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Inflammatory modulation of hematopoietic stem cells: viewing the hematopoietic stem cell as a foundation for the immune response

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Preface

Cells of the innate and adaptive immune systems are the progeny of a variety of hematopoietic precursors, the most primitive of which is the hematopoietic stem cell. Hematopoietic stem cells have been thought of generally as dormant cells that are only called upon to divide under extreme conditions, such as marrow ablation through radiation or chemotherapy. However, recent studies suggest that hematopoietic stem cells respond directly and immediately to infections and inflammatory signals. In this Review, we will summarize the current literature regarding the effects of infection on hematopoietic stem cell function and how these effects may have a pivotal role in directing the immune response from the bone marrow.

Introduction

Infection is a common natural stressor on the hematopoietic system. Immune cells are consumed in the fight against invading pathogens, either by mobilization to sites of infection or by apoptosis¹. Homeostasis within the hematopoietic system depends on replacement of these immune effector cells by hematopoietic precursors. For example, common myeloid progenitors (CMPs) in the bone marrow and peripheral blood can quickly produce large numbers of neutrophils in response to sepsis². The contribution of the most primitive of the hematopoietic progenitors, the hematopoietic stem cell (HSC), to hematopoietic homeostasis during infection, is a new area of investigation.

HSCs are rare, multipotent cells capable of generating all of the cells of the blood and immune systems over the lifespan of an organism. Usually residing in a quiescent state in the bone marrow, HSCs represent a reservoir of pluripotency that replenishes other hematopoietic populations as they are depleted by age or use. The factors that determine how the HSC population is maintained over the lifespan of an organism, or those that trigger differentiation into mature hematopoietic cell types, are the subject of ongoing investigation.

Recent studies, detailed in this review, have changed our fundamental understanding of HSC biology. These studies indicate that HSCs truly are first responders to infection, and that proinflammatory cytokines released during infection are critically important to HSC regulation. In the simplistic view, this cascade of proinflammatory cytokines may include: tumour necrosis factor α (TNF), interleukin-1 (IL-1) and IL-8 to activate dendritic cells, macrophages and neutrophils; IL-2 and interferon α (IFN α), IFN β and IFN γ to activate T cells and natural killer (NK) cells; and IL-4 and IL-6 to activate B cells. Aside from canonical effects on immune effector cells, these immune activators also mediate important changes in hematopoietic stem cell biology. Indeed, proinflammatory cytokines appear to be required for maintenance of the appropriate number, proliferation and differentiation of HSCs, both under homeostasis and in response to stress. Furthermore, dysregulation of such inflammatory cytokines and the consequent impact on the earliest hematopoietic progenitors may be a major contributor to hematological abnormalities in aging, cancer and bone marrow failure syndromes.

HSCs as a foundation for the immune response

Even though HSCs are long-lived and frequently dormant³, recent studies suggest that HSCs participate directly in the primary response to both acute and chronic infections. In one study, mice were infected with *Escherichia coli* by intrapulmonary injection, and the bone marrow was collected at subsequent time points for phenotypic analysis of hematopoietic cell types. Infection of mice led to an expansion of bone marrow lineage-negative SCA1⁺KIT⁺ (LSK) cells, a loose collection of hematopoietic stem and progenitor cells (HSPCs), even in the absence of a period of leucopenia (see Box 1)⁴. Notably, the stimulatory cytokine G-CSF was elevated in response to infection⁵. These findings suggest that bone marrow progenitors respond to the infection itself, rather than as a secondary response to peripheral cytopenia. Similar stimulatory effects on the LSK compartment have been observed in polymicrobial, viral, and Candidal models of systemic infection^{6–8}.

BOX 1

A word about HSCs

HSCs are defined experimentally in a variety of ways; hence it is useful to briefly discuss these definitions. HSCs are rare cells that exist at a frequency of less than 0.01% of bone marrow leukocytes and cannot be propagated by *in vitro* culture without differentiation. The gold standard for identification of an HSC is to conduct bone marrow transplantation and demonstrate generation of all blood lineages for at least 16 weeks in mice. An assay that can be used as an adjunct is to test the ability of bone marrow cells to form colonies in methylcellulose culture medium; however this method does not differentiate between HSCs and committed progenitor cells.

Transplantation or methylcellulose culture is impractical for many forms of experimental manipulation, so surrogate methods have been developed. Most commonly, mouse HSCs are defined immunophenotypically using cell surface markers. A cocktail of antibodies specific for antigens, termed ‘lineage markers’, that are characteristically expressed by differentiated blood cells can be used to separate out mature hematopoietic cell types.

The lineage marker-negative SCA1⁺ KIT^{hi} (LSK) fraction of bone marrow cells is enriched for hematopoietic stem and progenitor cells (HSPCs). Although the LSK population is commonly studied, it is heterogeneous and includes multipotent progenitors and other committed progenitors that do not maintain long-term reconstitution potential⁵⁵. In fact, only approximately one-tenth of LSK cells are HSCs⁵⁵. HSCs are more specifically selected within LSK cells based on expression of SLAM family members (CD150⁺ CD48⁻)⁵⁶ or by selection of the CD34⁻, FLT3⁻ fraction of LSK cells⁵⁷. Use of cell surface markers is convenient and allows manipulation of cells by flow cytometry. However, one must be aware of changes in cell surface marker expression due to environmental conditions that can change the population represented by those markers. For instance, SCA1 expression is known to be upregulated by interferon-gamma (IFN γ)⁵⁸. Therefore, unless definitions are strictly employed, the expression of SCA1 can be misleading as a marker for the HSPC population. As an alternative or adjunct to immunophenotypic methods, HSCs can also be isolated based on functional properties such as their ability to efflux Hoechst 33342 or rhodamine-123 dye⁵⁹. The so-called 'side population' of cells that efflux Hoechst or rhodamine-low cells are highly enriched for HSCs and can be further purified with additional selection of cell surface markers. These dye efflux methods are potentially less susceptible to environmental changes or antibody avidity to cell surface markers^{60,61}.

The literature regarding HSPC responses to infection is highly contradictory, in part due to the lack of uniformity in which methods and, hence, which cell types are being examined. Clarity will be gained by studies that carefully define the HSPC subpopulation being addressed.

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1: hematopoietic tree; 2: "The push:" HSCs divide in direct response to infectious stimuli (including pathogen-associated molecular patterns (PAMPs) or inflammatory cytokines elicited by the pathogen versus "the pull:" HSCs divide in response to a depletion in committed progenitor populations in the bone marrow; 3: Infections may influence HSC biology by a number of potential mechanisms. Infection of HSCs themselves may occur; pathogen-derived products may be sensed by HSCs; proinflammatory cytokines may signal to HSCs; and changes in cell-cell interactions in the niche may occur in response to infection; 4. Inflammatory signals likely promote differentiation of HSCs at the expense of self-renewal activity, leading ultimately to depletion of the HSC population.

Recently, inflammatory conditions have been shown to increase proliferation and self-renewal among LSK cells. The rate and extent of cell division can be determined precisely by measuring the dilution of a fluorescent dye (5-6-carboxyfluorescein diacetate succinimidyl ester, or CFSE), over time. When mice containing CFSE-labeled bone marrow cells were exposed to lipopolysaccharide (LPS) over an 8-day period, LSK cells were noted to expand in number and to display accelerated dye dilution, indicating a higher rate of cellular proliferation. These findings suggest that inflammation triggered by LPS exposure can activate proliferation of hematopoietic progenitors^{9,10}.

Studies focusing on the LSK compartment of bone marrow are limited by the inherent heterogeneity of that cell population, as well as effects of inflammation on common phenotypic stem cell markers (See Box 1). Since the LSK compartment is a diverse mixture of HSCs and committed progenitors, it is erroneous to infer that effects on the LSK compartment apply to the HSC subpopulation. In order to delineate effects of inflammation in HSCs, we recently reported results of bone marrow analysis following *Mycobacterium avium* infection using more discriminating purification schemes. By including additional immunophenotypic criteria such as CD34⁻, CD48⁻, CD150⁺ and FMS-related tyrosine kinase 3 (FLT3, also known as FLK2)⁻ or the functional capacity to efflux the vital dye Hoechst 33342 to identify the so-called ‘side population’, it is possible to exclude committed progenitor populations and enrich more specifically for HSCs (see BOX 1). Using these techniques, we found that HSCs proliferated in response to chronic *M. avium* infection¹¹. Notably, *M. avium* infection leads to a chronic indolent infection during which peripheral blood counts, as well as the numbers of committed progenitors, are never very low throughout the 4-week course of infection. Thus, the finding of increased proliferation of HSCs in this infection implies that HSCs divide as part of the primary immune response rather than simply to replace depleted progeny pools. In other words, in addition to the “pull” effect that peripheral cell deficiencies may have on HSCs, they can also be “pushed” towards cell division and differentiation by infectious states.

HSCs may sense infectious agents directly

Having established that HSCs respond to infectious states even in the absence of peripheral cytopenia, we turn to the question of how the infectious state is sensed by the HSC. One may imagine a number of different scenarios: the HSC itself may be infected, leading to direct effects on intracellular organelles. Alternatively, pathogenic organisms may signal directly to the HSC, such as by releasing pathogen-associated molecular patterns (PAMPs) that bind to receptors contained in or on the HSC. A final possibility is that alterations in the HSC environment as a result of infection, including the cellular composition or the cytokine environment, may lead to indirect changes in the HSC. These indirect changes could be mediated by a broad range of factors such as cell-cell interactions, miRNAs, and epigenetic factors. Certainly, the above-mentioned scenarios are not mutually exclusive.

The possibility that HSCs are affected by direct invasion of pathogenic bacteria has not been borne out experimentally. In animal models, HSCs are not commonly infected by pathogens themselves. Even when animals are systemically infected, or when purified HSCs are incubated with high concentrations of bacteria *in vitro*, HSCs have been found to be resistant to infection by a variety of intracellular pathogens including respiratory viruses, Salmonella, Listeria, and Yersinia^{12,13}. In our studies utilizing *M. avium* infection, we have not observed viable bacteria within the HSCs. Hence, changes in the proliferative rate of HSCs or their capacity to engraft and repopulate bone marrow following transplantation are probably not attributable to the presence of pathogens in the intracellular space.

On the other hand, hematopoietic progenitors do possess the machinery to directly sense infectious particles. Toll-like receptors (TLRs), the major cell surface receptors for detecting pathogen associated molecular patterns (PAMPs), are present on the surface of

hematopoietic progenitors¹⁰. Ligation of TLR4 on early hematopoietic progenitors leads to proliferation and differentiation of these cells, with increased differentiation of common myeloid progenitors and preferential differentiation of lymphoid progenitors into dendritic cells¹⁰. TLR4 is the main receptor for LPS, and TLR4 was required for expansion of the LSK bone marrow compartment in mice infected with *Pseudomonas aeruginosa*¹⁴. Furthermore, long term stimulation of TLR4 led to impaired long-term self-renewal and lower CLP numbers in mice treated with low dose LPS¹⁵. Studies addressing the cell autonomous functions of TLRs on HSPCs and the degree to which HSCs react in a TLR-specific way to different infectious agents will be of great interest in the future.

HSC responses to infection may also be mediated by changes in the immediate microenvironment. A range of studies has indicated that HSC fate is strongly influenced by other cells in its specialized microenvironment, termed the HSC niche. For instance, retention of the HSC to an area close to an osteoblast, which produces important regulatory cytokines, may be required for maintenance of HSC quiescence¹⁶. Infection likely leads to changes in the HSC niche that contribute to changes in HSC biology. For instance, exposure to TLR ligands suppresses maturation of osteoclasts in the bone marrow niche, a condition which may lead to mobilization and differentiation of HSPCs^{17,18}. Similarly, G-CSF represses CXCL12 production by osteoblasts and leads to increased mobilization of HSPCs¹⁹. Complement activation can lead to HSC mobilization indirectly by promoting granulocyte egress^{20,21}. Recently, bone marrow-resident macrophages have been shown to govern HSC retention within the bone marrow niche²². It is interesting to speculate that these macrophages may be recruited to sites of infection and that egress of these cells from the HSC niche may, secondarily, result in HSC proliferation and mobilization. These questions remain experimentally untested.

Indirect HSPC responses to infection are mediated by inflammatory cytokines

Aside from direct sensing of pathogens, quantitative changes in the HSPC compartment and in HSC activity in response to infection appear to be mediated by inflammatory cytokines. Expansion of LSK cells in response to *E. coli* infection is mediated by LPS via TNF and nuclear factor kappa B (NF κ B) signaling⁵. In a recent study, HSCs (defined as FLT3⁻ CD150⁺CD48⁻ CD34⁻ LSK cells) were found to proliferate and to demonstrate functional deficits due to combined exposure to IL-6, TNF and the chemokine CCL2, all of which are proinflammatory cytokines induced by LPS exposure²³. Inhibition of these three cytokines rescued the quantitative and functional effects of LPS treatment on HSCs. The finding that ablation of a single signaling pathway during polymicrobial sepsis, such as induced by cecal ligation and puncture, indicates that there are probably multiple redundant cytokine pathways for HSC activation during infection²⁴.

In our recent study using *M. avium* infection in mice, we reported that HSC activation could be triggered by IFN γ signaling. Further, this activation could be recapitulated by short-term exposure of purified HSCs to IFN γ *in vitro*, showing that HSC activation can be triggered directly by an interferon-mediated immune response to infection, regardless of the chronicity of the infection or the presence of other cell types¹¹. The finding that IFN γ can

activate HSC proliferation was surprising in light of long-standing studies showing that patients with bone marrow failure syndromes frequently have high IFN γ levels²⁵. Very high IFN γ levels are thought to trigger apoptosis of hematopoietic cells, leading to hypocellularity of the bone marrow. Indeed, exposure of human CD34⁺ HSCs to IFN γ can lead to expression of pro-apoptotic genes in *in vitro* studies²⁶. Furthermore, IFN γ exposure leads to reduced colony formation in cultured human bone marrow cells^{27,28}.

However, apoptosis may not be the only mechanism for bone marrow failure in the setting of elevated IFN γ levels. A number of studies suggest that the level of interferon as well as the particular cell type under examination may be pivotal for the nature of the response. For example, two groups found that *in vitro* IFN γ treatment caused an increased number of viable cells and colonies formed from human CD34⁺ cells^{29,30}. Similarly, hematopoietic progenitors were stimulated to proliferate and have increased colony-forming ability in a mouse model of Plasmodium infection³¹ or when mice were injected with recombinant IFN γ ³². In our studies, *in vitro* treatment of mouse HSCs with IFN γ led to increased cellular proliferation, rather than apoptosis¹¹. Treatment of mouse HSCs with IFN α or polyinosinic-polycytidylic acid (polyI:C), which triggers an IFN α response, has a similar activating effect³³. Indeed, experiments using chimeric mice with HSCs isolated from wild-type mice and mice lacking the IFN α receptor indicated that HSCs could be selectively activated via IFN α signaling. These findings highlight the direct, cell-autonomous impact of interferon signaling on HSC function. Indeed, the weight of evidence indicates that IFNs are activating rather than inhibitory for highly purified HSCs, although this may not be true for more differentiated hematopoietic progenitors. Further studies are required to understand the potential interaction between types I and II IFN in HSC activation, as well as the dual action of inhibitory and activating effects. The duration and context of interferon exposure on HSCs are likely to be pivotal in guiding HSC responses; and these factors are also important areas for future investigation.

The potency of interferons in mediating HSC activation is underscored by the finding that stringent regulation of the interferon response is required for normal bone marrow function. When type I interferon responses are excessive, as in interferon-regulatory factor 2 (IRF2)-deficient mice, HSCs hyperproliferate and slowly decline in number over time, suggesting that overexposure to interferon signaling impairs HSC self-renewal processes^{33,34}. Similarly, mice lacking the adenosine to inosine editing enzyme ADAR1 display global upregulation of types I and II interferon-inducible transcripts and loss of HSC populations³⁵. This phenomenon also occurs in mice lacking immunity-related GTPase M (IRGM1); these mice display unrestricted IFN γ signaling and impaired HSC function³⁶. Collectively, these studies indicate that chronic hyperstimulation by interferons leads to HSC exhaustion. On the other hand, low steady-state levels of interferon may have a different effect and, indeed, may be necessary to maintaining the stem cell pool. Longer-term studies to investigate the potential mechanisms of this phenomenon are needed.

Immunomodulation of HSPCs directs the immune response from the early progenitor stage

Following the early progenitor stage, the hematopoietic tree is traditionally thought to divide along two main branches: myeloid and lymphoid. Despite improved knowledge about lineage differentiation and the *in vitro* conditions that can lead to production of specific cell types³⁷, the factors that influence lineage fate decisions *in vivo* remain poorly understood. Because responses to infectious diseases tend to depend asymmetrically on either myeloid or lymphoid cells, it is tempting to speculate that infections can influence the differentiation pattern even of cells as primitive as hematopoietic stem cells.

Indeed, some studies suggest that immune-mediated effects on HSPCs may be important in influencing the balance of myeloid versus lymphoid hematopoietic differentiation. For instance, in a mouse model of malaria infection, IFN γ signaling led to induction of an IL-7R⁺ KIT^{hi} myelolymphoid progenitor population that generated mainly myeloid cells to combat the infection³¹. Extramedullary HSPCs in tissues such as kidney have been found to be responsive to TLR ligands and to generate myeloid lineage cells in those tissues upon TLR stimulation³⁸. Hence pathogen-associated or cytokine-mediated responses may help HSPCs tailor their output to the infection at hand.

Lineage biasing of HSC output may be mediated by differential responses of HSC subtypes. Whereas HSCs have previously been treated as a homogeneous group, the field is now evolving towards a finer understanding of the HSC population. Namely, a variety of subsets exist within the HSC population, termed myeloid-biased or lymphoid-biased HSCs, which, while retaining the capability to fully reconstitute all blood lineages, have the tendency towards either myeloid or lymphoid development^{39,40,41}. Cytokine-mediated selection of one HSC subset over another might be a clever way for the immune system to preferentially manufacture myeloid or lymphoid effector cells as appropriate for the situation. It will be of great interest in coming years to understand how infectious states may specifically select for proliferation of one HSC class over another.

Some researchers have suggested that cytokine signaling to HSCs may be a mechanism for recruiting HSPCs and immune effector cells to distant sites of infection. Infections appear to increase the number of HSCs that can be detected in the peripheral circulation^{42,38}. In one study, schistosomal infection appeared to alter the splenic microenvironment, making it more hospitable for HSPC homing⁴³. However, it is unclear whether this phenomenon is important for immunity or if it is simply a byproduct of altered retention in the bone marrow niche. A recent study utilized a mouse model of pneumonia to investigate the effects of cytokines released in the lung on bone marrow progenitors. Interestingly, exposure of bone marrow cells to cytokine-laden serum from these mice appeared to influence leukocyte migration to the infected lungs and, ultimately, the outcome of the infection¹². These findings suggest that serum factors condition bone marrow cells to home to sites of infection. To our knowledge extramedullary hematopoiesis is not a common or important phenomenon in the absence of conditions that disrupt the bone marrow itself. Further studies are necessary to establish whether there is any role for HSCs at sites of infection.

Immunomodulation of HSCs has functional consequences

That HSCs can be activated to divide and differentiate in response to infectious or inflammatory stimuli has important functional consequences for this stem cell population. Quiescence is an important characteristic of HSCs, and the total HSC population is best preserved when quiescent or even dormant³. In one study of *Pseudomonas* sepsis in mice, LSK cells of infected mice did not engraft well after transplantation into lethally irradiated mice, indicating a deficit in stem cell function¹⁴. Similarly, HSCs from mice infected with *M. avium* are highly defective in post-transplantation engraftment¹¹. The functional defect of HSPCs from infected mice is consistent with the general finding that frequently dividing stem cells are functionally impaired in their ability to engraft in the setting of bone marrow transplantation⁴⁴, suggesting impaired self-renewal capacity.

Indeed, disruption of HSC quiescence through immune activation can have long-term consequences for the hematopoietic system. In fact, several recent studies have shown self-renewal defects of the HSC population in the setting of chronic inflammation. When genes that normally suppress interferon signaling are disrupted, the mice are exposed to high levels of interferon signaling and HSC populations are depleted over time^{34,36}. Similarly, HSCs were diminished in mice chronically exposed to LPS¹⁵. Conversely, if chimeric mice containing wild-type or IFN α receptor deficient HSCs were chronically exposed to IFN α , only the IFN α -receptor deficient HSCs survived³³. Collectively, these findings indicate that immune-mediated bone marrow exhaustion can result from persistent activation of quiescent HSCs, thus depleting the total population of HSCs over time. This finding strikes at some of the core questions in stem cell biology: are we born with a finite number of stem cells, and are these cells limited by a finite number of cell divisions? Further studies regarding the effects of infection on the stem cell population over time promise to shed further light on these fundamental questions in the field.

Inflammatory mediators as part of homeostatic function

The finding that inflammatory signals activate HSCs suggests a previously unconsidered possibility: do inflammatory signals contribute to HSC biology at baseline, even in the absence of infection? As our knowledge of the human microbiome expands, we now understand that humans are rarely if ever in a state that is devoid of inflammatory stimulation⁴⁵. Instead, we live among latent bacteria and viruses that provide a constant stimulus for some degree of inflammatory signaling. Recent studies regarding immune-mediated effects on HSCs have led to evidence that baseline inflammatory signaling is important for HSC function, even under homeostatic conditions. For example, mice lacking the TNF α receptor p55 have increased marrow cellularity with age accompanied by a relative decrease in HSC function, based on transplantation assays^{46,47}. Similarly, bone marrow from mice lacking IFN γ or TLR4 show increased engraftment after transplantation when compared to bone marrow from a wild type donor, likely because these cells are in a more quiescent state at baseline¹¹. These findings suggest that the number or proliferative status of HSCs can be influenced by inflammatory or microbial signaling, even in the absence of active infection. Thus, a low-level inflammatory response may be required for maintenance of a normal stem cell compartment. Indeed, immune activators such as IFN γ

may serve as a biological rheostat for the hematopoietic system, providing a sensitive and rapid method for the modulation of hematopoietic output.

Therapeutic applications of immune-mediated HSC activation

There are enormous clinical implications to the understanding of immunomodulatory effects on HSC number and function. A wide variety of infectious diseases lead to bone marrow failure syndromes, and the pathophysiology of such syndromes has been poorly understood. Specifically, acquired aplastic anemia follows an infectious process such as Epstein-Barr virus (EBV) or cytomegalovirus (CMV) infection in one-third of cases⁴⁸. HIV infection is frequently accompanied by bone marrow suppression even in the absence of marrow suppressing drugs such as zidovudine⁴⁹. Finally, bone marrow suppression in tuberculosis has frequently been ascribed to underlying hematologic disorders or an infiltrative process; but the mechanism of this effect has not been thoroughly studied⁵⁰. Inflammatory cytokines released during these infections likely contribute to bone marrow failure in each case, and further understanding of this effect is paramount. For instance, interferons may lead to apoptosis of hematopoietic progenitors in some circumstances, but proliferation of those cells in others. The concentration and timing of interferon exposure is likely to be pivotal in the final outcome, and further studies are needed to clarify these effects. Improved understanding of the effects of immune activators on HSCs will lead to new approaches to address acquired aplastic anemia syndromes and pancytopenia in the setting of infectious diseases.

Hematopoietic stem cell transplant recipients represent another group of patients who may benefit from further knowledge about inflammatory signaling and HSCs. In the initial engraftment period, recipients of hematopoietic stem cell transplantation are extremely immune suppressed and hence prone to a wide range of infectious agents⁵¹. How the lack of cytokine signaling combined with potentially severe infections in the immediate post-transplant period affect HSC engraftment is now beginning to be better defined. Again, immunomodulatory approaches in such situations may improve HSCT outcome by preserving HSC function despite deleterious consequences of inadequate or overabundant cytokine signaling.

Finally, studies regarding the interaction between inflammatory signaling and HSCs may yield advances in the treatment of hematologic malignancies. Oncologists are increasingly focused on the role of cancer stem cells in driving persistent or residual disease despite aggressive chemotherapy. If inflammatory cytokines such as TNF α or interferons can drive differentiation and reduce longevity of HSCs, these cytokines can be applied therapeutically to increase the chemosensitivity of cancer stem cells³³. Such an application has recently been shown in combination treatment for chronic myelogenous leukemia^{52,53}.

Future directions and open questions

In summary, recent work has indicated that HSPCs are activated to proliferate in response to infections and the inflammatory cytokines that are elicited to combat them. Furthermore, new studies suggest that inflammatory cytokines are important for regulation of the appropriate number and function of HSCs at baseline. Different hematopoietic cell types

respond quite differently to inflammatory cytokines, likely in a concentration-dependent manner. In order to eliminate confusion about the effects of inflammatory signals on HSPCs, it is of critical importance to carefully define the particular HSPC subset being described using a combination of cell surface markers and functional properties.

Studies using these techniques have allowed us to view the HSC in a new light – rather than as a dormant cell called upon to proliferate in times of extreme hematopoietic stress, we now view the HSC as a cell whose activity is constantly modulated by the shifting inflammatory environment. Furthermore, preliminary studies indicate that inflammatory signals can influence the pace and direction of HSC differentiation, suggesting that these signals play a direct and vital role in the primary immune response to infection.

These ideas open up a great number of experimental opportunities. First, the specificity of the HSC response to different inflammatory cytokines elicited by different pathogens remains to be explored. Inflammatory cytokines other than the interferons are likely to be active in influencing HSC activity. Moreover, HSCs may respond differently to type I versus type II IFNs, elicited to different degrees by various pathogens, thereby tailoring their response to the type of infection at hand. Similarly, signaling through various TLRs may lead to pathogen-specific responses by HSCs. Second, effects of inflammation on the HSC niche remain undefined, but are likely to prove extremely influential in the HSC response to infection. Recent work has shown that stromal cells of the bone marrow niche play a pivotal role in regulating HSC quiescence⁵⁴. Thus, it will be of great interest to determine how the cellular composition of the niche changes with infection, and for how long those changes persist. Further investigation in this area will also elucidate the degree to which inflammatory signaling to the HSC is locally versus systemically generated. Finally, it will be of great interest to understand how chronic inflammatory conditions affect the human HSC population over the long term. Specifically, we speculate that long term activation of these cells during chronic inflammation may lead to depletion of the HSC population or long term functional deficits such as seen in some acquired bone marrow failure syndromes. Human studies to augment the findings in animal models will be of importance in all of these avenues of investigation.

Glossary terms

common myeloid progenitors (CMPs)	A progenitor that gives rise to megakaryocyte and erythrocyte progenitors (MEPs) or granulocyte and macrophage progenitors (GMPs) and, subsequently, the mature progeny of those cells
common lymphoid progenitor (CLP)	A progenitor that is committed to the lymphoid lineage that can give rise to all lymphocyte subsets, including T cells, B cells and natural killer cells
lineage-negative SCA1⁺KIT⁺ (LSK) cells	A commonly used population of bone marrow cells which, while enriched for hematopoietic stem and progenitor cells (HSPCs) also contains a heterogeneous mix of multipotent progenitors and other committed progenitors

Hoechst 33342	A lipophilic fluorescent stain for labeling DNA which is excited by UV light and is effluxed by hematopoietic stem cells
acquired aplastic anemia	A syndrome of bone marrow hypocellularity combined with peripheral pancytopenia

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Biographies

Margaret Goodell received her PhD from the University of Cambridge in England before pursuing postdoctoral work at the Whitehead Institute at MIT and Harvard Medical School. She studies the genetics and epigenetics of hematopoietic stem cell regulation at Baylor College of Medicine, where she is the Vivian L. Smith Professor of Regenerative Medicine and Director of the Stem Cells and Regenerative Medicine Center.

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Online Summary

- Hematopoietic progenitors proliferate during infections, even in the absence of peripheral cytopenia.
- Hematopoietic stem cells respond to both direct and indirect signals during infection.
- Direct signaling to HSCs during infection may occur via pattern recognition receptors such as Toll-like receptors.
- Indirect signaling to HSCs during infection is mediated by inflammatory cytokines, the most extensively characterized of which are the interferons.
- Baseline interferon signaling and tight regulation of this signaling are imperative to maintain HSC and peripheral cell populations.
- Cytokine-mediated activation of HSCs impairs their self-renewal capacity.