) into cellular intrinsic molecular circuits determines whether HSCs remain quiescent or engage in multilineage