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Hematopoiesis in Steady-State versus Stress: Self-Renewal, Lineage Fate Choice, and the Conversion of Danger Signals into Cytokine Signals in Hematopoietic Stem Cells

Lisa Borghesi

Long-term hematopoietic stem cells (LT-HSCs) replenish the innate and adaptive immune compartments throughout life. Although significant progress has defined the major transcription factors that regulate lineage specification, the architectural proteins that globally coordinate DNA methylation, histone modification, and changes in gene expression are poorly defined. Provocative new studies establish the chromatin organizer special AT-rich binding protein 1 (Satb1) as one such global regulator in LT-HSCs. Satb1 is a nuclear organizer that partitions chromatin through the formation of cage-like structures. By integrating epigenetic and transcriptional pathways, Satb1 coordinates LT-HSC division, self-renewal, and lymphoid potential. Unexpected among the assortment of genes under Satb1 control in hematopoietic stem cells (HSCs) are cytokines, a finding that takes on additional importance with the provocative finding that short-term HSCs and downstream multipotent progenitors are potent and biologically relevant cytokine secretors during stress-mediated hematopoiesis. Together, these studies reveal a new mechanism of fate regulation and an unforeseen functional capability of HSCs. The Journal of Immunology. 2014, 193: 2053–2058.

The molecular mechanisms that regulate long-term hematopoietic stem cell (LT-HSC) quiescence, self-renewal, and differentiation are incompletely understood. Major progress has identified key transcriptional and signaling regulators of self-renewal and lymphomyeloid potential (1–3), organized their activity into defined modules (4–7), and resolved how shared transcription factors govern distinct lineage fate programs depending on timing and context of expression (8). However, major questions remain. For example, although the changes in histone marks and DNA methylation that regulate gene suites are increasingly well resolved (9–12), how these epigenetic processes are tandemly coordinated in LT-HSCs is not known. Moreover, the classical model for LT-HSC biology is changing with the realization that hematopoietic stem and progenitor cells (HSPCs) directly sense pathogens through TLRs, functionally responding with accelerated differentiation, lineage-biased output, and even lineage fate redirection at the single-cell level (13–15). Thus, rather than simply replenishing downstream immune cell subsets, LT-HSCs and their immediate progeny detect pathogen signals actively and respond by selective differentiation. Even more important, a new study shows that TLR activation elicits cytokine secretion by short-term (ST)-HSCs and multipotent progenitors (MPPs), with the magnitude and diversity of cytokine production rivaling those of mature immune cell subsets under the same conditions (16). The revelation of this previously unrecognized paracrine function firmly changes our view of hematopoietic stem cells (HSCs) as passive responders to the depletion of mature immune cell subsets and instead positions HSCs as central players in directing hematopoietic outcomes in the contexts of infection and inflammation.

Epigenetic and transcriptional regulation of HSCs

Blood cell replenishment throughout life depends on two functional features of LT-HSCs: quiescence (i.e., activation status) and fate choice (i.e., the balance between self-renewal and differentiation). During hematopoiesis, self-renewing LT-HSCs give rise sequentially to ST-HSCs and then MPPs, the last of which has limited, if any, self-renewal potential but retains the ability to produce all blood cell subsets (17, 18). MPPs, in turn, produce lymphomyeloid primed MPPs (LMPPs) that have robust lymphoid and myeloid potential but have lost megakaryocyte and erythroid potential (19–21). Total LMPPs also contain the rag1+ early lymphoid progenitor (ELP) subset (22). LMPPs/ELPs differentiate to intermediates with select lineage-development capabilities. Common lymphoid progenitors produce B cell, NK cell, and dendritic cell lineages and, with poor efficiency, T cells (23, 24). Reciprocally, early thymic progenitors that transit from bone marrow to thymus have robust T cell potential and retain some NK cell, dendritic cell, and myeloid potential, but little B cell
activity (25). Granulocyte-macrophage progenitors rapidly produce granulocytes and macrophages (26, 27). Disruption in the balance between self-renewal and differentiation is associated with both hematopoietic failure and leukemic transformation. The dynein-binding protein Lis1, for example, is required for both maintenance of the stem cell pool and for leukemic propagation. Conditional ablation of Lis1 increases the frequency of asymmetric HSC divisions, ultimately leading to a striking bloodless phenotype, whereas deletion of Lis1 in human leukemia blocks oncogenic growth (28).

HSCs are marked by high levels of DNA methylation and bivalent histone marks, a chromatin signature that is thought to reflect developmental genes poised for expression. DNA methylation threshold is clearly important because HSCs from mice with reduced DNA methyltransferase I undergo erythromyeloid differentiation at the expense of lymphoid development (10). During cell fate specification, bivalent marks at cis-regulatory regions are resolved with lineage-appropriate genes gaining the presence of additional activating H3K4me3 modifications and lineage-inappropriate genes repressed by H3K27me3 modifications (12). PU.1, E2A, and Ikaros are among the major transcription factors that regulate lineage-specification programs. PU.1 is an ETS-related family member, Ikaros is a zinc-finger protein 1 family member, and E2A belongs with the E family of helix-loop-helix proteins. These three transcription factors act to promote the expression of lineage-specific genes and repress alternate fates, with transcriptional activity exhibiting striking dose dependence. Disrupted expression of any single factor due, for example, to genetic haploinsufficiency is mechanistically associated with malignancy (29–31). Significant progress has established the coordinated action of PU.1, E2A, and Ikaros within defined, interacting networks.

PU.1 has a major role in HSC lineage potential, with high, intermediate, and low levels of PU.1 promoting myeloid, lymphoid, and erythroid fate, respectively (32, 33). Concordantly, PU.1-deficient mice die embryonically as a result of a gross failure of hematopoiesis (34). At high doses, PU.1 drives expression of major myeloid-associated genes, including the c-fms cytokine receptor and FcεRI/IIIII, an outcome that is reinforced by a feed-forward mechanism involving the activities of Egr and Nab2, which coordinate macrophage versus neutrophil output (7). PU.1 activity needs to be actively restrained to foster lymphopoiesis, and one requisite modulating influence is exerted by the transcriptional regulator Gfi1 (33, 35). Gfi1 physically displaces PU.1 from autoregulatory elements, thereby alleviating PU.1 autoamplification (7). At these intermediate levels, PU.1, along with Ikaros, drives major lymphoid-associated genes, such as the Fk3 cytokine receptor (36). Consistent with the role of Gfi1 in restraining PU.1 activity, Gfi1-deficient animals have increased myeloid precursors and complementary reductions in B and T cell development. Mechanistically, PU.1 mediates these diverse lineage-specific effects through both nucleosome remodeling and H3K4me1 histone methylation at distinct target genes, depending on whether PU.1 is activated in the context of myeloid-associated (e.g., Gfi1 or C/EBPPB) or lymphoid-associated (e.g., E2A) cofactors, respectively (37).

E2A and Ikaros regulate HSC self-renewal, as well as promote lymphoid fate decisions (38–43). E2A preserves HSC self-renewal potential, in part, through directly activating the p21 (CIP1/WAF1) cell cycle inhibitor (42, 43). Mice deficient in the E2A isoform E47 exhibit decreased p21, a loss of G0 quiescence, and hyperproliferation that ultimately leads to HSC exhaustion (43, 44). Mice bearing a point mutation in Ikaros have a diminished LT-HSC compartment that fails to expand in response to supportive Notch signals (45). Both E2A-deficient and Ikaros-deficient mice exhibit a complete block in B cell development due to a paucity of LMPPs, as well as aberrant T cell development accompanied by spontaneous T cell leukemias (38, 46). E2A, along with PU.1, regulates expression of IL-7R, a key cytokine receptor in lymphoid formation (47). Additionally, Ikaros directly engages Gfi1 regulatory targets, thereby reinforcing Gfi1-mediated restraint of PU.1 levels to within the lymphoid-supportive range (7). E2A remains important during B and T cell commitment. Within B cell precursors, E2A activates EBF and Pax5, along with FOXO1, to promote rag gene expression, V(D)J recombination, and formation of the BCR (5, 6, 48, 49). E2A occupancy is associated with increased H3K4 monomethylation across a spectrum of lineage-specific cis-regulatory regions (5). Within T cell precursors, E2A signals are delivered in the context of Notch1, which has the effect of simultaneously antagonizing the B and myeloid fates while activating T cell–specific genes, including Gata3, TCF1, and Bcl11b (4, 8). Conversely, E protein activity needs to be restrained for the formation of innate lymphoid cells, including NK cells and the cytokine-producing innate lymphoid cell subsets (50).

Together, these findings establish the regulatory circuitry that guides HSCs to the lymphoid versus myeloid lineage fates. What remains unknown is how the epigenetic and transcriptional pathways are themselves regulated.

The global chromatin organizer special AT-rich binding protein 1 in hematopoiesis

Two new publications identify the organizer special AT-rich binding protein 1 (Satb1) as coordinating HSC activity at the levels of DNA methylation, histone modification, and gene transcription. The global chromatin organizer Satb1 was first cloned two decades ago based on the ability to bind to the matrix attachment region 3′ of the IgH locus (51). The nominal allusion to special binding refers to the ability of Satb1 to preferentially recognize dsDNA in base unpairing regions, stretches of DNA that become unpaired when subject to negative superhelical strain. The tendency to unpair, or unwind, is observed in DNA sequences flanking the IgH enhancer and intronic κ enhancer (iEκ) among other genes, augmenting lineage-specific expression (51–53). The geometry of Satb1 nuclear distribution is striking (Fig. 1A, upper panel). Satb1 has a dense cage-like distribution that folds chromatin into organized domains, an effective visual indicator for understanding Satb1 activity. Similar structures are apparent in mouse and man (54, 55). Satb1 effectively serves as a physical platform for coregulating target DNA sequences and chromatin-modifying enzymes (54). The functional implication is that Satb1 links nuclear architecture, chromatin structure, and domain organization of DNA sequences.

As a result of particularly high expression in thymus, the biological function of Satb1 was first extensively characterized in T cell precursors. Satb1-deficient mice exhibit a disordered pattern of gene expression in thymocytes, including dramatic
changes in the expression of the cytokine receptor chains IL-7R and IL-2Rα and γc, effectively disrupting T lineage fate specification (56). At the IL-2Rα promoter, for example, Satb1 normally represses expression to physiologic levels by recruiting the NURD chromatin-remodeling complex, along with the ISWI and ASF1 nucleosome-mobilization complexes, leading to histone H4 hypoacetylation and long-range (>7-kb) nucleosome repositioning (57). Consequently, Satb1-deficient progenitors have a partial block at DN3, at the double positive to single positive transition, and a bizarre accumulation of peripheral T cells marked by diffuse, rather than discrete, CD4+ or CD8+ flow cytometry profiles. Beyond thymocytes, Satb1 has broad activity across hematopoietic and nonhematopoietic lineages in both mouse and man, including roles in B cell formation (56), globin expression in erythroid progenitors (58), Wnt/β-catenin-mediated Th2 differentiation (55), and fate determination in embryonic stem cells (59).

Satb1 regulates LT-HSC self-renewal and lineage potential through the global coordination of DNA methylation, histone activation, and gene transcription

Provocative new studies by Will et al. (60) and Satoh et al. (61) revealed a new mechanism of regulation of HSC fate. Satb1 directs not only the major opposing functions of self-renewal versus differentiation, but also lymphoid potential.

The goal propelling Will et al. (60) was to distill how quiescence, division, and lineage commitment are coordinated in HSCs. To avoid high perinatal lethality, the investigators used reconstitution chimeras in which Satb1-deficient progenitors were engrafted into wild-type hosts. Posttransplantation, Satb1-deficient HSCs (CD150+LSK, lineage-, Sca-1-, c-kit+) exhibited a loss of G0 quiescence and poor performance in competitive serial-transplantation assays, indicating a loss of self-renewal potential. At the single-cell level, Satb1-deficient HSCs, but not MPPs, had enhanced myeloid potential, suggesting that Satb1 restrains differentiation specifically in the former hematopoietic subset. Moreover, Satb1-deficient HSCs also exhibited more symmetric differentiation divisions and fewer symmetric self-renewal divisions due, in part, to increased levels of the cell polarity factor Numb (3). Satb1 binding to promoter regions of Numb and another regulator of self-renewal, Myc, in an HSC cell line decreased the abundance of permissive H3K4me3 histone marks. Genome-wide assays revealed a pattern of DNA cytosine methylation in Satb1-deficient HSCs resembling that of wild-type MPPs, a cellular state poised for differentiation. In the absence of Satb1, >50 genes were identified with paired changes in both transcription and flanking methylation. Genes with altered expression include major regulators of cell cycling (Rbbp9, Kdm3a, Chaf1a, Btg) and cell organization and polarity (Ipt1, Tnib), as well as a pleiotropic regulator (Myc). Thus, within HSCs, Satb1 is the first well-characterized chromatin remodeler that coordinates the expression of genes at the levels of transcript expression, histone modification, and DNA methylation (Fig. 1A, lower panel).

Using an independent approach, Satoh et al. (61) sought to identify initiators of lymphoid potential that may act in advance of, or in parallel with, defined transcription factors. The investigators identified Satb1 in a comparative array between rag-1 HSCs (self-renewing, multipotent) and rag1+ ELPs (nonrenewing, high lymphoid potential, poor erythromyeloid potential). The detection of Satb1 along with a cohort of lymphoid-specific genes suggested that lymphoid lineage fate specification may be initiated before the ELP stage. Sorted
Satb1-deficient HSCs had poor lymphoid differentiation in defined in vitro cultures or following in vivo transfer to wild-type hosts, whereas myeloid potential was comparable to controls. Reciprocally, ectopic expression of Satb1 in HSCs conferred enhanced B and T cell output, in terms of both numbers and kinetics. These finding suggest that Satb1 regulates lymphoid lineage decisions in single HSCs. Moreover, enforced Satb1 also significantly restored the B lymphoid potential of HSCs from 2-y-old mice, an age at which lymphoid potential is severely crippled. Although overexpression of Satb1 in this model did not cause oncogenic transformation, single nucleotide polymorphisms in a Satb1 binding site in a PU.1 distal enhancer are mechanistically linked to acute myeloid leukemia (31, 62).

The molecular basis for Satb1-mediated regulation of lymphoid potential is both a little perplexing and potentially exciting. One possibility is that Satb1 augments expression of lymphoid-specific transcription factors (i.e., Ikaros, E2A, EBF, Notch1), while restraining myeloid-specific factors (i.e., PU.1). An alternative, but not mutually exclusive, possibility is that Satb1 changes the developmental context in which these transcriptional programs exert activity, thereby influencing fate choice. A comparative microarray between wild-type and Satb1-overexpressing HSCs (flk2−/− LSK) revealed that lymphoid potential appears to be enhanced without detectable change in the expression of the canonical transcriptional regulators PU.1, Ikaros, E2A, Notch1, and C/EBPs (61). Rather, other lymphoid-associated transcription factors and genes (Sp4, Maf, Fos, Id3, CD86, rag1) are upregulated, and a major myeloid associated factor (Csf3r) is downregulated, following Satb1 transduction. It is clear that the biological impact of transcription factors depends on the exact cellular and temporal context in which they are expressed. For example, a shared set of E proteins drives different fate decisions during B versus T cell development, due, in part, to the distinct transcriptional and signaling environment in which they operate (8). Unexpectedly, an unforeseen third possibility arises. Transcripts for stem cell factor and IL-7, cytokine genes traditionally associated with stromal cells, are upregulated in Satb1-transduced HSCs (61). HSCs are not traditionally recognized for their cytokine-secretion potential, and protein levels of these cytokines were not examined in these experiments. However, a major new study reveals the potential of HSC subsets and downstream MPPs to elaborate large amounts of cytokines that functionally influence hematopoiesis (16), immediately raising the potential impact of these striking observations.

**LT-HSCs directly respond to danger and potently secrete cytokines that influence hematopoiesis**

LT-HSCs and their downstream progeny express TLRs and can respond directly to pathogen. Following TLR stimulation, murine HSCs (14, 15), as well as human HSCs (63–67), proliferate and preferentially undergo myeloid-specific differentiation. Direct sensing of TLR ligand is thought to enable HSCs to immediately replenish innate immune cells that are rapidly depleted during infection, so-called “demand adapted hematopoiesis” (68). Indeed, circulating HSCs that have exited the marrow undergo rapid myeloid differentiation in response to TLR4 ligand, suggesting that both bone marrow–resident HSCs and migratory HSCs directly respond to TLR danger signals (14). The functional significance of TLR sensing by HSCs and HSPCs is just beginning to be explored.

Using a powerful microfluidic-based proteomic platform to measure cytokine production by single cells, Zhao et al. (16) showed that HSPCs secrete large amounts of cytokines following TLR2 and TLR4 stimulation (Fig. 1B). At the single-cell level, 50–70% of ST-HSCs and MPPs each produced cytokines, including IL-6, TNF-α, GM-CSF, and IL-1β. Moreover, the magnitude and breadth of cytokine production exceeded that of splenic myeloid and lymphoid cells under parallel conditions. Cytokine production depends on NF-κB, a transcription factor central to inflammation in multiple immune cell types. LT-HSCs had relatively lesser capabilities for cytokine production under these in vitro on-chip conditions, with 0.8 or 7% of single HSCs identified as cytokine producers using the CD150−CD48− LSK or flk2−/−CD34− LSK phenotypic definitions, respectively. Stem cell factor and IL-7, the cytokines detected following enforced expression of Satb1 in HSCs (61), were not among the 12 cytokines assayed in the microchip panel, and they remain to be further investigated. The investigators hypothesized that HSPC-produced cytokines may influence the developmental trajectory of neighboring HSPCs in bone marrow niches. This functional property of HSPCs may be advantageous in the context of infection, because responses to locally sourced cytokine could circumvent the lag time needed to accrue long-distance signals from systemically derived cytokines or from migratory WBCs activated at distal effector sites (16, 68). Within total HSPCs, LT-HSCs bear receptors for IL-6, TNF, and IFN-γ (69–72), whereas total LSKs additionally express the receptor for GM-CSF (73). Studies with neutralizing Abs and gene-knockout mice prompted the investigators to focus on IL-6. In vivo, under a neutropenic regimen, HSPC-derived cytokines enhance myelopoiesis in an IL-6–dependent manner. The revelation of HSPCs as potent cytokine producers raises major new questions about the biological contribution of this pathway to hematopoietic activity under homeostasis and stress. The functional impact of HSPC-derived cytokines is likely to be highest when downstream cytokine-producing cells are depleted due to infection or inflammation.

**Conclusions**

Together, these findings establish the epigenetic modifier Satb1 as a major participant in HSC self-renewal and lineage potential and reveal a new function of ST-HSCs and MPPs as biologically relevant cytokine secretors. Correlative evidence additionally connects Satb1 to the regulation of cytokine gene transcription in LT-HSCs, a finding that is poised for further exploration. These new and unexpected discoveries raise several major questions. First, how is Satb1 itself regulated? At a biochemical level, Satb1 is modified by phosphorylation and acetylation (74, 75) and is susceptible to caspase-mediated cleavage (76). However, little else is known about the pathways that govern expression. Satb1 transcript is reduced in Ikaros-deficient HSCs (77), and whether this regulation is direct or indirect awaits investigation. Likewise, other pathways that influence the developmental stage–specific expression of Satb1 remain to be resolved. Second, unsupervised clustering identified two major cytokine clusters, a lymphoid-associated profile and a myeloid-associated profile (16), raising the prospect that HSPC subsets may contribute to the
functional heterogeneity of distinct bone marrow niches (78). Barcoding technology, recently used to trace the developmental fate of single LMPPs in vivo (79), may be similarly useful for tracking the functional capability of single HSPCs in terms of cytokine potential, lineage potential, and possible relationships between the two. Finally, the discovery of HSCs and MPPs as biologically relevant cytokine producers fundamentally changes our understanding of hematopoiesis. HSPCs are much more versatile than previously appreciated, with the ability to both respond to danger signals and to direct hematopoietic outcomes in a paracrine manner. HSPCs have long been known to circulate through blood and lymph, raising the possibility of TLR-directed hematopoiesis at localized sites of infection (14). Future studies are likely to reveal important contributions of circulating HSPCs not only in serving to locally replenish immune cells consumed by infection but also in coordinating TLR ligand–specific hematopoiesis.

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