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Inflammatory Modulation of Hematopoiesis: Linking Trained Immunity and Clonal Hematopoiesis with Chronic Disorders

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Abstract

Inflammation-adapted hematopoietic stem and progenitor cells (HSPCs) have long been appreciated as key drivers of emergency myelopoiesis, thereby enabling the bone marrow to meet the elevated demand for myeloid cell generation under various stress conditions, such as systemic infection, inflammation, or myelosuppressive insults. In recent years, HSPC adaptations were associated with potential involvement in the induction of long-lived trained immunity and the emergence of clonal hematopoiesis of indeterminate potential (CHIP). Whereas trained immunity has context-dependent effects, protective in infections and tumors but potentially detrimental in chronic inflammatory diseases, CHIP increases the risk for hematological neoplastic disorders and cardiometabolic pathologies. This review focuses on the inflammatory regulation of HSPCs in the aforementioned processes and discusses how modulation of HSPC function could lead to novel therapeutic interventions.

INTRODUCTION: HEMATOPOIETIC STEM CELLS AND THE BONE MARROW NICHE IN HEMATOPOIESIS

Bone marrow hematopoietic stem cells (HSCs) are capable of self-renewal and generation of differentiated progeny, hence sustaining the continuous production of billions of mature blood cells daily (1, 2). Mouse HSCs are primarily characterized by the presence of c-Kit and stem cell antigen-1 (Sca-1) on their cell surface and the differential expression of receptors of the signaling lymphocytic activation molecule family ($CD150^+$, $CD48^-$), but they lack mature hematopoietic cell markers ($Lineage^-$ or Lin^-) and CD34 (1). Human HSCs, in contrast, are Lin^- cells expressing CD34 and CD90 but lacking CD38 and CD45RA (1). In the traditional tree-like hematopoietic hierarchy, HSCs are located at the top, giving rise next to multipotent progenitors (MPPs), followed by a stepwise restriction of lineage potential at every branching point downward (2). However, emerging studies revealed an extraordinary functional heterogeneity within the pool of hematopoietic stem and progenitor cells (HSPCs), with lineage decisions also occurring at earlier stages, including at the HSC level (3, 4). For example, recent studies identified HSC clones that are primed for generating megakaryocyte progenitors, without losing their multipotency (5, 6).

The hematopoietic homeostasis is regulated by a complex interplay of cell-intrinsic processes within the HSPCs, including their cellular metabolism, but also cell-extrinsic influences, such as hormonal or neuronal signals (7–9). A central role in HSPC homeostasis is attributed to the specialized bone marrow microenvironment, the so-called HSPC niche. The cellular constituents of the niche include endothelial cells (ECs), mesenchymal stromal cells (MSCs), and pericytes with perivascular location, osteoblasts, bone marrow adipocytes and neuronal cells, as well as HSC-derived cells such as megakaryocytes, macrophages, or T regulatory cells (Tregs); moreover, the niche includes several extracellular matrix proteins (10–12). Additionally, the activity from sympathoadrenergic nerves or even nociceptive neurons in the bone marrow can regulate the niche and thereby HSC functions, including their mobilization and hematopoiesis (8, 11, 13, 14). The location of the HSPCs and their juxtaposition to the niche constituents represent definitive criteria for genuine niches (15). Imaging studies of the bone marrow identified a fraction of quiescent HSCs in the proximity of arterioles (16), consistent with the reduced permeability of the latter, thus facilitating HSC maintenance at conditions of low reactive oxygen species (ROS) (17). Moreover, HSCs may reside close to megakaryocytes (18) or they are distributed in the perisinusoidal space (19). Single-cell RNA sequencing (scRNAseq) analysis identified three bone marrow EC subsets among a total of 17 bone marrow stroma cell subpopulations and demonstrated higher expression of niche factors, such as *Kitl* and *Cxcl12*, in arterial rather than sinusoidal ECs (20). A small $Apelin^+$ EC subset is critical for HSC maintenance at steady state and bone marrow regeneration after transplantation (21). Among niche stromal cells, MSCs comprise several overlapping cell populations with perivascular localization, such as leptin receptor $(LEPR)^+$ cells, Nestin (*Nes*)-GFP^+ cells, $NG2^+$ cells, and CXCL12-abundant reticular (CAR) cells that have been identified based on different approaches, including the use of genetically modified reporter mice (10). A high level of heterogeneity with four distinct $LEPR^+$ MSC populations with differential expression of *Cxcl12*, *Kitl*, or *Il7* was recently demonstrated by scRNAseq (22). A novel approach combining scRNAseq and spatially resolved transcriptomics showed molecular, cellular, and spatial organization of bone marrow niches and differentiated CAR cells into two populations, osteo-CAR and adipo-CAR, with perivascular niche properties (23). Moreover, single-cell protein expression analysis of bone marrow stroma cells by mass cytometry identified a $CD73^+NGFR^{high}$ subpopulation expressing high levels of hematopoietic cytokines under steady state and upon irradiation-induced myelosuppression, hence supporting normal and stress-associated hematopoiesis (24).

A common feature in many forms of hematopoietic stress, including inflammation, infection, or myelosuppressive insults, is the driving of HSCs out of their quiescent state to produce offspring, thereby allowing the bone marrow to meet the increased demands for myeloid cell production, a process termed emergency myelopoiesis (25). Besides emergency myelopoiesis, inflammation-adapted HSPCs engage in further processes, such as the induction of innate immune memory or the emergence of clonal hematopoiesis, and may promote the chronification of inflammatory, cardiometabolic pathologies (25, 26). The inflammatory modulation of hematopoietic progenitors in the aforementioned processes and its ramifications are the focus of this review.

INFLAMMATION-ADAPTED HEMATOPOIETIC STEM AND PROGENITOR CELLS IN EMERGENCY MYELOPOIESIS

Demand-adapted or emergency myelopoiesis represents a crucial homeostatic mechanism of the host in response to stressful conditions, such as disseminated infection and myeloablation from chemotherapy or irradiation. Under such hematopoietic stress, the hematopoietic system swiftly adapts to the enhanced need for myeloid cells, especially neutrophils, by shifting from steady-state myelopoiesis to strongly augmented production of myeloid cells through enhanced proliferation and myeloid-biased differentiation of HSPCs and increased proliferation of lineage-committed granulocyte/macrophage progenitors (GMPs) in the bone marrow (25, 27). Furthermore, single-cell analyses have identified a committed progenitor within the heterogeneous population of GMPs, termed proNeu1, which is committed to exclusively generating neutrophils through an intermediate proNeu2 subset (28). Also supporting demand-adapted myelopoiesis are proNeu2-derived committed neutrophil precursors (termed preNeu), which proliferate upon systemic infection or tumoral stress and produce nonproliferating immature neutrophils that migrate to sites of injury or inflammation (29). In what appears to be a potential feed-forward loop, neutrophils and their bone marrow precursors can enhance emergency myelopoiesis through cross talk with their progenitors or niche cells. Specifically, ROS produced by $Gr1^+$ myeloid cells within the bone marrow during systemic inflammation trigger myeloid progenitor cell expansion and differentiation, while tumor necrosis factor (TNF) from neutrophils acts on the endothelium to promote vessel and hematopoiesis regeneration in bone marrow transplantation (30, 31). Emergency myelopoiesis is normally a protective response. However, under extreme inflammatory conditions, for instance in severe COVID-19 cases, the process may be dysregulated, leading to unfavorable modifications of the myeloid compartment, including dysfunctional mature neutrophils and increased immature neutrophil numbers (32).

Integral to the HSPC response in emergency myelopoiesis is the activation of HSPCs by cytokines and growth factors or by pathogen-associated molecular patterns that interact with their cognate pattern-recognition receptors on HSPCs. Consequently, HSCs switch from a quiescent to a proliferative state and undergo myeloid differentiation (25). As the actions of cytokines and growth factors on HSCs were recently reviewed in detail (25), a select few are mentioned here only briefly. Interleukin (IL)-1β directly stimulates HSC proliferation and myeloid skewing, thereby facilitating bone marrow recovery upon acute stress (33). Conversely, chronic IL-1β exposure leads to HSC exhaustion (33). Additionally, acute exposure to interferon α (IFNα) drives cell-cycle entry and expansion of quiescent HSCs, while chronic exposure to IFNα diminishes HSC self-renewal (34). The growth factor macrophage colony-stimulating factor (M-CSF) acts directly on HSCs, instructing their myeloid differentiation (35). Moreover, the granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor is upregulated on HSCs and MPPs in response to systemic inflammation, thereby driving a pronounced increase in myelopoiesis in response to GM-CSF (36).

In stress-associated hematopoiesis, the bone marrow niche may display dramatic remodeling induced by different stress types, such as radiation or aging (37, 38). Bone marrow stroma remodeling also influences the amplitude of emergency myelopoiesis in the context of infection, as illustrated by the effects of Toll-like receptor (TLR) ligands on niche cells besides HSPCs. HSPCs express TLR4 and can directly respond to pathogen-derived lipopolysaccharide (LPS) with enhanced proliferation (39), although sustained endotoxemia blocks the self-renewal and hence repopulation capacity of HSCs (40). TLR4 in nonhematopoietic bone marrow cells also plays a central role in emergency myelopoiesis (41). Intravital microscopy and positron emission tomography/magnetic resonance imaging (PET/MRI) revealed that, upon LPS administration, bone marrow ECs proliferate and increase the expression of αvβ3 integrin, leading to enhanced vascular permeability. These endothelial adaptations are linked to neutrophil egress and consequent HSPC proliferation (42). Moreover, systemic LPS-induced TLR4/MyD88 signaling in bone marrow ECs leads to production of G-CSF and consequent neutrophil generation (43). Furthermore, upon sterile tissue injury, IL-1/MyD88-induced G-CSF upregulation triggers HSPC expansion and emergency myelopoiesis (44).

Besides instructing myeloid progenitors toward granulopoiesis (45, 46), G-CSF is a major player in HSPC mobilization (27). Mobilized HSPCs migrate to the spleen and other organs where they differentiate to mature cells and contribute to the clearance of local or systemic infections (47, 48). In *Escherichia coli* infection, HSPC mobilization is mediated by a cooperative action between TLR4 and nucleotide-binding oligomerization domain-containing protein 1 (NOD1) in radio-resistant niche cells, leading to induction of G-CSF expression, while TLR4 signaling on hematopoietic cells is dispensable (48). Indeed, transfer of mobilized HSPCs limits infection in recipient mice infected with *E. coli* (48). G-CSF may also affect other niche cells in the context of mobilization. For instance, G-CSF downregulates the expression of essential HSC retention molecules (*Angpt1*, *Kitl*, *Cxcl12*, and *Vcam1*) in *Nes*-GFP$^+$ MSCs (49) or may deplete endosteal macrophages (50), thereby supporting bone marrow egress of HSPCs. Besides macrophages, bone marrow dendritic cells are perivascularly localized and may act as niche cells by promoting HSPC retention. Dendritic cell ablation in mice leads to enhanced bone marrow endothelial expression of CXCR2 and its ligands (CXCL1/CXCL2), thereby resulting in elevated bone marrow vessel permeability and HSPC mobilization (51).

An important aspect in stress-adapted hematopoiesis is the transition of HSCs from quiescence to an activated state, which is facilitated by their metabolic plasticity. HSCs can switch from glycolysis (typically utilized by quiescent/self-renewing HSCs) to mitochondrial oxidative phosphorylation, accompanied by an increase in mitochondrial mass that enables the HSCs to meet the increased bioenergetic demands required for their proliferation, differentiation, and lineage commitment (9, 52). Interestingly, at least upon acute bacterial infection, where a quick host reaction is crucial, the initial increase in HSC mitochondrial mass results from exogenous mitochondria transfer from bone marrow stromal cells through a ROS- and phosphoinositide 3-kinase-dependent mechanism that regulates the opening of connexin channels (53).

Although the inflammatory adaptation of HSPCs is crucial for emergency myelopoiesis and hence host survival under acute stress, certain adaptations, discussed below, may have long-lived effects on HSPC function.

INFLAMMATION-ADAPTED HEMATOPOIETIC STEM AND PROGENITOR CELLS IN TRAINED IMMUNITY

Earlier microbial or inflammatory challenges can imprint a form of epigenetic memory in innate immune cells, enabling them to respond faster and stronger to future (even unrelated) challenges. This state of enhanced immune responsiveness, which represents nonspecific innate

immune memory and is irrespective of adaptive immunity, is designated trained innate immunity (TII) (54, 55) (**Figure 1**). TII can be instigated by vaccines, such as the mycobacterial bacillus Calmette-Guérin (BCG), and by microbes or isolated microbial products, such as β-glucan derived from fungal cell walls. The induction of TII is shaped by metabolic and epigenetic adaptations that are often functionally connected. For instance, the Krebs cycle metabolite α-ketoglutarate acts as a cofactor that enhances the function of key epigenetic enzymes, such as the ten-eleven translocation (TET) family of methyl-cytosine dioxygenases, whereas the acetyl coenzyme A (acetyl-CoA) regulates histone acetylation, a histone mark associated with enhanced gene transcription (54, 55).

Initiation of Trained Immunity in the Bone Marrow

Recent studies in mice showed that TII not only directly affects mature innate immune cells but is also initiated at the level of their bone marrow progenitors, a process that is required for long-lived TII. Sustained immunometabolic, epigenetic, and transcriptional adaptations of HSPCs leading to their proliferation and myeloid differentiation undergird the induction of TII, which gives rise to increased myeloid cell numbers (25, 56–58). Importantly, moreover, bone marrow–based TII can promote enhanced responsiveness to ensuing challenges not only by the HSPCs themselves but also their trained myeloid progeny (25, 54, 55, 59) (**Figure 1**). Therefore, trained HSPCs form the basis for long-term adaptation of mature myeloid progeny to enhanced immune responsiveness. Human relevance for the concept that TII is initiated at the bone marrow HSPC level was provided by analysis of HSPC responses in BCG-vaccinated healthy human volunteers: BCG vaccination induced a sustained transcriptomic myeloid bias in human HSPCs associated with a persistent (≥3 months) heightened responsiveness of peripheral innate immune cells to heterologous stimuli (60). The epigenetic basis of TII in bone marrow hematopoietic progenitors was substantiated by findings that deficiency in Set7, a histone-lysine *N*-methyltransferase that mediates methylation of histone H3 at lysine 4 (H3K4me), resulted in defective β-glucan-induced TII accompanied by reduced expression of *Csf2* (encoding GM-CSF) and *Il1b* in the bone marrow (61); GM-CSF and IL-1β are two cytokines that drive TII-mediated modulation of hematopoietic progenitors, as outlined below (56).

Distinct cytokines can contribute to the induction of TII in the bone marrow, although whether and how the different cytokine signaling pathways interact to cooperatively promote TII are currently poorly understood. In mice, TII induction by systemic β-glucan administration drives IL-1β- and GM-CSF-mediated expansion of myeloid-skewed HSPCs in the bone marrow, including $CD41^+$ long-term-HSCs (LT-HSCs). Moreover, β-glucan-induced TII is linked to metabolic rewiring of HSPCs, including augmented cholesterol biosynthesis and accumulation in HSPCs (which alters the physicochemical properties of the cell membrane), thereby facilitating increased signaling via the common β-subunit of the IL-3/GM-CSF receptor (IL-3Rβ; CD131) and driving myelopoiesis (56). The long-term influence of TII on HSCs was established by transplantation studies. Upon transplantation to lethally irradiated mice, LT-HSCs from β-glucan-trained mice give rise to an increased proportion of myeloid cells (with a corresponding reduction in B cell frequency) in the recipients' blood, compared to LT-HSCs transplanted from untrained mice (56). In addition to IL-1β, type I IFNs are also critical in the induction of TII by β-glucan, although it is not clear whether IL-1β and type I IFNs act on different or overlapping cellular targets. Specifically, β-glucan-administered mice display type I IFN-dependent epigenetic rewiring of GMPs in the bone marrow, resulting in the generation of trained neutrophils with augmented tumor-killing capacity (59).

A key role for IL-1β in the induction of TII is also supported by human studies. Indeed, BCG vaccination in humans induces epigenetic reprogramming of monocytes and protects against experimental viral infection in a manner that is correlated with upregulation of IL-1β and with

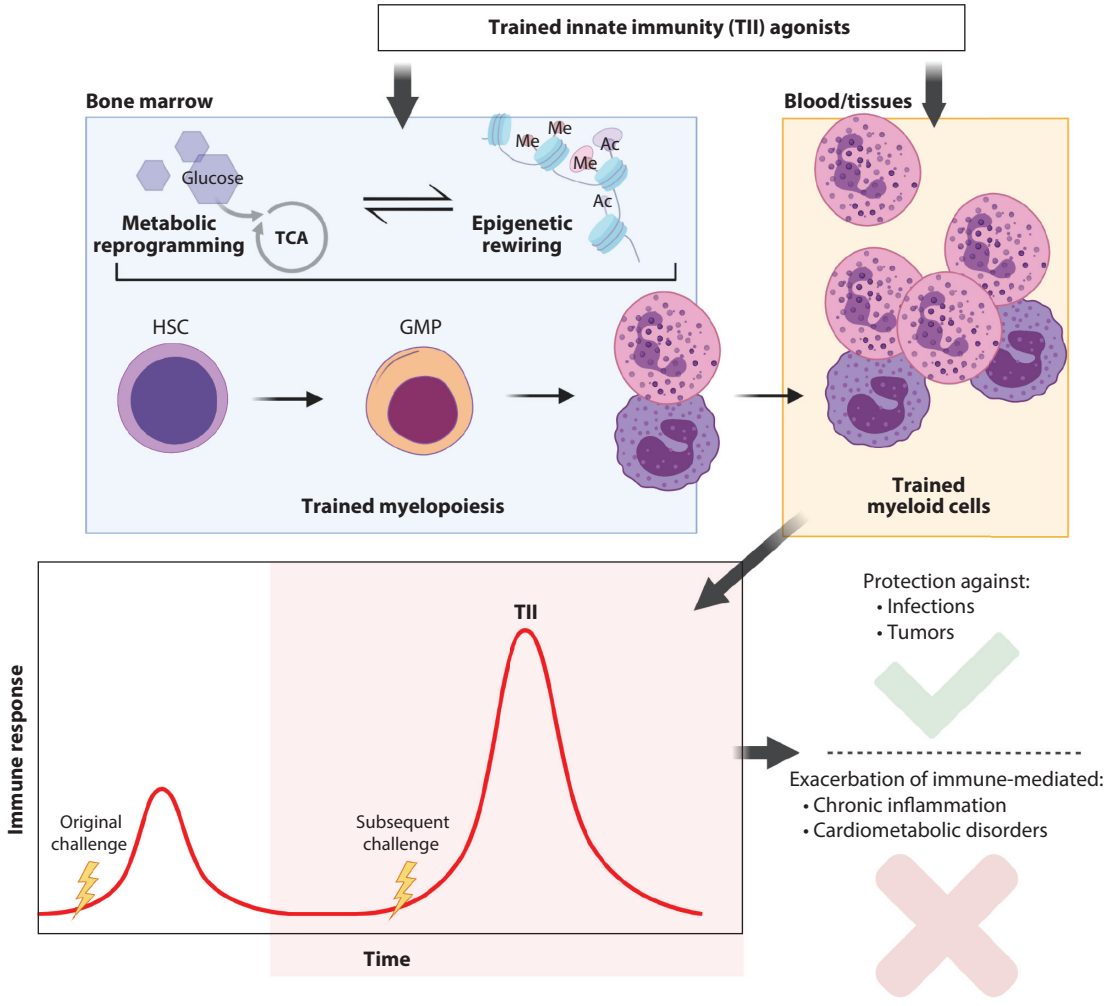

Figure 1

Induction and consequences of TII. TII can be triggered by different agonists, such as a Western diet, vaccines (e.g., the BCG vaccine), infections, and purified agonists (e.g., the fungal cell-wall constituent β-glucan). Although these stimuli can act directly on mature myeloid cells in the periphery (54), long-term TII is initiated at the level of HSPCs in the bone marrow (56, 58). TII agonists stimulate the production of certain inflammatory cytokines in the bone marrow, such as IL-1β (not shown), which in turn induce long-term metabolic, epigenetic, and consequent transcriptional adaptations in HSPCs (similar adaptations occur when TII agonists act directly on mature myeloid cells; not shown). The inflammation-adapted HSCs proliferate and preferentially give rise to hyper-reactive or trained myeloid cell populations (56, 58). This myeloid differentiation bias involves expansion of myeloid-biased HSPCs and myeloid progenitors, e.g., GMPs. The trained/memory phenotype enables innate immune cells (e.g., neutrophils, monocytes/macrophages) to respond faster and stronger to secondary challenges with the same or heterologous stimuli. This state of enhanced immune responsiveness leads to increased protection against infectious or tumoral challenges but could also exacerbate chronic inflammatory and cardiometabolic conditions (25, 54, 55, 59). Abbreviations: Ac, acetylation; BCG, bacillus Calmette-Guérin; GMP, granulocyte/macrophage progenitor; HSC, hematopoietic stem cell; HSPC, hematopoietic stem and progenitor cell; IL-1β, interleukin-1β; TCA, tricarboxylic acid cycle; TII, trained innate immunity. Figure adapted from images created with BioRender.com.

genetic polymorphisms in the IL-1 pathway (62). In contrast, intravenous BCG injection in mice induces IFNγ signaling-dependent proliferation and myeloid-biased differentiation of HSPCs through a myelopoiesis-biased transcriptional program. This leads to the production of epigenetically reprogrammed macrophages that confer enhanced protection against *Mycobacterium tuberculosis* infection in mice, irrespective of adaptive immunity (58). Similarly, acute LPS exposure trains mouse HSCs; LPS-exposed HSCs harbor open chromatin sites in regulatory regions associated with myeloid differentiation (63). These LPS-induced epigenetic changes in HSCs persist for several weeks and confer protection against subsequent infection with *Pseudomonas aeruginosa* (63).

Beneficial Versus Maladaptive Trained Immunity

The notion that TII has evolved as a mechanism that confers broad-based protection against reinfection is supported by adequate evidence from human and animal studies (54). As alluded to above, vaccination of human volunteers with BCG afforded protection against subsequent infection with an attenuated vaccine strain of yellow fever virus, as shown by decreased viremia correlating with increased IL-1β levels (62). Upon training with low-dose *Candida albicans* or purified β-glucan, mice lacking adaptive immunity exhibited elevated production of cytokines and phagocytic killing, which correlated with increased protection against reinfection with *C. albicans*, as compared to untrained controls (64). By inducing expansion of bone marrow HSPCs and increased myelopoiesis, β-glucan also induced protective TII against lung infection by *M. tuberculosis* (65). In the same study, β-glucan failed to induce TII in IL-1 receptor-deficient mice, thereby exhibiting increased mycobacterial burden and lethality as compared to wild-type mice (65), hence underscoring the importance of IL-1β in β-glucan-induced TII (56).

The concept that TII can mediate broad-spectrum, nonspecific protection against multiple unrelated bacterial infections was demonstrated in mice trained by systemic injection of zymosan (β-glucan-containing fungal cell wall preparation) or of heat-killed *C. albicans*. The trained mice were protected against distinct infections, such as *E. coli* peritonitis, *Citrobacter rodentium* enteritis, *P. aeruginosa*–induced pneumonia, lethal listeriosis, and staphylococcal infection (66). Again, the protective effect against listeriosis required intact IL-1 signaling during the induction of TII, as shown by pharmacologic IL-1 receptor antagonism (66). Training with β-glucan also conferred protection against *Leishmania braziliensis* parasitic infection in a manner dependent on both IL-1 and IL-32 (67).

Using a mouse model of polymicrobial sepsis, a recent study investigated potential induction of TII in sepsis survivors (68). The authors demonstrated a sustained shift toward myelopoiesis (indicated by the increased frequency of GMPs in the bone marrow) and a trained phenotype in bone marrow monocytes. Their findings thus highlight the complexity of postseptic sequelae, which has been hitherto dominated by the concept of sustained postseptic immunosuppression (68). However, it is uncertain at present whether postseptic TII can provide beneficial responses to future systemic infections and/or septic challenges. Additionally, many vaccines may have intrinsically incorporated TII-inducing capacity (69, 70). In support of this hypothesis, epidemiological studies have associated specific vaccinations (e.g., BCG) with off-target protection against unrelated infections (71, 72). For further studies supporting the protective potential of TII against infections, the reader is referred to a recent review (54).

TII can also be protective in further disease settings. The β-glucan-induced training of HSPCs and the increment of myelopoiesis confers a beneficial outcome against chemotherapy-induced myelosuppression in mice (56). TII induced by nanobiologic therapy promotes antitumor effects via epigenetic reprogramming of HSPCs (73). Moreover, training of mice with β-glucan leads to

diminished tumor growth by generation of neutrophils with an antitumor phenotype (59). This antitumor effect of β-glucan-induced TII was mediated via type I IFN signaling-dependent transcriptomic and epigenetic rewiring in bone marrow GMPs that gave rise to trained neutrophils with a ROS-producing antitumor phenotype, as assessed by single-cell epigenomic analysis. The antitumor activity of trained granulopoiesis was consistently transmissible via bone marrow transplantation to nontrained recipient mice (59). Via a similar mechanism, BCG-dependent induction of TII in humans also promotes long-lasting epigenetic rewiring of neutrophils associated with enhanced antimicrobial function, including increased ROS production (74). Interestingly, the off-target beneficial effects of BCG vaccination are therapeutically utilized in bladder cancer, with neutrophils being the primary effectors (75, 76).

Intriguingly, TII might also promote maladaptive immune responses that can aggravate chronic inflammatory and metabolic diseases, including atherosclerosis and primary aldosteronism, whereby myeloid cells play a key role in pathogenesis (25, 57, 77–80). Aberrant TII in the bone marrow could be induced by a variety of inflammatory stimuli, including damage-associated molecular pattern molecules, leading to the perpetuation of inflammation in both quantitative and qualitative ways, i.e., by boosting the production of myeloid cells, which also have increased inflammatory potential (25). An exemplar of aberrant TII in myeloid progenitors with pathological consequences was provided by a preclinical study that modeled metabolic syndrome–induced inflammation. Specifically, when a hypercaloric Western-type diet was fed to atherosclerosis-prone, low-density lipoprotein receptor–deficient (*Ldlr*$^{-/-}$) mice, IL-1-dependent transcriptomic and epigenetic reprogramming of GMPs was observed, resulting in the generation of myeloid progeny with enhanced proinflammatory potential (57). Consistent with the long-term effects of TII, this maladaptive rewiring of GMPs persisted even after the mice were switched back to a normal diet (57). A recent clinical study has shown that peripheral blood mononuclear cells from patients with atherosclerosis have increased cytokine production capacity upon ex vivo stimulation more than in their counterparts from healthy controls; moreover, transcriptomic analysis of bone marrow progenitors of patients with atherosclerosis revealed enrichment for neutrophil- and monocyte-related pathways, suggesting a myeloid differentiation bias (81).

Moreover, maladaptive training of HSPCs may provide a basis for mechanistic understanding of comorbidities (82), such as the augmented cardiovascular risk in patients with periodontitis or rheumatoid arthritis (82, 83). In this regard, systemic inflammation associated with experimental arthritis in mice causes an enhancement in membrane cholesterol of bone marrow HSPCs, resulting in increased myelopoiesis. The resulting increased abundance of circulating myeloid cells is associated with increased macrophage burden in the atherosclerotic lesions of Apoe$^{-/-}$ mice subjected to K/BxN-induced arthritis (84). Maladaptive TII may not necessarily involve aberrantly activated inflammatory responses. Myocardial infarction in mice was shown to epigenetically reprogram Ly6C^{high} monocytes in the bone marrow toward an immunosuppressive phenotype, which were recruited to breast tumors favoring their growth (85). Consistent with this, in early-stage breast cancer patients, post–cancer diagnosis cardiovascular events (e.g., myocardial infarction or stroke) were correlated with an elevated risk of cancer recurrence and cancer-specific mortality (85). Further investigations are needed to support the role of maladaptive TII in comorbidities.

CLONAL HEMATOPOIESIS AS BOTH A DRIVER AND CONSEQUENCE OF INFLAMMATION

The concept that advanced age is linked to chronic low-grade inflammation, known as inflammaging, is well established epidemiologically; however, the underlying causes are poorly understood (86–88). Aging enriches for myeloid-biased HSCs at the expense of lymphoid-biased HSCs (89);

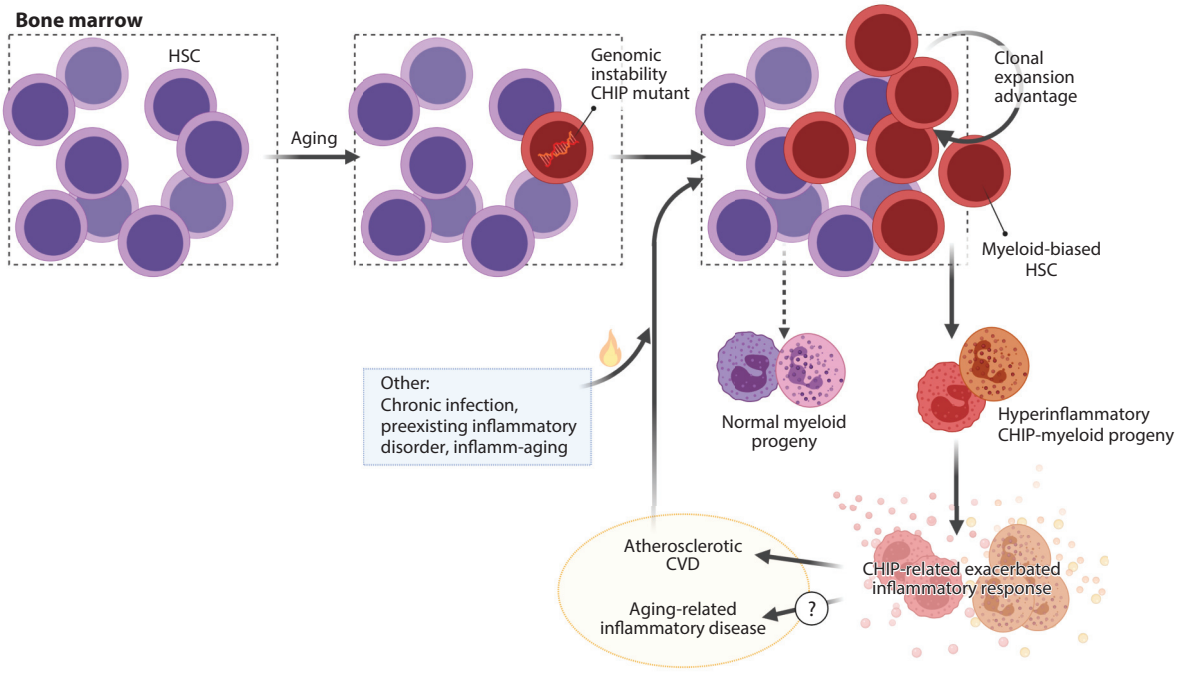

Figure 2

Reciprocal reinforcement between inflammation and clonal hematopoiesis of indeterminate potential (CHIP)-associated hematopoietic stem cell (HSC) clonal expansion. With aging, HSCs may acquire somatic mutations that confer increased self-renewal capacity and myeloid bias, leading to the generation of mutant myeloid progeny with increased proinflammatory potential. In the absence of overt hematological malignancy, this condition is known as CHIP (97). Inflammation promotes apoptosis resistance in CHIP-mutant clones, while suppressing normal HSC clones that may undergo exhaustion (125, 126). Therefore, the competitive expansion advantage of CHIP-mutant clones over normal clones is accentuated in the setting of inflamm-aging or preexisting chronic infection or inflammatory disorder. The generation of hyperinflammatory myeloid cells elevates systemic inflammation that in turn may contribute to the pathogenesis of atherosclerotic cardiovascular disease (CVD) (99, 112) and perhaps other inflammatory conditions that emerge as comorbidities (25). These diseases increase the inflammatory burden and hence foster further clonal expansion of CHIP-mutant HSCs, which in turn can contribute to a vicious cycle that accelerates progression of the comorbid conditions. Figure adapted from images created with BioRender.com.

additionally, aged HSCs display diminished self-renewal (90–92). Aged LT-HSCs are enriched for CD61^{high} LT-HSCs, a myeloid-biased subset that expands with age and is highly responsive to proinflammatory stimuli (92). A mechanism by which aging may further contribute to aberrant, inflammatory myelopoiesis and hence chronic inflammation is through clonal hematopoiesis of indeterminate potential (CHIP) (93–95). CHIP results from the progressive accumulation with age of somatic mutations in HSPCs that confer to the mutant cell a proliferative advantage that allows its clonal expansion. This, in turn, prompts the generation of mutant progeny cells, which comprise an outsized fraction of leukocytes in the periphery (95, 96) (**Figure 2**). In CHIP, the acquisition of somatic mutations occurs in individuals without hematological malignancy (95, 97); however, epidemiological studies indicate that CHIP is linked to an increased risk of hematologic malignancies and atherosclerotic cardiovascular disease (CVD) and type 2 diabetes (96, 98–101). However, on the basis of the biological properties of CHIP clones, CHIP might also contribute to aging-related inflammatory disorders (25). Moreover, such association between CHIP and chronic

inflammatory disorders may be bidirectional, as supported by evidence outlined below (**Figure 2**). It is therefore conceivable that CHIP may be both triggered by and contribute to maladaptive TII.

CHIP-Related Mutations and Inflammatory Disease

With a variant allele fraction threshold set at 2%, CHIP-mutant clones are rare in individuals younger than 40 but are detected in >10% of individuals older than 70 (95, 97, 98). CHIP clones may have substantial downstream effects, as a median of 18% of peripheral blood cells may carry a specific CHIP mutation, i.e., arise from a single mutated HSC (96). CHIP mutations occur most frequently in three genes encoding epigenetic regulators: *TET2* (*ten-eleven translocation methylcytosine dioxygenase 2*), *DNMT3A* (*DNA methyltransferase 3A*), and *ASXL1* (*associated sex combs-like 1*) (94–96, 98, 102). *TET2* and *DNMT3A* control cytosine demethylation and methylation, respectively, and together account for almost two-thirds of acquired CHIP mutations, which typically cause loss of function of the mutant allele (96, 98, 102). *ASXL1* plays a critical accessory role in the trimethylation of lysine 27 of histone 3 by physically interacting with the histone methyltransferase complex PRC2 (polycomb repressive complex 2) (103).

In humans and mice, loss-of-function mutations in *TET2* are associated with enhanced HSPC expansion and a myeloid-differentiation bias (104–106). Loss-of-function mutations in *DNMT3A* appear to affect all hematopoietic lineages. CHIP-related *DNMT3A* mutations are associated with multipotent lineages in humans, and genetic ablation of *Dnmt3a* in mice increases the risk for both myeloid and lymphoid malignancies (106–109). In patients with aortic valve stenosis, those individuals with CHIP-related *DNMT3A* mutations displayed proinflammatory $CD4^+$ T cell polarization, whereas those with CHIP-related *TET2* mutations exhibited increased numbers of proinflammatory monocytes (105). ASXL1 balances self-renewal and differentiation of HSCs, whereas hematopoietic-specific deletion of *Asxl1* in mice causes multilineage cytopenias and dysplasia with elevated HSPC numbers, thus resembling human myelodysplastic syndrome (110).

Most human studies on CHIP and associated cardiovascular risk have focused predominantly on mutations in *DNMT3A* and *TET2*, although the latter has been studied more extensively in preclinical mechanistic models, which provided evidence for a causal link between TET2 loss-of-function and accelerated atherosclerosis (99, 111, 112). TET2 catalyzes the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine, which is ultimately converted to unmodified cytosine (113, 114). TET2, therefore, essentially functions as a demethylase, and its inactivation leads to DNA hypermethylation within active enhancers and promoters (113, 114). By preventing aberrant DNA methylation in CpG islands and promoters, TET2 homeostatically modulates the self-renewal, expansion, and differentiation of HSCs. As alluded to above, TET2 inactivation can lead to clonal HSC dominance through increased self-renewal and a myeloid differentiation bias in humans and mice (104, 115, 116). Moreover, noncatalytic functions of TET2, such as its ability to recruit histone deacetylase 2 (HDAC2) for transcriptional regulation, also contribute to HSPC homeostasis (117, 118).

As most human CHIP-*TET2* mutations involve loss of function, $Tet2^{-/-}$ mice are a relevant model to study CHIP-associated pathology. Consistent with the association of CHIP-*TET2* mutations with human coronary heart disease, $Ldlr^{-/-}$ mice, which were reconstituted partially or completely with bone marrow cells from mice with homozygous or heterozygous *Tet2* null mutations, displayed accelerated atherosclerosis featuring macrophages with enhanced production of IL-1β and IL-6 (99, 112). Even when only a fraction of transplanted HSCs bore the TET2 deficiency (10% $Tet2^{-/-}$ and 90% wild-type bone marrow cells), the recipient mice displayed preferential clonal expansion of $Tet2^{-/-}$ hematopoietic cells with a mild myeloid skewing (99), consistent with clinical studies on CHIP-*TET2* mutations and their impact on hematopoiesis (106, 119).

Moreover, TET2-deficiency-driven CHIP increases macrophage-derived IL-1β levels in white adipose tissue and aggravates insulin resistance in obese or aged mice (120), consistent with the association between CHIP and type 2 diabetes (101).

Individuals with CHIP owing to mutations in *TET2* (as well as in *DNMT3A* or *ASXL1*) have normal total and differential leukocyte counts (112). Similarly, *Ldlr*$^{-/-}$ mice reconstituted with TET2-deficient bone marrow cells have normal blood cell counts (99, 112). Thus, the acceleration of atherosclerosis in these mice (relative to *Ldlr*$^{-/-}$ mice reconstituted with wild-type bone marrow cells) was attributed to the generation of TET2-deficient myeloid progeny with increased proinflammatory potential rather than leukocytosis (99, 112). Activated TET2-deficient macrophages produce increased levels of IL-6 through a mechanism that is independent of the catalytic function of TET2; specifically, TET2 is required for recruitment of HDAC2 to the *Il6* promoter, thereby restraining *Il6* transcription via histone deacetylation (118) (**Figure 3**). Moreover, activated TET2-deficient macrophages exhibit increased production and release of IL-1β attributable to both NLR family pyrin domain-containing 3 (NLRP3) inflammasome activation and increased *Il1b* transcription (99, 121) (**Figure 3**). The mechanism whereby TET2 deficiency increases macrophage *Il1b* transcription is also independent of the TET2 catalytic activity, involving decreased HDAC-mediated histone deacetylation (99) (**Figure 3**).

Notably, transplantation of *Tet2*$^{-/-}$ bone marrow cells into nonpreconditioned wild-type mice resulted in spontaneous development of age-related cardiac dysfunction typified by increased fibrosis and hypertrophy (122). This study thus suggests that loss of TET2 in bone marrow may cause CVD even in the absence of known predisposing risk factors (122).

Impact of the Inflammatory Bone Marrow Microenvironment on CHIP Clones

In CHIP, the bone marrow microenvironment appears to be poised for increased inflammatory responses upon infectious or inflammatory insults. For instance, given that macrophages are a critical component of the HSC niche (50), the enhanced proinflammatory nature of *TET2*-mutant macrophages (99, 112) may promote inflammatory responses within the niche in response to infection or other challenges. CD4^{+}CD25^{+}FoxP3^{+} Tregs, which are integral to the immune privilege of the HSC niche (123), may also be affected by loss-of-function *TET2* mutations, as TET family enzymes contribute to Treg stability and suppressive function (124). Therefore, it is likely that *TET2*-mutant Tregs may not efficiently prevent aberrant inflammatory responses in the bone marrow.

This inclination for enhanced inflammation may contribute to further expansion of CHIP clones (**Figure 2**). Indeed, by inducing Src homology 2–containing protein tyrosine phosphatase 2 (SHP2) and signal transducer and activator of transcription 3 (STAT3) signaling, IL-6 promotes B cell lymphoma 2 (BCL-2)- and myeloid RNA regulator of Bim-induced death (*Morrbid*)-mediated antiapoptotic pathways and supports the survival of TET2-deficient HSPCs (125) (**Figure 3**). TNF also promotes resistance to apoptosis of *TET2*-mutant human and *Tet2*$^{-/-}$ mouse HSPCs (126). Intriguingly, TET2-deficient HSPCs thrive under inflammatory conditions, while TET2-sufficient HSPCs are suppressed in terms of self-renewal (125). Thus, *TET2*-mutant clones appear to have a competitive advantage over normal clones for expansion in an inflammatory environment, as in the setting of inflamm-aging (93, 125–127) (**Figure 2**). Consistently, systemic inflammation induced by TLR2 ligands or disseminated intestinal bacteria promotes IL-6-dependent preleukemic myeloproliferation in *Tet2*$^{-/-}$ mice (128). Interestingly, the surface expression of IL-6Rα in *Tet2*$^{-/-}$ GMPs is upregulated, in a cell-intrinsic manner (independently of microbial challenge), thus rendering TET2-deficient mice more sensitive to the myeloproliferative effects of IL-6 (128). The potential involvement of IL-6 with CHIP-associated pathology is supported by a

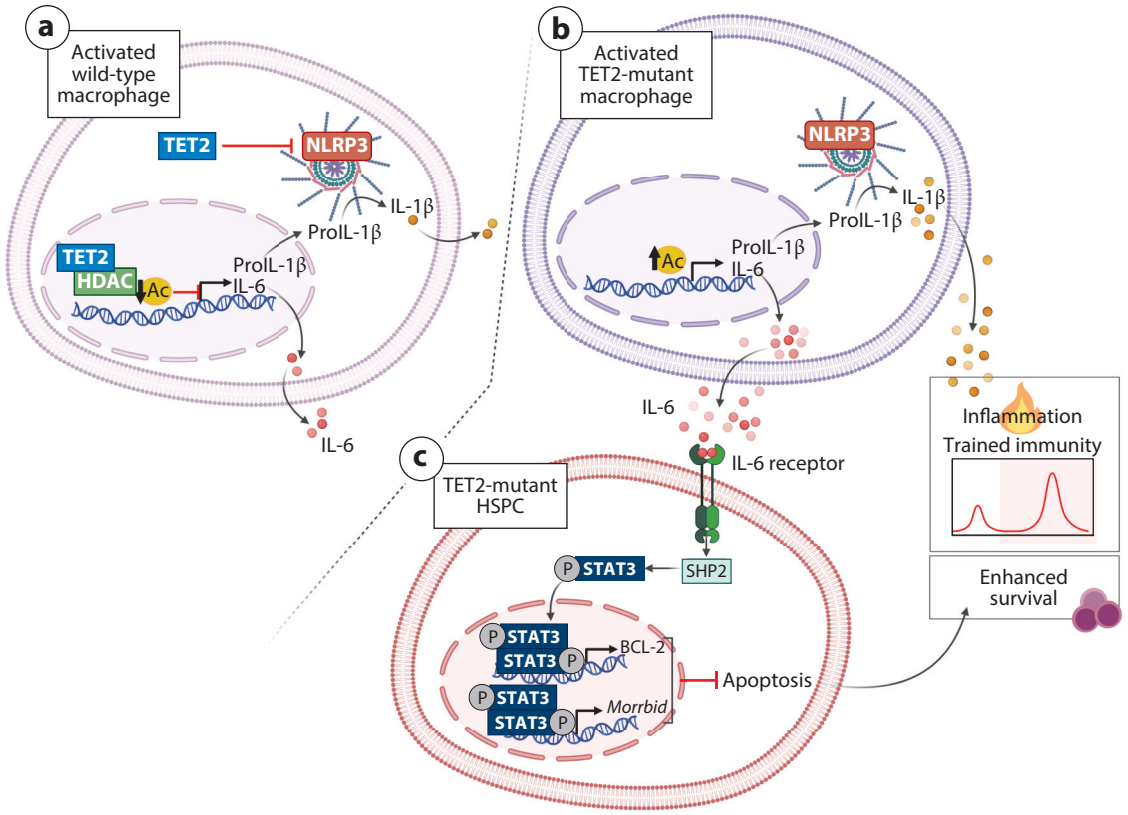

Figure 3

TET2 regulation of proinflammatory cytokines and HSPC survival. (*a*) In wild-type macrophages, TET2 promotes the recruitment of histone deacetylase to the IL-6 promoter and downregulates *Il6* transcription through histone deacetylation (118). Through a similar mechanism at the IL-1β promoter, TET2 downregulates *Il1b* transcription (99). TET2 also restrains the expression and activity of the NLRP3 inflammasome, thereby downregulating the release of mature IL-1β by activated macrophages. Ac denotes acetylation (of histones). (*b*) In TET2-deficient macrophages (or with TET2 loss-of-function mutation), IL-1β is overproduced upon infection or inflammation and exacerbates inflammatory conditions, as in the setting of atherosclerosis (99). It is hypothesized that IL-1β produced by *TET2*-mutant macrophages in the bone marrow might also contribute to induction of TII at the HSPC level (56). (*c*) In the setting of TET2-associated CHIP, IL-6 released by TET2-deficient macrophages in the bone marrow can act on TET2-deficient HSPCs and promote their survival at the expense of normal HSPCs, which are suppressed. The mechanism involves IL-6-induced SHP2 and STAT3 signaling, which upregulates the expression of BCL-2 and the long noncoding RNA *Morrbid*, which in turn promote antiapoptotic pathways and support the survival of TET2-deficient HSPCs (125). Abbreviations: BCL-2, B cell lymphoma 2; CHIP, clonal hematopoiesis of indeterminate potential; HDAC, histone deacetylase; HSPC, hematopoietic stem and progenitor cell; IL-1β, interleukin-1β; IL-6, interleukin-6; *Morrbid*, myeloid RNA regulator of Bim-induced death; NLRP3, NLR family pyrin domain-containing 3; P, phosphorylation; ProIL-1β, IL-1β promoter; SHP2, Src homology 2–containing protein tyrosine phosphatase 2; STAT3, signal transducer and activator of transcription 3; TET2, ten-eleven translocation methylcytosine dioxygenase 2; TII, trained innate immunity. Figure adapted from images created with BioRender.com.

prospective study. Although carriers of large *DNMT3A*- or *TET2*- associated CHIP clones (variant allele fraction >10%) displayed an increased risk for CVD, a subset of those individuals who also carried an *IL6R* mutation (p.Asp358Ala; associated with reduced surface expression of the IL-6 receptor) exhibited fewer CVD events (129).

In conclusion, whereas inflammation may induce exhaustion in normal HSPC clones (25, 27), it can promote the proliferation of mutant HSPC clones (125–127). CHIP-related *TET2*

mutations lead to the expansion of HSPC clones with a myeloid differentiation bias and increased proinflammatory potential (99, 104, 112, 115, 116). Therefore, at least TET2-associated CHIP may potentially increase susceptibility to different myeloid cell-driven inflammatory diseases that may emerge as comorbidities (**Figure 2**). The inflammation-induced expansion of mutant HSPC clones may also predispose the affected individual to the onset of a myeloid malignancy (125–127), as discussed below.

Inflammation and CHIP-Associated Hematological Neoplasia

CHIP increases the risk for hematological neoplastic disorders, which may develop with the accrual of additional mutations. For instance, a cell from a CHIP clone may acquire a loss-of-function mutation in a critical transcription factor regulating HSPC differentiation (e.g., *RUNX1* or *CEBPA*) and/or further mutations, resulting in development of myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) (95, 130). In MDS, increased HSC self-renewal is accompanied by inhibition of progenitor differentiation, leading to bone marrow dysplasia and peripheral blood cytopenia (95). In myeloproliferative neoplasms (MPNs), the accrued mutations lead to expansion of the HSPC pool and overproduction of blood cell lineages, including leukocytosis. JAK2(V617F), the product of a mutation that leads to constitutive activation of the Janus kinase 2 (JAK2) and stimulates HSC renewal and lineage differentiation, is a common mutation associated with MPNs and also observed in CHIP (95, 130). Overall, mutations associated with malignant clones involve genes that encode for epigenetic regulators (e.g., *DNMT3A*, *TET2*, *ASXL1*, *IDH*, *MLL*), transcription factors (e.g., *RUNX1*, *NPM1*), tyrosine kinases (e.g., *FLT3*), tumor suppressors (e.g., *TP53*), and splicing factors (e.g., *SF3B1*) (130). Although MPN, MDS, and AML can evolve in a stepwise fashion from CHIP, these malignancies can also develop de novo from normal hematopoiesis (130).

As discussed above, mutant preleukemic HSCs have a competitive growth advantage in an inflammatory bone marrow microenvironment (**Figure 2**), which is also conducive for the accumulation of additional mutations, thereby promoting the emergence of malignant HSCs, the leukemic stem cells (130, 131). Studies in humans have revealed a significant association of chronic immune stimulation (owing to chronic infections or autoimmune and inflammatory diseases) with MDS, MPN, and AML (132). In this regard, the promoting effect of inflammation on premalignant HSC clone proliferation may increase the likelihood for DNA replication-associated mutations (133). In the bone marrow niche, MSC-mediated inflammation through the release of S100A8/A9 alarmins induces TLR4-dependent genotoxic stress in HSPCs, leading to DNA damage, cell-cycle arrest, cell death, and progression to leukemia in a mouse preleukemic model (134). This inflammatory pathway was shown to be of human relevance, because in low-risk MDS patients, S100A8/9 was aberrantly overexpressed in MSCs and activation of the S100A8/9-TLR axis predicted leukemic progression (134). The capacity of S100A9 to induce NADPH oxidase-dependent ROS production, NLRP3 inflammasome activation and pyroptosis in HSPCs from MDS patients may also contribute mechanistically to the MDS phenotype (135). Interestingly, the cell-intrinsic response of MDS-associated HSPCs to inflammatory stimuli, which involves TLR signaling through the noncanonical nuclear factor-kappa B (NF-κB) pathway, protects these HSPCs from the detrimental effects of chronic inflammation in comparison to normal HSPCs (136).

The importance of the bone marrow niche, and specifically of MSCs, in HSC homeostasis and prevention of myelodysplasia is also supported by findings that dysfunction of the mesenchymal osteolineage cells is sufficient to drive the development of myelodysplasia and subsequent emergence of secondary AML in mice (137). Intriguingly, in a mouse model of MPN, leukemic myeloid

cells remodel the niche by stimulating MSCs [via cell–cell interactions and secreted factors such as C-C motif chemokine ligand-3 and thrombopoietin] to differentiate into functionally altered, proinflammatory osteolineage cells; these accumulate in the bone marrow niche and further impair normal hematopoiesis while favoring leukemic stem cell expansion and overproduction of leukemic myeloid cells (138). In the JAK2(V617F) MPN model in mice, mutant HSCs release IL-1β, which causes neuroglial damage that compromises the survival and function of Nestin$^+$ MSCs, thereby leading to the expansion of mutant HSCs, exacerbating MPN pathogenesis (139). Importantly, sympathetic nerve fibers (which normally innervate Nestin$^+$ MSCs and promote their HSC-supporting ability) are consistently reduced not only in mice expressing the JAK2(V617F) mutation in HSCs but also in the bone marrow of MPN patients (139). Similarly, in the MLL-AF9 AML model, AML development disrupts the sympathetic nerve fibers and consequently the normal function of Nestin$^+$ MSCs, leading to a remodeled bone marrow niche that supports leukemic stem cell development at the expense of healthy HSCs (140).

INFLAMMATORY MODULATION OF HEMATOPOIETIC PROGENITORS IN CARDIOMETABOLIC DISEASES

As discussed above, the inflammatory modulation of the bone marrow and hematopoietic progenitors therein is associated with increased myelopoiesis and enhanced numbers of circulating myeloid cells with elevated inflammatory responsiveness. This concept has emerged as a central mechanism contributing to chronicity and progression of cardiometabolic diseases (25, 26, 141).

An adipose tissue–bone marrow connection has been described in obesity. Diet-induced obesity results in the expansion of myelopoiesis and generation of macrophages with increased inflammatory capacity (142). Elevated production of proinflammatory adipose tissue macrophages is also observed in mice subjected to a high-fat diet after bone marrow transplantation from obese mice, as compared to bone marrow from lean mice, indicating that the myeloid bias of bone marrow HSPCs from obese mice is long lived and transmissible via transplantation (142). The obesity-driven myeloid bias of bone marrow progenitors is mediated via the TLR4/MyD88 and Toll/IL-1 receptor domain-containing adapter protein-inducing interferon-β signaling cascade (143). The obesity-induced increased myelopoiesis, leading to neutrophilia and monocytosis, is driven by a pathway that involves S100A8/A9-dependent TLR4 and inflammasome activation in adipose tissue macrophages, culminating in enhanced IL-1β production, which promotes myeloid progenitor expansion, as shown by adipose tissue transplantation experiments (144). Obesity-induced cell-intrinsic changes in HSCs include upregulation of the transcriptional repressor Gfi1, which promotes HSC quiescence and integrity (145, 146). Obesity thus drives aberrant HSC responses upon hematopoietic stress, including increased reconstitution potential upon primary HSC transplantation and exhaustion upon subsequent transplantations (146). In diabetic mice, hyperglycemia-driven enhanced myelopoiesis in the bone marrow is mediated by neutrophil-derived S100A8/A9 that stimulates the expansion of myeloid progenitor cells. The resulting neutrophilia and enhanced numbers of circulating Ly6C^{high} monocytes promote atherosclerotic lesion development. Consistently, in type 1 diabetes patients, the concentration of S100A8/A9 in the plasma associates with leukocyte counts and coronary artery disease (147).

An adipogenic differentiation bias is observed in bone marrow MSCs in obesity (148) and in type 2 diabetes (149). Specifically, diet-induced obesity in mice triggers alterations of bone marrow niche cells, including reduced osteoblastic and increased adipogenic differentiation of MSCs, which are mediated by gut microbiome changes in high-fat diet-fed mice. The microbiome-related effects of diet-induced obesity on the bone marrow niche are associated with diminished bone marrow regeneration upon chemotherapeutic stress and contribute to the obesity-induced

bone marrow myeloid bias (148). In human and mouse diabetes, bone marrow adipocytes drive further adipogenic differentiation of bone marrow MSCs in a paracrine manner, dependent on the chemokine MCP-1 (149). Hence, adipocytes in the bone marrow and marrow adipose tissue provide an additional layer of cross talk among systemic metabolic dysregulation in obesity, diabetes, and the bone marrow niche. Bone marrow adipocytes, representing ~5% of $LEPR^+$ cells within the bone marrow, are a substantial source for stem cell factor and critical for regeneration of hematopoiesis after irradiation (150). Aging and a high-fat diet increase the adipocyte abundance in the bone marrow (151), and bone marrow adipocyte–derived stem cell factor promotes HSPC expansion and their myeloid and lymphoid differentiation in diet-induced obesity (152).

Capillary rarefaction accompanied by dysfunction of sorted $CD146^+$ pericytes is found in the bone marrow of diabetic patients. Bone marrow MSCs from diabetic patients display decreased ex vivo proliferation, migration, and defective angiogenesis-promoting properties, due to reduced secretion of fibroblast growth factor 2 (FGF2) and C-X-C motif chemokine ligand 12 (CXCL12) (153). The bone marrow pericyte dysfunction in diabetes may contribute to maladaptive remodeling of the bone marrow microvasculature in diabetes (153). Similarly, mouse experiments in models of obesity and streptozotocin-induced diabetes showed HSPC expansion and augmented myelopoiesis associated with reduced expression of *Cxcl12* in bone marrow endothelium. Moreover, epithelial growth factor receptor (EGFR) signaling in bone marrow ECs plays a protective role by reducing HSPC proliferation and myelopoiesis in diabetic mice, as shown by experiments with mice bearing endothelial-specific *Egfr* inactivation (154).

Leptin is a hallmark adipokine of the adipose tissue and its secretion increases in obesity (155). Adipose tissue–derived leptin acts on $LEPR^+$ stromal cells in the bone marrow to reduce expression of factors promoting HSPC quiescence and retention, such as *Cxcl12*, *Vcam1*, and *Angpt1*, thereby leading to enhanced HSPC proliferation and leukocyte generation. Exercise downregulates leptin levels, thus reducing HSPC proliferation and myelopoiesis and inflammatory cell generation in the context of atherosclerosis, without impairing emergency hematopoiesis (156). Therefore, a leptin-dependent pathway may link sedentary lifestyle to enhanced myelopoiesis and inflammatory cell generation that is reversible by exercise (156).

Significant evidence suggests that inflammatory adaptation of HSPCs in the bone marrow toward enhanced myelopoiesis contributes to atherosclerosis and CVD (25, 26, 81, 157, 158). Altered cholesterol metabolism in HSPCs may provide the link between hypercholesterolemia and increased myelopoiesis in atherosclerosis (25, 159). Increased cellular cholesterol levels in HSPCs, associated with inactivation of cholesterol efflux mechanisms (ATP-binding cassette transporters ABCA1 and ABCG1 or apolipoprotein E), promote the surface expression of CD131 (IL-3Rβ chain) on HSPCs. The resulting enhancement in IL-3- and GM-CSF-mediated signaling in HPSCs triggers their proliferation and myeloid bias, and hence increased myelopoiesis, neutrophilia, and monocytosis, leading to atherosclerosis progression (159–161). Augmented cholesterol accumulation and CD131 signaling in HSPCs also underlie the enhanced myelopoiesis in the context of TII, indicating that trained myelopoiesis may also link hypercholesterolemia with vascular inflammation (55, 56). Furthermore, apoA-I binding protein facilitates cholesterol efflux from the hemogenic endothelium in zebrafish, which leads to activation of the main transcriptional regulator of cholesterol synthesis, sterol regulatory element-binding protein 2 (Srebp2), and subsequently of NOTCH signaling, leading to increased HSPC generation. This mechanism may contribute to hypercholesterolemia-mediated HSPC expansion in adults, as suppression of Srebp2 inhibited HSPC expansion in hypercholesterolemic, Western diet–fed $Ldlr^{-/-}$ mice (162). Moreover, $CD34^+CD45^+$ HSPC numbers in healthy humans correlated with cholesterol levels, while circulating HSPCs from individuals with high low-density lipoprotein displayed an upregulation of SREBP2 and NOTCH1, compared to individuals with low low-density lipoprotein (162).

Together, cholesterol metabolism in HSPCs promotes their adaptations induced by and contributing to perpetuation of cardiovascular inflammation.

Acute ischemia of the myocardium leads to increased myelopoiesis in the bone marrow and at extramedullary sites (163, 164). HSPCs mobilized from the bone marrow into the spleen after myocardial infarction, due to elevated sympathoadrenergic activity, facilitate augmented extramedullary myelopoiesis (165). Moreover, IL-1β upregulation post–myocardial infarction drives myelopoiesis in the bone marrow and enhanced monocyte generation in the spleen (164, 166). Myocardial infarction also triggers the expansion of a myeloid-biased subpopulation of $CCR2^+$ HSPCs with superior proliferative capacity but reduced self-renewal capacity as compared to HSCs (163). Conversely, GM-CSF, which is produced locally in the infarcted myocardium of mice and humans, acts distally in the bone marrow to activate the expansion of a myeloid-biased MPP subset (MPP3) expressing CD131 (167). Augmented myelopoiesis is also observed in $Apoe^{-/-}$ mice upon chronic angiotensin II infusion and development of aneurysm of the abdominal aorta in an IL-27 signaling-dependent manner (168).

In conclusion, cardiometabolic diseases promote the adaptation of HSPCs toward heightened myelopoiesis. This HSPC adaptation may further propagate inflammation by creating a feed-forward loop between the bone marrow and the cardiometabolic disorder (25).

SUMMARY AND OUTLOOK

Inflammation-adapted HSPCs are critically involved in emergency myelopoiesis, trained immunity, and the development of CHIP and thus may not only confer protection in acute infections or other conditions but may also contribute to chronification of inflammatory disorders, as outlined above (25, 26). The enormous complexity of HSPCs and niche biology requires that future studies focus on understanding how HSPCs and multiple niche components interact in the context of demand-adapted myelopoiesis and in different forms of hematopoietic stress. For instance, a cooperative network comprising different bone marrow niche cells (megakaryocytes and ECs), HSPCs, growth factors, and cytokines facilitates the response of HSPCs to acute thrombocytopenia (169). Therefore, understanding the adaptation of HSPCs and the niche, as well as the interactions thereof by infectious and inflammatory stimuli and the consequences for emergency myelopoiesis, will likely require systems biology approaches in future investigations.

The retention of innate memory for enhanced immune responsiveness in bone marrow progenitors and their myeloid progeny may not only exacerbate an existing chronic inflammatory disorder but may also underlie the comorbid connection between distinct inflammatory diseases (79, 82, 157). The inflammatory adaptation of HSPCs in cardiometabolic disease contributing to disease chronicity may actually represent maladaptive TII, given the significant resemblances between the two processes (25). Thus, blocking IL-1β and/or other cytokines involved in maladaptive TII may reduce the risk of multiple comorbidities. Targeting inflammatory cytokines, such as IL-6 and IL-1β, may also attenuate the impact of CHIP on promoting CVD. The notion that IL-1β is implicated in the innate immune training of HSPCs in the bone marrow, in enhanced myelopoiesis in cardiometabolic disease (157), and in CHIP-related exacerbated cardiovascular inflammation, is consistent with the successful application of IL-1β blockade in the CANTOS trial for the treatment of atherosclerosis (170). Importantly, patients with *TET2* mutant clones appear to respond better to anti-IL-1β treatment than those with non-*TET2* mutant clones associated with CHIP (171).

Population-level prevention of CHIP-related comorbidities could, at least in principle, be achieved by identifying at-risk individuals with CHIP-mutation-positive clones and treating them with inhibitors of inflammatory pathways implicated in the expansion and pathological

consequences of CHIP-mutant clones. In the same vein, because IL-1β, IL-6, IL-8, TNF, and IFNs are commonly increased in hematologic malignancies and may contribute to their pathogenesis, pharmacologic targeting of these inflammatory pathways could prevent or mitigate the oncogenic potential of underlying mutations associated with MDS, MPN, and AML (131, 172). In this context, the JAK2 inhibitor ruxolitinib inhibited neutrophil extracellular trap (NET) formation and thrombosis in a JAK2(V617F)-driven MPN mouse model (173), perhaps because JAK2 inhibition may have prevented the generation of altered hematopoietic cell types that could contribute to excessive thrombotic activity. Moreover, approaches to inhibit noncanonical NF-κB signaling might attenuate MDS progression (136).

Further studies on the long-term alterations of HSPCs in cardiometabolic and other chronic inflammatory diseases may also provide a mechanistic basis for the reciprocal connection between aberrant myelopoiesis, as present in CHIP, and enhanced risk for these inflammatory disorders (25, 112). The overlaps and distinctions between processes, such as HSPC adaptations in cardiometabolic and other inflammatory diseases, maladaptive trained immunity, and CHIP, as well as their potential cross talk, should be addressed in future studies.

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