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Inflammatory signals regulate hematopoietic stem cells

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Abstract

Hematopoietic stem cells (HSCs) are the progenitors of all blood and immune cells; yet their role in immunity is not well understood. Most prior studies have focused on the ability of committed lymphoid and myeloid precursors to replenish immune cells during infection. Recent studies, however, have indicated that HSCs also proliferate in response to systemic infection to replenish effector immune cells. Inflammatory signaling molecules including interferons, tumor necrosis factor- α , and Toll-like receptors are essential to the HSC response. Observing the biology of HSCs through the lens of infection and inflammation has led to the discovery of an array of immune-mediators that serve crucial roles in HSC regulation and function.

Hematopoietic stem cells respond to infectious stimuli

The hematopoietic stem cell (HSC) is a rare, multipotent cell that gives rise to the many cell lineages that constitute the blood and immune systems (Figure 1). Residing in a specialized niche of the bone marrow, HSCs generally remain in a quiescent, or dormant, state. They can be stimulated, however, under conditions of stress to proliferate and differentiate to replenish cellular progeny lost to cell death or apoptosis. For example, treatment of animals with the chemotherapeutic agent 5-fluorouracil causes the HSC population to expand many-fold¹. Similarly, HSCs proliferate in response to hemorrhage in order to restore the erythrocyte population².

Prior work on the effects of infection on the hematopoietic system has mostly focused on immune effector cells and their immediate progenitors. For instance, severe infection (e.g. Gram negative sepsis or lipopolysaccharide (LPS) treatment) causes T cell apoptosis, and common lymphoid progenitors (CLPs) proliferate as a compensatory response^{3,4}. Thus, changes in the hematopoietic stem and progenitor cell (HSPC) compartment have been viewed as a reactive homeostatic response to replenish immune cells, which is termed “emergency granulopoiesis” or “emergency lymphopoiesis” Changes to the HSPC compartment as a primary or direct response to infection has not commonly been considered.

New evidence, however, indicates that HSCs respond to cytokine signaling directly, *in lieu* of sensing a depletion of downstream cells. Indeed, systemically infected host organisms can

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utilize the HSC to generate an appropriate immune response and maintain homeostatic conditions in the peripheral blood. Recent studies suggest that HSCs might directly sense pathogens via toll-like receptor (TLR) signaling. Furthermore, the cytokines interferon (IFN) α/β , IFN γ , and tumor necrosis factor alpha (TNF α) directly regulate HSC function. In this review, we summarize these recent discoveries that change our understanding of how inflammatory signaling affects the HSC, and we highlight open questions in this complex field.

Hematopoietic progenitor responses are pathogen-specific

Previous studies to examine the effects of infection on HSPC function vary widely in terms of the pathogen utilized and the hematopoietic cell types analyzed (Table 1). Model pathogens used in experimental infections of mice include bacteria as diverse as *Escherichia coli* and *Mycobacterium avium*, viruses such as vaccinia virus, and fungal pathogens such as *Candida albicans*. These pathogens differ in terms of their target cells, infection kinetics and location, and the host immune response elicited. Thus the variation in HSPC response to different pathogens is expected. These studies also vary according to their definition of, and methodology for, isolating HSPCs. Many studies use the designation LSK (lineage marker-, cKit+, Sca-1+) to denote the stem cell compartment, even though this population contains many different cell types, including committed progenitors that are clearly not stem cells^{5,6}. These varied approaches make it difficult to compare one study to another or to make generalizations about the effects of “infection” on “HSPCs.” In addition, the difficulty in phenotypic identification of HSCs in the context of inflammation, and the need for functional verification of flow cytometric findings (see HSC identification text box for further discussion) lend a sense of confusing disarray to the field.

Technical limitations notwithstanding, numerous studies show that HSPC respond to systemic infection by expansion, mobilization, and differentiation. Infection of cultured human HSCs and a murine *in vivo* infection model have both shown that *Escherichia coli* stimulates expansion and mobilization of hematopoietic progenitors (CD34-positive cells in the human; LSK cells in the murine system)⁷⁻¹⁰. Similarly, the LSK compartment is stimulated in models of systemic polymicrobial, viral, and *Candida* infection^{11,12}. Vaccinia virus infection increases immature myelocytes in peripheral blood, suggesting that this infection triggers mobilization and differentiation of common myeloid progenitors due to infection¹¹.

In contrast, some other pathogens diminish HSPC numbers. *Ehrlichia chaffeensis* or *Anaplasma phagocytophilum* infection led to anemia, thrombocytopenia, and reduction in bone marrow cellularity in infected mice^{13,14}. The number of committed progenitors in the bone marrow were reduced, including erythroid and granulocyte/monocyte progenitors^{13,14}. Infectious particles were not detected in the bone marrow, indicating the effects were secondary to systemic infection, rather than from infection of hematopoietic progenitors themselves¹³. *Listeria monocytogenes* infection resulted in reduced numbers of monocyte progenitors and more primitive progenitor populations¹⁵. Similar suppressive effects on committed hematopoietic progenitors in the bone marrow occurred in association with some viral infections. Murine cytomegalovirus infection suppressed the absolute number and cellular proliferation of myeloid progenitors (CFU-GM, BFU-E, CFU-GEMM)¹⁶. Collectively, these suppressive effects on committed hematopoietic progenitors might reflect a skewing of the immune system towards a myeloid or lymphoid response, depending upon the type of infection. Of note, whereas suppressive effects were observed on committed progenitors in these models, the specific effects on pluripotent hematopoietic progenitors were not investigated.

Few studies have investigated the impact of systemic infection specifically on HSPC function. In a polymicrobial sepsis model using cecal ligation and puncture, both long-term and short-term HSC compartments expanded, and long-term HSCs from septic mice retained sufficient reconstitution potential in transplant assays to allow survival of lethally-irradiated recipients¹⁷. Of note, the study did not compare engraftment efficiencies between HSCs from infected and uninfected mice. Similarly, the LSK compartment increased within the first 24 hours of sepsis in mice infected with *Pseudomonas aeruginosa*. *Pseudomonas*-infected mice became neutropenic and displayed reduced bone marrow cellularity, and the LSK compartment of infected animals did not engraft well after transplantation into lethally irradiated recipient mice, indicating a deficit in stem cell function. Thus, the authors concluded that sepsis leads to expansion of LSK progenitors associated with reduced stem cell activity and differentiating capabilities¹⁸.

These findings in *Pseudomonas*-infected mice were consistent with a recent study of the progenitor compartment of mice chronically infected with *Mycobacterium avium*. In this study, the progenitor compartment was defined as the side population (SP) of cells undergoing Hoechst dye 33342 efflux, a population of cells that has been shown to be highly enriched for the stem and progenitor population within bone marrow¹⁹. Expansion of the SP was accompanied by an increase in cell cycle activity and a significant functional impairment in the HSPC compartment as determined by transplantation assays²⁰. It was further demonstrated that this functional deficit occurs at the level of the long-term (LT)-HSC by conducting transplantation assays with highly purified LT-HSCs.

Notably, *Mycobacterium avium* was not detected in HSCs from chronically infected mice²⁰. Similarly, CD34-positive HSCs have been found resistant to infection by *Listeria monocytogenes*, *Salmonella enterica ser. typhimurium*, and *Yersinia enterocolitica*²¹. Thus infection of HSCs themselves is unlikely to explain the numerical and functional changes seen in the cells during infection. Instead, the effects on hematopoietic progenitors, and HSCs in particular, are probably mediated by inflammatory signals generated by the immune system. Furthermore, these inflammatory signals are likely specific to the type of pathogen. For example, *E. coli* stimulates LSK cells through lipopolysaccharide (LPS) via TNF α and NF κ B signaling⁷⁻¹⁰. Likewise, vaccinia virus and *Candida albicans* infection both lead to LSK expansion via a MyD88-dependent pathway, implicating TLR signaling in this process^{11,12,22}. The effects of these pathways on the HSC are just beginning to be understood, as outlined in the sections that follow.

IFN signaling stimulates HSC proliferation

IFNs have emerged as a major axis for HSC regulation^{20,23,24}. Initially discovered as factors that impair viral replication²⁵, the IFNs are subdivided into two groups: type I IFN, including IFN α and IFN β , which bind the IFN α receptor, and the type II IFN γ , which binds the IFN γ receptor^{26,27} (Figure 2). Type I IFNs are induced by viral infection and can be synthesized by most types of virally-infected cells²⁸. In contrast, Type II IFN is induced by mitogenic or antigenic stimuli, but can only be produced by some immune effector cells such as NK and T cells^{28,29}. Type II IFN coordinates the immune response against a wide variety of intracellular pathogens including some viral infections, *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Leishmania major*²⁹.

Two studies recently reported that treatment of mice with IFN α stimulates dormant HSCs *in vivo*^{24,30}. Treatment of mice with poly(I:C), a synthetic double-stranded RNA that mimics viral infection, activated HSCs to proliferate in an IFN α -receptor-dependent fashion²⁴. Additionally, injection of mice with purified IFN α stimulates HSCs to enter the cell-cycle via both direct and indirect mechanisms, as demonstrated by treating mice chimeric for

wild-type and *Ifnar1*^{-/-} bone marrow. These effects were dependent upon intact *Stat1* signaling, indicating that IFN α signals the HSC to proliferate via its canonical pathway²⁴. Confirming these findings, the IFN α -dependent transcriptional regulator *Irf2* was found to be a critical factor for limiting excessive IFN signaling in HSCs^{30,31}. HSCs from *Irf2*^{-/-} mice exhibit impaired function in transplant assays, a defect rescued by the addition of a mutation in the *Ifnar1* locus. These results indicate that *Irf2* is a negative regulator of IFN α signaling in the HSC, and that chronic unabrogated IFN α signaling can lead to HSC exhaustion^{30,31}. Thus, it appears that IFN α/β is an important molecule to stimulate the HSC to proliferate in response to viral infection, but excessive signaling through this pathway might be detrimental to HSC function.

Based upon both *in vitro* and *in vivo* studies, IFN γ has been classically understood as a hematopoietic inhibitor. IFN γ maintains homeostasis of downstream immune effector cells such as activated T cells and myeloid cells during infection by inhibiting their overproduction^{32,33}. With regard to HSCs, IFN γ suppresses human bone marrow colony formation^{34,35} and inhibits growth of human CD34⁺ bone marrow cells by inducing differentiation and apoptosis^{36-39;40 41}. Similarly, IFN γ attenuates survival of the immortalized myeloblast-like 32D cell line⁴², and it induces differentiation of a chronic myeloid leukemia (CML) cell line and CD34⁺ cells from CML clinical samples⁴³. IFN γ is thought to be a major contributor to some bone marrow failure syndromes such as acquired aplastic anemia in humans^{44,45}. Several studies, however, found alternate effects of IFN γ on the HSC. *In vitro* IFN γ treatment increases viable cells and colony-formation from human CD34⁺ cells^{46,47}, and in one study, was found to stimulate growth of blood-derived hematopoietic progenitors but inhibit growth of those derived from bone marrow⁴⁸.

In contrast to its previously understood role, recent publications show that IFN γ , like type I interferons, can stimulate the HSC to proliferate. Upon exposure to IFN γ , the bone marrow LSK compartment expands and has greater colony-forming potential²³. Furthermore, IFN γ biases the progenitor compartment towards myeloid differentiation. In a *Plasmodium chabaudi* murine model of malaria infection, a myelolymphoid IL-7Ra⁺ cKit^{hi} progenitor is generated in response to IFN γ stimulation after infection. Chimeric transplantation and infection experiments indicated that IFN γ is a major modulatory cytokine in the development of these cells, which can go on to produce myeloid cells that restrict parasite abundance during the infection⁴⁹.

As already discussed, *Mycobacterium avium* infection stimulates HSCs to proliferate and differentiate in an *Ifngr1*- and *Stat1*-dependent fashion²⁰. This study showed that like IFN α , IFN γ also stimulates murine HSCs proliferation *in vivo*²⁰. HSCs of IFN γ -deficient animals are more quiescent and perform better in transplantation assays, indicating that IFN γ signaling is also important for HSC function under homeostatic conditions²⁰. We suspect that IFN α and IFN γ serve redundant functions in HSC activation, as they are differentially induced based upon the type and chronicity of the infection but act through related pathways (Figure 2). Additional studies examining the interplay between IFN α and IFN γ in regulation of the HSC will be necessary to fully appreciate their individual roles in this process. Collectively, these reports show IFNs can directly stimulate HSCs; and although intact IFN signaling is critical for a proper host response, excessive chronic signaling can have negative effects on HSC function.

Indeed, HSCs might be driven to exhaustion by chronic IFN stimulation, leading to HSC anergy or death over the long-term²⁰. The effects of excessive IFN signaling have been linked to several clinical scenarios in humans. For example, polymorphisms associated with high production of IFN γ are more frequent in patients with aplastic anemia (AA), a syndrome characterized by pancytopenia and bone marrow hypocellularity, or paroxysmal

nocturnal hemoglobinuria (PNH), a syndrome of red blood cell breakdown associated with anemia^{44,45}. CD34+ HSCs from patients with these diseases show increased IFN signaling by microarray profiling⁴¹. Myelodysplastic syndrome (MDS), an HSC disorder consisting of ineffective hematopoiesis, cytopenias, and bone marrow failure, has also been associated with increased IFN signaling⁵⁰. Further study of these human syndromes will be necessary to understand the mechanistic contribution of IFN to HSC incompetence in bone marrow failure syndromes.

TNF α : a key HSC regulator?

Tumor necrosis factor alpha (TNF α) is a major proinflammatory cytokine first identified as a serum-derived factor causing necrosis of tumor cells⁵¹. When exposed to endotoxin or other bacterial antigens, stimulated macrophages produce TNF α , which mediates its signaling effects via two distinct receptors. The p55 receptor for TNF α (TNFRSF1A) is expressed on all nucleated cells, while the p75 receptor (TNFRSF1B) is present only on cells of hematopoietic lineages. TNF α binding activates nuclear factor- κ B (NF- κ B) and ATF2 to promote transcription of a wide range of inflammatory cytokines, chemokines, and proteases^{52,53} (Figure 2).

TNF α might also mediate important effects on the HSC. Like IFN γ , TNF α has been classically understood to inhibit growth and induce apoptosis of HSCs. TNF α was found to inhibit colony-formation of human bone marrow^{35,54}, and to inhibit *in vitro* growth of CD34+ human cells as well as their ability to reconstitute NOD-SCID mice^{34,55}. Additionally, TNF α inhibits proliferation of murine HSCs *in vitro*⁵⁶⁻⁵⁸. Some reports suggest that TNFRSF1A and TNFRSF1B play differential roles in mediating this inhibition, as use of agonistic antibodies against these receptors indicates that TNFRSF1A exclusively mediates the effects of TNF α upon committed progenitor cells, whereas TNFRSF1B is necessary to signal inhibition of primitive progenitors^{54,55,57}. One recent study, however, suggests an opposite effect for this cytokine; TNF α produced by CD8+ bone marrow-derived cells enhanced HSC clonogenicity, increased the proportion of multipotent progenitors *in vitro*, and prevented HSC apoptosis *in vitro* and *in vivo*⁵⁹. Further, *in vivo* treatment of mice suggests that TNF α stimulates peripheral mobilization of lymphocytes and bone marrow expansion of granulocytes, an effect that is abrogated in TNF α -/- mice⁶⁰. These results indicate that TNF α may have stimulatory effects on hematopoiesis in some contexts, similar to the IFNs.

Studies utilizing *Tnfrsf1a*-/- mice have shown that the TNF α pathway might be crucial for long-term HSC function. Unlike *Tnfrsf1b* knock-outs, *Tnfrsf1a*-/- mice have increased numbers of hematopoietic progenitors at baseline and develop increased marrow cellularity with age^{56,61}. This increase in progenitors corresponds to a relative decrease in HSC function, as whole bone marrow transplants show a loss of competitive repopulation ability and secondary transplants indicate impaired self-renewal of knockout HSCs^{59,61}. Thus it appears that TNF α signaling through TNFRSF1A is critical for the maintenance of HSCs. This finding contributes to a growing body of evidence in the field that baseline inflammatory signaling can be critical for the maintenance of proper HSC function, perhaps by providing just enough stimulation for baseline HSC cell division.

Like IFN, excessive TNF α signaling is associated with MDS and bone marrow failure. MDS patients exhibit increased TNF α mRNA and downstream signaling changes consistent with increased TNF α ^{50,62}. Polymorphisms in the TNF α promoter region that cause higher expression are also associated with MDS⁶³. Interestingly, the decreased HSC function associated with aging may also be due to increased TNF α inflammatory signaling and NF κ B protein activation⁶⁴⁻⁶⁶. We suspect that, like IFN γ , TNF α plays a major regulatory role in

the HSC response to infectious pathogens. However, further studies with highly purified HSCs are necessary to elucidate these effects of TNF α *in vivo*.

TLRs: do HSCs directly sense pathogens?

The Toll pathway was discovered in *Drosophila*, and represents the main antimicrobial defense pathway in flies⁶⁷. The thirteen TLRs identified in mice and humans play a critical role in the innate immune response by directly and specifically sensing microbial pathogens⁶⁸. TLRs generally function as homodimers, though heterodimeric binding has been reported, and they largely use the adaptor protein myeloid differentiation primary response 88 (MyD88) to activate production of inflammatory cytokines⁶⁸ (Figure 2). The TLR, IFN, and TNF α pathways can all lead to proinflammatory responses through NF- κ B signaling (Figure 2).

The Toll/Cactus pathway regulates hemocyte proliferation and density in *Drosophila* larva, as mutants in this pathway exhibit dysregulated hematopoiesis⁶⁹. It appears that the role of TLRs in regulating HSCs may extend to mammals, as murine and human hematopoietic progenitors have recently been found to express TLRs⁷⁰⁻⁷³. Murine progenitors express bacterial component-sensing receptors TLR2 and TLR4, and stimulation of these receptors provokes cell cycle entry and myeloid differentiation *in vitro* in a MyD88-dependent fashion⁷⁰. Human CD34+ progenitors express TLR4, TLR7, TLR8 and TLR9, and stimulation of TLR7 and TLR8 *in vitro* causes myeloid differentiation of these progenitors⁷¹⁻⁷³.

The expression of TLRs on HSCs suggests a possible early sensing mechanism for HSCs to directly detect infection and respond with immediate production of immune effector cells. Indeed, several of the infectious models described earlier (Table 1), including *Pseudomonas*, implicate intact TLR signaling as necessary to the HSC response to systemic infection¹⁸. Similarly, LPS, the most commonly studied ligand for TLR signaling, appears to stimulate the HSC *in vivo*. LPS induces an increase in HSC cycling as measured by spleen colony formation⁷⁴, increased colony-forming-units in blood, bone marrow, and spleen⁷⁵, and mobilization of cells out of the bone marrow⁶⁰. These effects are likely mediated by a combination of signaling pathways. For example, endotoxemia has been found to cause increased granulocyte colony-stimulating factor (G-CSF), a cytokine that is known to stimulate the bone marrow to produce myeloid cells⁷⁵. Additionally, there may be functional cross-talk between the TLR pathway and Wnt signaling, known to be critical for HSC maintenance, as they exhibit opposite effects on VCAM-1 expression on hematopoietic cells⁷⁶. It remains unclear whether direct HSC stimulation via TLRs occurs *in vivo*. Further studies are necessary to understand the penetration of TLR agonists into the bone marrow where the HSC resides and the specific contribution of TLR signaling to the HSC response to infections.

Interestingly, like IFN γ , TLR signaling may also play a role in HSC function under physiological conditions. HSCs from mice mutant for *Tlr4*, *Tlr9*, or *Myd88* exhibit an advantage in competitive transplant assays, though HSCs from *Tlr2*^{-/-} mice perform similarly to wild-type⁷⁷. It will remain to be seen whether these results are secondary to physiological modulation of HSCs by endogenous microbiota or if they are an artifact of the immunomodulatory effects of TLRs on HSCs in the inflamed environment of the lethally irradiated transplant recipient. Because TLR stimulation of downstream immune cells also causes production of powerful inflammatory cytokines like IFNs and TNF α , some effects attributed to TLR signaling may instead reflect indirect methods of HSC stimulation. Studying the effects of TLR stimulation on HSCs in mouse mutants lacking components of these other inflammatory pathways would offer insight into this mechanism.

Summary and future directions

Collectively, these studies indicate that the HSC directly responds to infection via multiple pathways. The pathways discussed here are critical to both immune effector cells and the HSC. Indeed, circulating cytokines appear to reach HSCs in their niche via the systemic circulation, leading to cell-autonomous effects including proliferation, proliferation, and mobilization (Figure 3). While initial studies have given us improved insight into the effects of infection upon the HSC, many questions remain. Whereas inflammatory cytokines appear to stimulate HSCs to proliferate in the short-term, little is known about the long-term effects of sustained inflammation. Might bone marrow failure syndromes seen in association with inflammatory signaling be the result of sustained effects that ultimately lead to apoptosis, exhaustion, or anergy? Alternatively, are gradients in cytokine levels enough to produce different outcomes – will increased proliferation of hematopoietic progenitors occur at some levels but apoptosis and hypocellularity at others? Is the HSC response to infection mediated only by systemic cytokine stimulation, or is there also a local signaling component mediated by direct interaction of HSCs with pathogens or effector cells? As this field progresses, the answers to these questions will offer invaluable insight into the role of the HSC in the stress response to infection and, conversely, into the role of inflammatory signals on HSC regulation.

The finding that HSCs respond to infectious stress has many implications. The studies described herein point towards a previously unrecognized role for HSCs in restoration of homeostasis in the blood system during infections. Further, these studies indicate that there are functional consequences for HSCs as they respond to chronic inflammatory stimuli. Specifically, HSCs appear to divide more frequently in response to infection, but subsequently perform less well in reconstitution experiments, indicating that there is a cost to the HSC in responding to infection. Understanding these consequences might elucidate the pathophysiologic mechanism behind cytopenias seen in the context of many chronic infections. Finally, there is a growing cohort of patients who receive immunomodulatory therapy, such as IFNs or anti-TNF, for conditions including hepatitis C, polycythemia vera, or autoimmune diseases. In order to optimize the use of such therapies, it is important to better understand the consequences of such immunomodulation on the function of hematopoietic progenitors.

Hematopoietic stem cell identification in the context of inflammation

Purification of long-term (LT)-HSCs, which make up only ~0.01-0.001% of total nucleated cells in the bone marrow, poses challenges even in the absence of HSC stressors. Several alternate schemes have been classically used to identify and purify LT-HSCs in the absence of infectious challenge, as reviewed in⁷⁸. The stem cell compartment can be enriched by identification of LSK (lineage marker-, cKit+, Sca-1+) cells, but only around 10% of these cells are LT-HSCs. The remainder includes short-term (ST)-HSCs, with the capacity to differentiate into all hematopoietic lineages over a relatively short period of time (~4 weeks), in addition to a variety of lineage-specific progenitors⁷⁸. Therefore, data from studies on LSK cannot distinguish between different responses by individual cell types within the population. The addition of further cell surface markers can help to differentiate between LT-HSC (Flk-2⁻CD34⁻LSK) and ST-HSC (Flk2-CD34+LSK) or MPP (Flk2+CD34+LSK)^{79,80}. Alternately, HSCs can be highly enriched by identification of the side population (SP) based on HSC-specific efflux of the vital DNA binding dye Hoechst 33342^{19,81}, a strategy which can be combined with LSK to identify LT-HSCs as SP^{KLS}. Finally, HSCs can be enriched using the SLAM markers (CD150⁺CD244⁻CD48⁻), best also used in conjunction with LSK⁸²,

⁷⁸. A variety of different marker combinations have been used to obtain similarly enriched LT-HSCs.

In analyzing HSCs in the context of inflammatory signaling, however, HSC identification becomes substantially more complex. Expression of the canonical stem cell marker Sca-1 is induced in hematopoietic cells by both IFNs and TNF α ^{83,84}, causing non-HSCs to be misinterpreted as HSCs during infection. Additionally, when HSCs are stimulated to proliferate after 5-fluorouracil treatment, the cKit marker is downregulated and Mac-1, one of the lineage markers, is upregulated ⁸⁵. Since infection appears to simulate an HSC response similar to the stress response to 5-fluorouracil, stem cell markers are also likely to be affected by infection, thus impacting the choice of markers and potentially leading to erroneous identification of HSCs. The difficulty of clear delineation between LT-HSC and ST-HSC in this context is compounded because these pools may be in a state of interchange, secondary to the proliferative stimuli, regardless of their marker status. We simply have a poor understanding of the relationships between these stem and progenitor cells in the context of proliferative stimulation. In the future, it will be critical to ensure that any claims regarding HSC status post-infection be validated using multiple phenotypic criteria and functional studies. HSPC populations should be assessed not only by flow cytometric analysis of cell surface markers but also by cell growth or, the gold standard, the ability to reconstitute bone marrow upon transplantation into a marrow-depleted recipient animal.

Outstanding Questions

What are the long-term functional consequences of inflammatory cytokine signaling on HSCs?

What is the impact of immunomodulatory therapy on HSC function?

What is the extent of the exposure of HSCs to cytokines in the bone marrow niche?

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Figure 1. Hematopoietic stem cells give rise to all the cell lineages of blood

Upon cell division, HSCs can achieve a variety of cell fates, including self-renewal or differentiation into cells with a more restricted differentiation potential. Long-term hematopoietic stem cells (LT-HSCs) are the most primitive, with a single cell able to entirely reconstitute the blood of an organism for an extensive period of time. Short-term HSCs (ST-HSCs) are similarly pluripotent, but their longevity is limited to a few months in the murine system⁸⁶. ST-HSCs give rise to multipotent progenitors (MPP), which are pluripotent cells that lack long-term reconstitution capability⁵. The MPPs in turn differentiate into precursors that are committed to the lymphoid or myeloid lineages. These precursors, termed the common lymphoid progenitor (CLP) and common myeloid progenitor (CMP), represent the first major branching of the hematopoietic tree. CLPs give rise to B, T, and NK cells. CMPs give rise to the committed progenitor cells MEPs (megakaryocyte-erythroid progenitors) and GMPs (granulocyte-macrophage progenitors), which give rise to downstream differentiated progeny. As an example of the shifts that can occur in hematopoiesis with infection, those alterations that occur with chronic *Mycobacterium avium* infection are demonstrated²⁰. The cell populations that increase with infection are shown with a green background, and those that decrease are shown with a red background.

Figure 2. Inflammatory cytokine signaling pathways promote transcriptional changes to drive immune responses in hematopoietic cells

IFN α/β and IFN γ activate parallel signaling pathways. IFN α/β binding to the type I IFN receptor activates TYK2 and JAK1 to phosphorylate STAT1 and STAT2, which heterodimerize, associate with IRF9, and bind to ISREs to activate transcription. Signaling through the type II IFN receptor occurs in a parallel pathway. When IFN γ binds its receptor, associated JAK1 and JAK2 kinases phosphorylate STAT1, which homodimerizes and translocates to the nucleus for transcriptional activation at GAS sequences. The TNF α and TLR signaling pathways act similarly to promote transcriptional changes; these pathways exhibit extensive crosstalk. TNF α receptor binding causes TRADD to recruit RIP and TRAF2, which activate IKK to phosphorylate I κ B α , thereby releasing NF- κ B. TNF α receptor signaling also activates MEKK1, which causes JNK to stimulate AP-1 and ATF2. AP-1 binds TPA DNA-response elements (TRE), while ATF2 binds cAMP-responsive elements (CRE). Infectious particles activate TLRs to signal through MyD88, which recruits IRAK which binds TRAF6 and activates NF- κ B and JNK pathways.

Figure 3. HSCs can be stimulated by cytokines via the systemic circulation

Upper panel: HSCs (blue) reside in the bone marrow niche amidst stromal cells (purple), separated from the bone matrix by osteoblasts (orange). Blood and circulating cytokines reach the bone marrow via small sinusoidal vessels. Lower panel: When systemic stresses, such as anemia or infection, stimulate production of cytokines, these factors travel in the blood to the HSC niche and can stimulate the HSC to proliferate, mobilize into the circulation or to differentiate into committed progenitors and immune effector cells.

Table 1

Pathogen	Host	Cell types examined	Mediators	Ref
Stimulatory Effects	<i>Escherichia coli</i>	Cultured human HSCs		
	<i>Escherichia coli</i>	Human CD34+	NF- κ B	8
	Polymicrobial	LSK	Plasma G-CSF, GM-CSF	7,9
	Vaccinia virus	CFU-GEMM, CD150-Flk2-LSK, CD150-Flk2+LSK	LPS, TNF α , IFI β , IFN γ	87,17
	<i>Candida albicans</i>	CMP, Lin-Sca1+Kit-	MyD88, TLR	88
	<i>Pseudomonas aeruginosa</i>	LSK		12,22
Inhibitory Effects	Thermal injury mouse model	LSK—expansion of HSCs with reduced functional capacity	TLR4	18
	<i>Mycobacterium avium</i>	SPKSL, KSL CD34-Flk2-	IFN γ	20
	<i>Listeria monocytogenes</i>	Monocyte precursors (cKit+CD115+ Fc γ R+CD127-)	MyD88, TLR	89
	<i>Ehrlichia chaffeensis</i>	Granulocyte /monocyte progenitors	CXCL12	90
	<i>Anaplasma phagocytophilum</i>	CFU-GM, BFU-E	TNF α , IL6	13
	Murine cytomegalovirus	CFU-GM, BFU-E, CFU-GEMM	NK cells	16