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Tuning of the Hematopoietic Stem Cell Compartment in its Inflammatory Environment

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Abstract

Purpose of Review The hematopoietic stem cell (HSC) compartment is the cornerstone of a lifelong blood cell production but also contributes to the ability of the hematopoietic system to dynamically respond to environmental challenges. This review summarizes our knowledge about the interaction between HSCs and its inflammatory environment during life and questions how its disruption could affect the health of the hematopoietic system.

Recent Findings The latest research demonstrates the direct role of inflammatory signals in promoting the emergence of the HSCs during development and in setting their steady-state activity in adults. They indicate that inflammatory patho-physiological conditions or immunological history could shape the structure and biology of the HSC compartment, therefore altering its overall fitness.

Summary Through instructive and/or selective mechanisms, the inflammatory environment seems to provide a key homeostatic signal for HSCs. Although the mechanistic basis of this complex interplay remains to be fully understood, its dysregulation has broad consequences on HSC physiology and the development of hematological diseases. As such, developing experimental models that fully recapitulate a normal basal inflammatory state could be essential to fully assess HSC biology in native conditions.

Keywords Hematopoietic stem cell · Inflammation · Aging · Obesity · Immunological history · Hematological disease

Introduction

Inflammation is defined as a protective immune mechanism and can be activated in response to acute events associated with infection/tissue injury or during ordinary physical/physiological disturbances that affect tissue homeostasis [1]. As inflammatory responses are triggered by a broad range of internal and environmental insults, they are extremely variable in quality, intensity, kinetics, and duration. These processes are ubiquitous, affecting all stages of life and all body tissues while interplaying with many biological processes. Although the innate and adaptive immune cells are the prime inducers and responders of inflammatory signals, inflammation also directly affects the bone marrow (BM) hematopoietic tissue that underlies the production of all blood cells and particularly the immune cells. Hematopoiesis originates within a small pool of self-renewing and multipotent hematopoietic stem cells (HSCs), which undergo a stepwise differentiation process to produce a series of intermediary progenitors. While these progenitors are proliferating to contribute to the daily blood cell production, HSCs remain mostly quiescent and are responsible for sustaining the hematopoietic activity during the entire life of the organism. Blood production is highly dynamic and is constantly adapted to accommodate environmental challenges and respond to the needs of the organism. Inflammation is viewed as a key interface between the hematopoietic system and the broad array of internal stresses and environmental disturbances. There is now a new understanding that this interplay involves the entire hematopoietic hierarchy including the quiescent and long-lived HSC compartment. In recent years, great progress has been made in the

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understanding of how inflammatory signals regulate HSC fate and modulates blood production. Here, we will review new studies that describe the interaction of the HSC compartment with its inflammatory environment during the different stages of life. We will focus on recent reports that suggest the basal inflammatory state in healthy individuals contributes to shaping the HSC compartment and toning its steady-state activity. Herein, we highlight how chronic inflammatory pathophysiological conditions or diverse immunological history could impact the overall fitness of the HSC compartment. Finally, we will discuss the potential mechanisms underlying the regulation of HSC activity by inflammatory signals and their implications for our understanding of hematological diseases.

Inflammation Signals as a Driver of Early Hematopoietic Development

During embryonic development, hematopoiesis occurs in discrete waves [2]. The first wave occurs in the yolk sac in vertebrates and only produces a limited set of primitive erythrocytes and macrophages. The second wave, which is the matrix of lifelong definitive hematopoiesis, is fueled by the activity of HSCs that emerged from the hemogenic endothelium lining the floor of the dorsal aorta. HSC emergence during this narrow developmental window represents a critical step for the establishment of a functional hematopoietic system. Several classical developmental pathways including Notch, Wnt, and bone morphogenetic protein (BMP) have been shown to contribute to HSC specification [3]. Recent studies in zebrafish and mouse embryos indicate that inflammatory signals, released by primitive macrophage and neutrophils, are essential cofactors of these pathways [4•]. In this context, pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and interleukin-1β (IL-1β), in addition to toll-like receptors (TLR) signaling are key determinants of HSC specification [5–8]. These results were surprising because the womb is traditionally considered as a sterile environment protected from the microbial environment. As such, the mechanisms triggering inflammatory signals in such a sterile environment remain to be defined. This inflammation could be linked to the release of damage-associated molecular patterns (DAMPs) by fast-growing embryonic tissues, which undergo cell remodeling and sustain oxidative stress. Alternatively, recent reports seem to revisit the sterile womb paradigm indicating some level of maternal microbial transmission before birth, therefore suggesting an early encounter between the hematopoietic system and the microbial environment [9]. Independent of the mechanisms, these reports indicate a co-development between the emerging hematopoietic system and its inflammatory environment. This co-development continues at birth when neonates are exposed to a microbial-rich environment and develop a gut flora. During this transitional period, newborns are highly susceptible to infections as their immune system progressively matures in contact with this new environment [10]. This neonatal period also coincides with profound changes in the HSC compartment that progressively develop its adult characteristics [11]. Notably, this includes the extinction of particular fetal HSC subsets responsible for the production of primitive innate-like lymphocytes [12] and a switch in the cycling activity of the HSCs that acquire their adult quiescent status [13]. Little is known about the environmental factors able to influence this critical fetal-adult transition but the inflammatory environment is certainly one of the potential candidates. Overall, these reports highlight how inflammatory signals could contribute to shaping the nascent hematopoietic tissue, a feature consistent with the evolutionary need to adapt the activity of this complex tissue to its environment.

Inflammatory Basal State and Adult HSC Homeostasis

Adult animals are in constant interaction with microbial organisms. These interactions include daily encounters with bacteria, viruses, and fungi found in food or in the environment, which can lead to sub-clinical immune responses. These interactions also include the complex system of microbes, often referred to as the microbiota, that exist on many body surfaces, including the skin, mouth, nose, vagina, and digestive tract [14, 15]. Most of these host-microbe relationships are not pathogenic and are actually part of the normal physiological functions. A majority of these microorganisms are commensal bacteria that reside in the colon which not only contribute to nutrient absorption and various metabolic functions but also regulate the innate and adaptive immune system [16••]. As such, the microbiota has been shown to play a protective role against infectious pathogens. Conversely, its perturbation (or dysbiosis) has been linked to various metabolic, inflammatory and autoimmune diseases including diabetes, inflammatory bowel disease, lupus, and allergy [17].

Recent studies have highlighted the impact of the inflammatory basal state on the hematopoietic system including the HSC compartment [18]. The first lines of evidence come from the study of germ-free (GF) mice. GF mice (also named axenic mice) are produced by hysterectomy re-derivation and maintained in strict sterile condition. They are by definition free of all microorganisms, including those typically found in the gut. Compared to the common specific-pathogen-free (SPF) laboratory mice that interact with non-pathogenic microbes and have a complete gut microbiota, GF mice display an immature adaptive immune system and an increased

susceptibility to infection [19]. Reports also indicate that GF conditions are associated with reduced mature myeloid compartments and reduced BM myeloid potential [20]. Notably, the number of granulo-macrophage progenitors (GMPs) in BM appears to be directly proportional to the microbiota complexity, suggesting its contribution to the dynamic production of these short-lived populations [21••]. This study also shows that the absence of microbiota reduces the size of the immature hematopoietic compartments (i.e., $Lin^{-}c\text{-}Kit^{+}Sca\text{-}1^{+}$, LSK population) that encompasses HSCs and multipotent progenitors. Interestingly, partial reconstitution of the microbiota fails to fully restore the size of LSK compartment suggesting the existence of specific qualitative and/or quantitative requirements for the microbiota to modulate the most immature hematopoietic fractions. These results were confirmed in more refined HSC compartment phenotypically defined as LSK $Flk2^{-}CD34^{-}$ or LSK $CD48^{-}CD150^{+}CD34^{-}$. Notably, studies in GF mice and in mice treated with a broad-spectrum antibiotic cocktail show that disruption of the microbiota results in a two-to-threefold reduction in the number of HSCs [22••, 23•]. Complementation experiments through microbiota transfer in GF mice or interruption of the antibiotic treatment seems to confirm that the microbiota provides a dynamic and reversible regulation of HSC number. However, the impact of the microbiota on HSC functions remains to be fully explored. One study shows that HSCs isolated from GF mice show improved reconstitution activity in transplantation assay [24] but this effect was not reproduced with antibiotics-treated mice [23•, 24]. This discrepancy could indicate that chronic but not acute alterations of the microbiota are able to modulate long-term HSC functions. Alternatively, this could highlight fundamental differences between the two models. Notably, it is unclear whether constitutive GF condition affects the initial maturation of HSC compartment in addition to its adult steady state function. In this context, development of experimental schemes based on (i) the use of auxotrophic bacterial strain to promote transient microbiota colonization [25] or (ii) the modulation of the timing/duration of the antibiotics treatment could prove informative. In addition, it is important to note that microbiota alterations in these models also affect nutrient absorption and therefore have important metabolic consequences that could disrupt the HSC compartment independently to inflammation. Despite their limitations, these recent studies suggest that signals derived from the microbiota provide, at steady state, a tonic stimulation not only to the mature immune cells and their immediate progenitors but also to the long-lived HSC compartment. However, more specific assays remains to be performed to validate this idea. Notably, it would be important to determine how the microbiota affects the continuum of dormant and active states in the HSC compartment during homeostasis [26••, 27, 28]. The impact of basal inflammatory states on the long-term HSC fitness remains to be studied through stringent serial transplantation assays and in aging condition. Finally, a full functional and molecular characterization of HSC pre- and post-microbiota complementation remain to be described.

Basal Inflammatory Signals Controlling HSC Fate

Several mechanisms have been proposed to mediate the effect of the basal inflammatory environment on the HSC compartment. It is well established that host interactions with pathogenic and commensal microbiota lead to the presence of immunogenic microbial compounds in the systemic circulation. Often referred as pathogen-associated molecular patterns (PAMPs), these molecules, which include lipopolysaccharide (LPS), RNA, and peptidoglycans, are present at low concentrations in healthy individuals [22••, 29]. As a normal part of the immune response, host cells recognize these molecules through numerous specialized pathogen-recognition receptors (PRRs) that include toll-like receptors (TLR), C-type lectin receptors, nucleotide-binding oligomerization domain-containing protein (NOD)-like receptors (NLR) and other RNA-sensing receptors. In the BM, these receptors are present on non-hematopoietic cells and on resident immune cells but also on the HSCs themselves. As such, PAMPs could alter the HSC function directly via intracellular signaling or indirectly via alterations of the BM niche and/or production of pro-inflammatory cytokines/chemokines (Fig. 1a) [30, 31]. Determining the ability of PRRs to sense the low PAMP concentrations present in non-infectious condition and evaluating their contribution to the HSC homeostasis at steady state remains challenging due to potential redundancy between these different signals and their potential complex effects on the various BM cell compartments. In addition, differences in the HSC definition has led to some degree of discrepancy in the published literature about the impact of these signals on the HSC compartment.

PAMP Receptors Seminal work by the Kincade group has shown that HSCs express TLR2 and TLR4 and respond to their respective agonists (Pam3CSK and LPS) [32]. HSCs also harbor low level of other TLRs but their impacts on HSCs have yet to be fully established [22••, 24]. Reports indicate that genetic disruptions of these signals by targeting individual TLR (including TLR2 or TLR4) or key components of their transduction pathways (such as the downstream adaptors proteins Myd88 and Trif) have no major consequences on HSCs at steady state [33, 34]. However, discordant studies have described an effect of these mutations on the HSC phenotype and functions [24, 35–37]. These studies suggest that disruption of these pathways could be associated with increased HSC quiescence and an overall competitive advantage upon transplantation. While such discrepancies remain to be explained, it is tempting to speculate that they could reflect

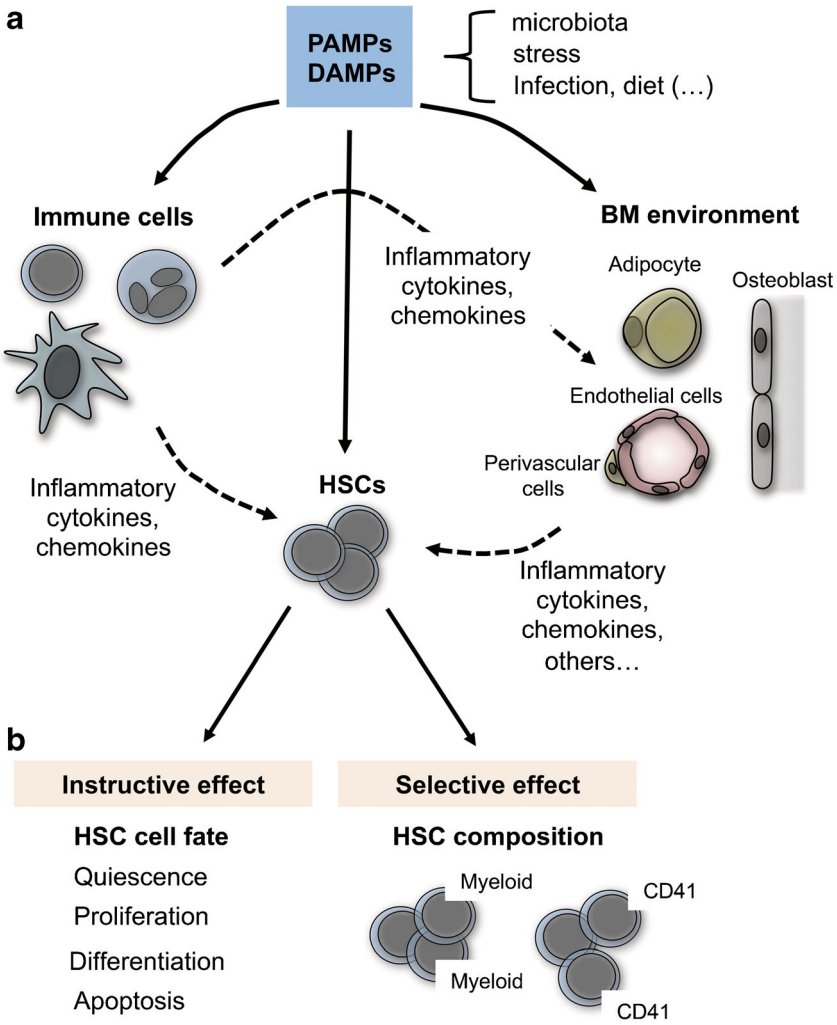

Fig. 1 Molecular network controlling the interplay between HSCs and its inflammatory environment. **a** Direct and indirect HSC response to inflammation. Low concentration of circulating pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) can directly affect the function of the HSCs, which express corresponding pattern recognition receptors (PRRs). PAMPs and DAMPs also alter the activity of immune cells and non-hematopoietic cells that form the bone marrow niche, leading to indirect modulation to the HSC compartment. **b** Consequences of inflammatory signaling on the HSC compartment. Direct and indirect inflammatory signals impact the HSC long-term fitness by instructing particular cell fate decision or promoting the selection of specific HSC subsets

some subtle environmental differences in research animal facilities and therefore might testify to the importance of these pathways in adapting the HSC compartment to changes in microbiota and/or hygienic conditions. In addition to TLR signaling, a recent study describes a key role of NOD-like receptor Nod1 on the HSC homeostasis [22••]. The authors found that HSCs express Nod1 and that the Nod1-deficient mice displayed a reduction in the size of the hematopoietic primitive compartment that mimics the phenotype observed in GF condition. At steady state, providing synthetic NOD1 ligand (NOD1L) to GF mice reverses their hematopoietic defect and particularly their reduced HSC number. Mechanistically, this study describes that NOD1L does not directly affect the hematopoietic compartments but provides a tonic signal necessary for the supportive function of the BM microenvironment. Nod1 signaling is particularly shown to stimulate the production of key hematopoietic cytokines (such as SCF, TPO, Flt3L) by mesenchymal stem cells (MSCs). However, it is interesting to note again some discordance in the literature as one study indicates that loss of the adaptor protein RIPK2 (receptor-interacting serine-threonine kinase 2) that is required for signal transduction downstream of the NOD-like receptors does not induce a major HSC phenotype at steady state [33].

Secondary Inflammatory Signals One the consequences of the presence of PAMPs in the circulation is a basal production of various secondary inflammatory cytokines by diverse immune and hematopoietic cells. Targeted disruption of some of these pathways at steady state show limited effects on the HSC compartment. As such, receptors for tumor necrosis factor (TNF) or interleukin-1 (IL-1) have been shown to be dispensable for maintaining HSC homeostasis [38, 39]. Although the observed effects are modest, HSCs seems to be susceptible to the basal concentration of both type I and type II interferons (IFN). As such IFN-α/β receptor (Ifnar)- or IFN-γ receptor (Ifngr1)-deficient mice showed reduced HSC numbers and lower proliferation rates [40, 41]. Consistent with these results, loss of Stat1, which represents a common node for IFN pathways, induces similar HSC defects and mimic some of the phenotype observed in antibiotic-treated animals [23•]. Finally, disruption of critical negative feedback mechanisms

for the IFN signals in *Irgm1*, *Irf2*, and *Adar1* mutant mice is associated with severe alteration of HSC compartment at steady state [42–44]. Altogether, these studies suggest that baseline IFN signaling is tightly regulated in HSCs and could be critical to the tonic stimulation necessary to maintain HSC homeostasis. The mechanisms driven by IFN in HSCs at steady state remain unknown. It would be particularly interesting to determine the impact of baseline IFN signaling on the HSC gene expression program and its role in tuning signal transduction pathways associated with other key hematopoietic cytokines [45].

Intracellular Molecular Integrators TLR, NLR, and IFN signaling converge on the intracellular activation of NF-κB. Early studies have demonstrated the role of the different NF-κB subunits as integrators of inflammatory signals in the hematopoietic system [46]. However, due to developmental defects in the mutant mice, most of these results were obtained using fetal liver cells and did not specifically address the role of these molecules in adult steady state. Recent publications have extended these results, demonstrating that both the canonical and the non-canonical NF-κB pathways are required to maintain adult HSCs [47–50]. Indeed, genetic disruption of these pathways by targeting of NF-κB subunits (e.g., RelA or RelB/NF-κB2) or their regulators (e.g., Ikkb or NIK) leads to complex alterations of the size and, in some cases, functions of the HSC compartment at steady state. Altogether, these studies suggest that a complex and subtle equilibrium of the different NF-κB subunits/pathways is required both intrinsically and in the BM environment to maintain HSC homeostasis. In the HSCs, these studies show that quantitative and qualitative dosage between NF-κB subunits could affect multiple HSC gene programs and control HSC fate. Although the NF-κB requirements for the HSCs are not completely understood, NF-κB appears to be one key regulator of the basal inflammatory tone in HSCs.

Pathophysiological Disruption of the Basal Inflammatory State

The steady-state hematopoietic activity of an individual could be affected by multiple chronic pathophysiological conditions. Natural aging or metabolic diseases such as obesity display similar hematological characteristics. These pathophysiological conditions are associated with functional declines of the immune system, which leads to increased susceptibility to infections, low response to vaccinations, and increased vulnerability to the development of autoimmunity and hematologic malignancies [51, 52]. Both conditions show hematopoietic skewing toward the myeloid lineage and reduction of the fitness of the HSC compartment [53, 54]. Along with common metabolic and hormonal characteristics, these conditions also share a common inflammatory component. Aged-associated inflammation (also termed inflamm-aging) and metabolic inflammation (also termed meta-inflammation) are chronic states associated with an increased basal level of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1Rα, even in the absence of infection [55, 56]. The low-grade inflammatory state of these conditions seems to be multifactorial and has been linked with the expansion of myeloid cells and adipocytes, which are key producers of inflammatory cytokines. Aging and obesity are also associated with increased gut permeability along with changes in the composition and the diversity of the gut microbiota [57, 58]. As such, both conditions show a higher concentration of microbial products in the blood circulation [59, 60]. Due to the complexity of phenotype associated with these conditions, the exact contribution of inflammation on the HSC phenotypes observed in aging and obesity remains to be fully explored. Importantly, both inflamm-aging and meta-inflammation arise through slow kinetics. The slow development of these specific basal inflammatory states raise the intriguing possibility of the concomitant development of yet to be defined adaptive or selective mechanisms aimed at preserving the HSC functions [53].

Infectious Inflammatory Response

Infectious episodes represent another type of normal recurrent alternations of the inflammatory basal state. Infections are associated with profound hematopoietic changes that contribute to the replacement of diminished immune effectors cells. Recent studies indicate that HSCs respond to inflammatory signals in case of acute and chronic infection and directly participate in the primary immune response [30]. In vivo injection of inflammatory mediators (including IFN-α/β, IFN-γ, IL-1, TNF-α, or PAMPs) [38–41, 61] and more physiological models mimicking bacterial and viral infections [33, 40, 62•] are invariably associated with HSC loss of quiescence, differentiation, and mobilization toward the periphery. Recent reviews provide excellent summaries of these studies [63•, 64, 65]. Overall, the type, intensity, and duration of the infection seem to dictate HSC fate in these models. Short-term inflammatory signals induce rapid HSC proliferation and differentiation that correlate with the increased hematopoietic production and promotes host defenses. This activation is transient allowing the HSC compartment to recover its quiescence after blood homeostasis has been re-established [28]. In contrast, sustained inflammatory signals, associated with chronic infection or autoimmune disease, alter HSC self-renewal activity and ultimately their long-term potential [38, 40, 41, 61]. Several mechanisms have been proposed to explain this negative impact of sustained inflammation (Fig. 1b). Reports have described how inflammation could disrupt key hematopoietic and non-hematopoietic elements of the BM niche that normally support HSC self-renewal, leading to

HSC mobilization to the periphery and promoting their differentiation [33]. In parallel, inflammation provides instructive signals that directly control HSC proliferation, differentiation, and apoptosis, all mechanisms that may contribute to the attrition of the HSC pool [32, 38, 66]. Finally, as the heterogeneity of the HSC compartment in term of levels of quiescence, self-renewal capability, and potential of differentiation is increasingly appreciated [28, 67], recent studies suggest that inflammatory signals selectively affect distinct HSC subsets. To some extent, this selectivity could contribute to the adaptation of hematopoietic lineage output. For instance, IFN-γ has been proposed to preferentially activate myeloid-biased HSCs, contributing to the refurnishing of the pool of innate immune cells [68]. Similarly type-1 IFN and TNF-α have been shown to promote a megakaryocytic program in one particular HSC subset, potentially contributing to a rapid platelet recovery upon infection [69••]. The mechanisms responsible for such selectivity are yet to be defined. HSC selection could be linked to the cell-intrinsic HSC ability to respond to inflammatory signals, affected by the differential expression of cytokines, chemokines, and pathogen-recognition receptors, or dependent on the presence of negative feedback responses able to modulate the transduction signals and protect some HSC subsets [42–44]. Alternatively, it could be associated with a localization of the HSC subsets in more or less immunologically sensitive BM niches. Independent of the underlying mechanisms, this selectivity could promote the exhaustion of particular HSC subsets and therefore affect the overall composition of the HSC compartment. This could explain how not only immunological status but also the accumulation immunological events could have long-term consequences on the fitness of HSC compartment and may be linked to alteration of the blood function and development of hematopoietic malignancies.

Conclusion

Point 1: Interplay between HSC Compartment and Basal Inflammatory State

Overall, the studies presented in this review highlight the ubiquitous nature of inflammation and its function in modulating the HSC activity at different stages of life (Fig. 2) and suggest a co-development of the HSC compartment with its inflammatory environment. During embryonic development, inflammatory signals collaborate with developmental pathways to stimulate the emergence of HSCs, which serve as the cornerstone of definitive hematopoiesis. At birth, encounter with the microbial environment and development of the gut microbiota contribute to shaping the immune system and may affect the maturation of the HSC compartment. In adults, several reports suggest that basal inflammatory signals are able to provide a tonic stimulation to the peripheral immune system and hematopoietic progenitors but also to the HSCs. Many questions remain about the mechanisms modulating the HSC activity in this context. A crosstalk between inflammation signals and key hematopoietic signaling pathways has been proposed [45]. We can also postulate that basal inflammation is necessary to maintain a threshold of activation by priming the signal transduction machinery but also its negative modulators [70]. Importantly, studies suggest that the in vivo inflammatory threshold required for activation is higher in HSCs than in downstream progenitors [21••, 32]. This could be linked to the intrinsic quiescent properties of the HSCs but could also be dependent on the location of HSCs receiving the inflammatory stimuli. In some instance, the BM niche has been shown to be essential in transducing the inflammatory information to the HSC compartment [22••, 33]. Alternatively, inflammatory sensing could occur during the daily HSC trafficking through the blood and peripheral tissues [71]. This immune-surveillance could be important for the PAMP sensing, as TLR activation in HSCs is at least partially dependent on the presence of the soluble CD14 co-receptor [32]. Further investigations will have to define the relative contributions of these different mechanisms and their overall impact on the health of the hematopoietic and immune systems.

Point 2: Dysregulated Inflammatory State and Hematopoietic Pathologies

Metabolic status, age, hygiene conditions, and immunological history are some of the multiple parameters that alter the basal inflammatory state and influence HSC homeostasis. Interestingly these factors have been associated with altered immunological functions but also with hematological pathologies including myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPNs), and acute myeloid leukemia (AML) [72–76]. Whether the lone effect of inflammation on HSCs is able to promote hematological diseases remains to be formally established. However, it is clear that inflammatory cytokines and signaling molecules can contribute to some important features of hematological disease initiation and development [77]. For example, inflammation is linked to genomic instability via DNA damage linked to the excessive production of reactive oxygen species (ROS) [78•] or via replicative stress linked to the aberrant activation of dormant HSCs [62•, 79]. Similarly, hyperactive inflammatory signaling has been shown to alter gene expression and RNA processing, resulting in impaired hematopoiesis and bone marrow failure [80, 81]. In addition to cell-autonomous alterations, the inflammatory environment could modulate HSC composition by balancing the representation of functionally distinct subsets. As such, inflammation could provide a selective landscape promoting the expansion of some HSC clones at the expense of others.

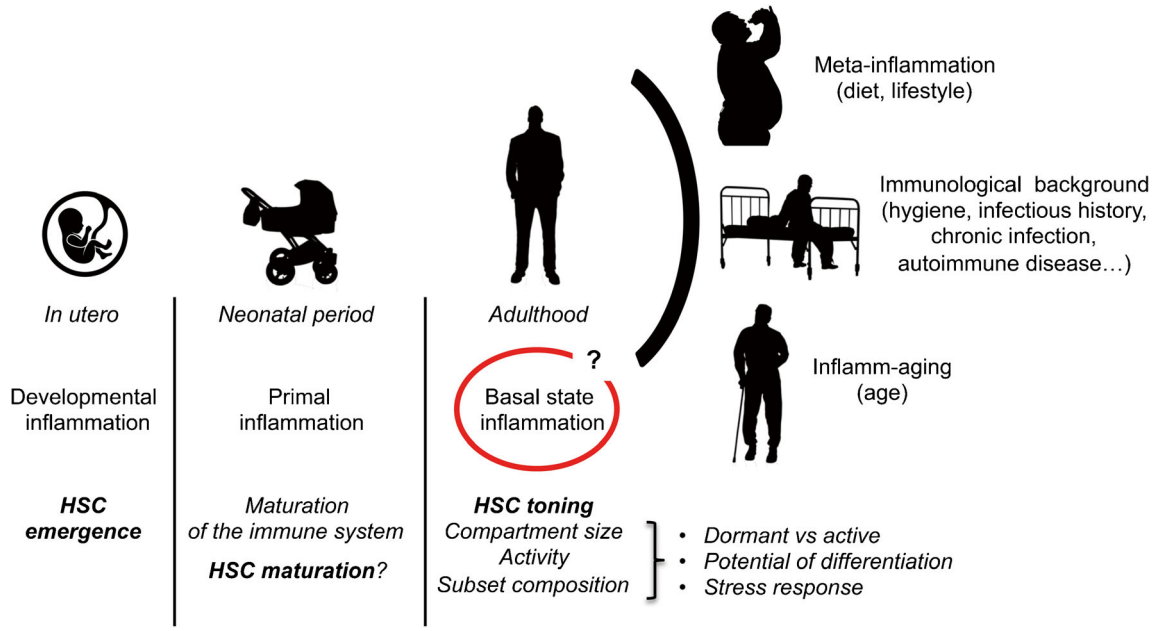

Fig. 2 Multiple types of inflammation modulate the HSC compartment during life. Inflammatory signals are key to the emergence of the HSCs in the embryo and may also contribute to their maturation after birth, underlying the tight relationship between the HSC compartment and its inflammatory environment. In adults, studies suggest that the presence of the microbiota and other physiological stresses maintain a basal inflammatory state that contributes to the toning of HSC activity. Chronic conditions associated with lifestyle choices, hygiene or age modify this steady state inflammatory landscape with consequences on the long-term health of the HSC compartment

Restriction of the clonal hematopoietic diversity, a phenomenon known as clonal hematopoiesis of indeterminate potential (CHIP) is a frequent feature of aging that may precede the emergence of hematological malignancies [82–85]. Associated with somatic malignancy-linked mutations (such as DNMT3A, TET2, ASXL1), CHIP is considered a pre-leukemic state even if it is not clinically predictive, with only a small fraction of patients with CHIP actually developing hematological disease [86, 87]. The mechanisms contributing to CHIP development are unknown. Notably, it is unclear if inflammation is a cause or a consequence of the CHIP phenomenon. On one hand, CHIP could act as a source of

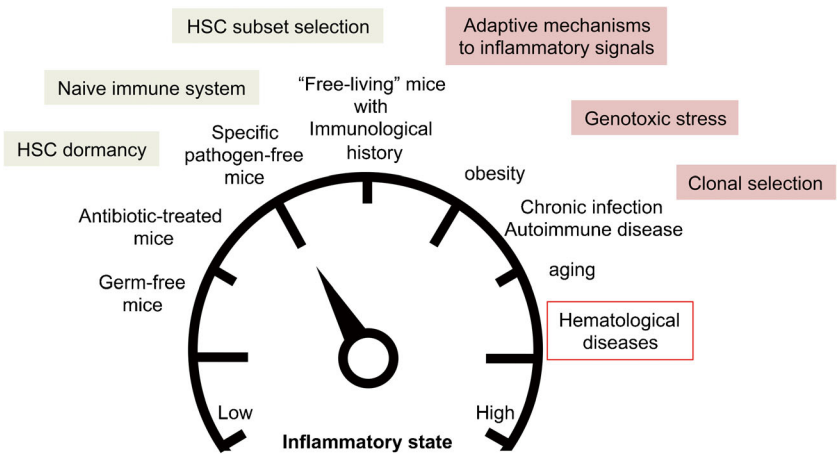

Fig. 3 Modeling the impact of the inflammatory landscape on the HSC compartment and its potential long-term contribution to hematological diseases. Use of animal models with low inflammatory background such as germ-free mice, antibiotic-treated mice, or specific pathogen-free mice could affect key feature of the HSC compartment at steady state and alter their response to stress. Normalization of the inflammatory state to mimic the situation occurring in "free-living" animals could provide a more accurate picture of the HSC biology in physiological conditions. Finally modeling the alterations of the inflammatory landscape in common pathophysiological conditions such as obesity, chronic infection, and aging could provide crucial information on the long-term contribution of inflammatory signals in dysregulating HSC molecular programs, promoting genomic instability and selecting pre-leukemic clones

inflammation as CHIP-associated mutations in genes such as *TET2* and *DNMT3A* could promote the development of an inflammatory state [88, 89]. In this context, recent studies show an association between clonal hematopoiesis and cardiovascular diseases and provide a new perspective on the broad consequences of the alteration of HSC fitness on the health of non-hematopoietic tissues [90••, 91••]. On the other hand, it has been proposed that inflammation could, through repeated cycles of infection, systemic inflammation, or other environmental exposures, confer a competitive advantage to mutant cells [92]. Whether and how inflammation differentially impacts fitness of normal HSCs and HSCs harboring CHIP mutation are crucial open questions [93]. Similarly, whether other inflammation-associated lifestyle factors such as smoking, alcohol consumption, or diet influence such clonal expansion could constitute a clinically relevant line of investigation [84].

Point 3: Integrating the Inflammatory State in Experiment Models

This review focuses on the broad influence of inflammation in the HSC compartment and its function. However, it is important to note that most of our experimental results are obtained in laboratory mice living in aberrant hygienic conditions. SPF mice develop an aberrant microbiota and therefore present an abnormal basal inflammatory state [94••]. In addition, these mice have a reduced immunological history that is normally associated with the occurrence of infectious episodes in "free-living" animals [95••]. As consequences, SPF mice present an immature immune system more related to human newborn than to adult [94••]. These conditions could impact the early maturation of the HSC compartment at birth and its activity in adults. As microbiota and basal inflammation appears to directly affect the size and composition of the HSC compartment but also tone its basal activity, these models may provide a distorted view of the HSC activity at steady state. We can envision that the overall inflammatory environment affects HSC state of dormancy and modulate HSC activity at steady state. This may play out in the controversy questioning the extent to the HSC contribution to the steady state hematopoietic output [96–99]. Basal inflammation could also tone the HSC response to stress. The absence of normal stimulation may limit the number of HSCs in a recently proposed primed "G_{Alert}" phase of quiescence, a phenotype accompanied by a more robust functional response to stress [100, 101]. Similarly, recent reports indicate that sporadic inflammatory events could lead to long-lasting modifications of the HSCs and their downstream progenitors, suggesting that these hematopoietic compartments could maintain a memory of the past inflammatory challenges [102, 103]. Finally, inflammation and immune signaling are now viewed as an inherent part of the pathogenesis of the hematopoietic malignancies. As such, we can postulate that the specificity of the basal inflammatory state and immunological history of SPF mice could have a direct impact on the modeling of hematopoietic malignancies [104••]. In this context, analysis of "dirty" mice presenting a normal inflammatory environment along with GF and SPF models could help to better define the influence of basal inflammatory signals on the HSC compartment at steady state. Similarly, exploring the impact of the inflammatory environment on the HSC compartment in diverse pathophysiological conditions could improve our understanding of the long and complex path from health to disease in the hematopoietic system (Fig. 3).

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Compliances with Ethical Standards

Conflict of Interest Vinothini Govindarajah and Damien Reynaud declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
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