Neutrophils as regulators of the hematopoietic niche

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Abstract

The niche that supports hematopoietic stem and progenitor cells (HSPC) in the bone marrow is a highly dynamic structure. It maintains core properties of HSPC in the steady state, and modulates their proliferation and differentiation in response to changing physiological demands or pathological insults. The dynamic and environment-sensing properties of the niche are shared by the innate immune system. Thus, it is not surprising that innate immune cells, including macrophages and neutrophils, are now recognized as important regulators of the hematopoietic niche, and ultimately of the stem cells from which they derive. This review synthesizes emerging concepts on niche regulation by immune cells, with a particular emphasis on neutrophils. We argue that the unique developmental, circadian and migratory properties of neutrophils underlie their critical contributions as regulators of the hematopoietic niche.

Introduction

Neutrophils are innate, polymorphonuclear leukocytes that act as the first line of host defense against invading pathogens. Central to their function is their ability to be recruited to sites of infection, to recognize and phagocytose microbes, and to kill pathogens through a combination of cytotoxic mechanisms (reviewed in [1]). These include the production of reactive oxygen species (ROS), the release of antimicrobial peptides, and the extrusion of their nuclear contents to form extracellular traps (NETs). Beyond their prominent immune roles, recent years have seen a remarkable emergence of unexpected non-immune functions of neutrophils in homeostasis as well as in diseases with an important inflammatory component, including systemic lupus and cancer [2].

A wealth of recent studies have begun to dissect the function of immune cells, including neutrophils, in the bone marrow. These studies most prominently highlight the diversity of properties of a cell type that not long ago was regarded as purely cytotoxic and pro-inflammatory. Here, we review fundamental aspects of neutrophil and bone marrow niche biology, and discuss the functional interplay between neutrophils and other immune cells within these niches that help to preserve HSPC. We finally consider temporal regulation of the hematopoietic niche driven in part by the unique circadian properties of neutrophils, as this highlights novel layers of interaction between immunity and hematopoiesis.

Developing neutrophils and neutrophils in development

Neutrophils are short-lived cells, as they are generally believed to circulate for only 6-12 h in mice and humans [3] [4]. Their short lifespan in circulation demands constant production and release from the bone marrow, with an estimated production rate in humans of around 10^{10} cells per day [5]. Given their indispensable anti-microbial roles but potential toxic activity in tissues, both excessive and deficient production of neutrophils can have major detrimental consequences for the organism. Indeed, neutrophil homeostasis is tightly regulated through a balance between granulopoiesis, storage and egress from the bone marrow, intravascular margination, clearance, constitutive death by apoptosis [6], and elimination through phagocytosis in specific organs [5,7].

Neutrophils are formed within the bone marrow through a series of progressively differentiated precursors in a process termed granulopoiesis. The most immature long-term or short-term stem cells give rise to multipotent progenitors, common myeloid progenitors and granulocyte-macrophage progenitors (GMPs). Only recently, GMPs have been shown to produce neutrophil-committed proliferative precursors (NeP and pre-Neu) that differentiate into non-proliferative immature neutrophils, and give rise to the mature neutrophils which are released into the bloodstream [8,9] (Figure 1).

The ultimate elimination of neutrophils is as important as their production, and these two processes must be tightly coordinated to maintain a constant supply and steady number of neutrophils in blood [10]. This is important because overproduction of neutrophils can aggravate cytotoxic damage in healthy tissues as seen in many inflammatory diseases, whereas neutropenia inevitably results in recurrent infections and, paradoxically, chronic inflammatory states [11]. A key mechanism regulating neutrophil homeostasis was reported in a seminal study by Ley and colleagues, and involves the IL-23/IL-17/G-CSF feedback circuit [12]. Senescent neutrophils that migrate to peripheral tissues are phagocytosed by tissue-resident phagocytes, including macrophages and dendritic cells [12] in a process that relies, at least partially, on the Liver X receptors (LXR) [13]. Activation of LXR in engulfing phagocytes inhibits transcription of *Il23*, a cytokine that boosts granulopoiesis by promoting the production of IL-17, which in turn induces the production by stromal cells of G-CSF, the main granulopoietic factor [13]. This homeostatic loop becomes evident in mice deficient in adhesion molecules, in which neutrophils have impeded egress from blood into tissues and consequent reduced uptake by tissue phagocytes, leading to unleashed production of IL-23 and IL-17, and therefore supra-physiological levels of G-CSF that

drive the overproduction and release of neutrophils into blood [12]. The importance of this study was not only the identification of a mechanism for homeostatic regulation of neutrophil numbers, it also provided the first link between neutrophils and functional regulation of hematopoiesis. The receptor CXCR2 is not only needed for the normal release of neutrophils from the bone marrow into blood, but also for their migration into tissues. Deficiency in Cxcr2 or its ligand CXCL5 produced by intestinal cells also results in dysregulation of the IL-17/G-CSF axis and microbiota composition, resulting in elevated medullary granulopoiesis and neutrophilia [12,14]. Interestingly, studies in antibiotic-treated mice demonstrated a reciprocal regulation, whereby the microbiota is an important innate stimulus for IL-17-producing cells in the intestine and G-CSF production, thereby participating in neutrophil production and immune competence of the organism [15].

The crosstalk between mature immune cells and HSCs is already evident from embryonic life, a stage at which specific populations of primitive immune cells have an essential role in determining HSC fate. For instance, yolk-sac derived macrophages that migrate to the fetal liver around embryonic day 10.5 contribute substantially to the first wave of hematopoiesis [16]. The fetal liver serves as the main hematopoietic organ during embryonic development until HSCs move to the bone marrow, which becomes the primary site of hematopoiesis from the perinatal period onwards. It is striking that yolk-sac-derived macrophages persist in functionally distinct tissues in the adulthood such as in brain (microglia), epidermis (Langerhans cells) and lung (alveolar macrophages) [16], among many other tissues, implying that early dissemination of immune cells is important for prenatal and adult life in hematopoietic and non-hematopoietic organs. Also during embryonic life, a subset of primitive neutrophils that lie in the dorsal aorta of the zebrafish embryo was shown to play an important role in determining HSC fate [17]. These cells were shown to be the main source of TNFα, a cytokine needed for the emergence and specification of HSC in the embryo, thereby providing an example of early immune-driven determination of HSC fate in development [17].

The hematopoietic bone marrow niche

HSPC proliferate and differentiate in a highly-regulated manner, thus giving rise to all immune subsets in the bone marrow, or after migrating into extramedullary hematopoietic or lymphoid organs. Regulation of hematopoiesis requires a highly dynamic and tightly regulated orchestration of stem cell-intrinsic programs [18]. Notably, the realization that HSPC lost repopulating ability when placed outside the marrow led

to the formulation of the “niche” concept, which proposed a specific stem cell-supportive environment inside the medullary space [19,20]. This supportive niche is comprised by a plethora of cellular components, which regulate HSPC activity by supplying growth regulators and retention factors. The specific location of HSC in the vast medullary space has been controversial (reviewed in [21]); many studies pointed to localization close to the endosteal region [22-25], whereas others studies suggested that HSC localized randomly in the bone marrow or were perisinusoidal [26]. It has become increasingly clear that the vast majority of HSC in the marrow localize adjacent to blood vessels, therefore proximal to perivascular cells. Endothelial cells are key sources of CXCL12 and the cytokine stem cell factor (SCF) that maintain HSPC [27-30]. In addition to the endothelium, rare populations of perivascular cells are also key sources of CXCL12 and SCF. Aided by the use of multiparametric imaging with different markers and lineage-specific reporter genes, the complex heterogeneity that exists among stromal cells is now being clarified. Based on the brightness and morphology of Nestin-GFP$^+$ cells, two subsets of mesenchymal progenitor cells were identified [24]. Nes-GFP-bright cells are scarce and associate with arterioles, while the GFP-dim cells are more abundant, reticular shaped, and associate with sinusoids. Interestingly, quiescent HSC preferentially localize near arteriolar cells. Nes-GFP bright cells express the pericyte markers NG2$^+$ and α-smooth muscle actin and produce abundant CXCL12 needed for HSC localization and quiescence [31]. In contrast, Nes-GFP dim cells, which can be also identified by expression of the leptin receptor (LepR$^+$), are an important source of SCF that help maintain constant numbers of HSC in the bone marrow [31].

Besides cells of mesenchymal origin, early findings provided strong evidence that the nervous system also regulates the hematopoietic niche and HSPC properties [32-34]. Sympathetic nerves that align with the medullary vasculature regulate the expression of stromal CXCL12 and thereby the traffic of HSPC in and out of the bone marrow under homeostasis or stress conditions [34-36]. Specifically, release of the neurotransmitter noradrenaline by sympathetic nerves signals stromal cells through the β3-adrenergic receptor, leading to rapid downregulation of *Cxcl12* expression. Interestingly, studies showed that noradrenaline secretion follows a circadian pattern controlled by the core genes of the molecular clock, which elegantly explained the diurnal release of HSPC into blood [34]. GFAP+ non-myelinating Schwann cells that ensheath sympathetic nerves are also functional regulators of HSC proliferation by providing active TGFβ1 [37].

Niche regulation by hematopoietic descendants

In addition to stromal niche components and sympathetic nerves, a growing list of hematopoietic cells that descend from HSPC have been shown to influence HSC homeostasis and fate, including macrophages, megakaryocytes (MK), regulatory T cells (Tregs) and neutrophils. Bone marrow-resident macrophages were the first among this progeny shown to favor retention of HSPC by reinforcing the function of Nestin^{+} cells and osteoblasts [38-40], an effect that opposes the niche-inhibiting and mobilizing effects of the sympathetic nervous system. Experiments in which CD169^{+} macrophages were acutely depleted demonstrated that their elimination was sufficient to induce HSPC egress into the bloodstream [38]. Interestingly, macrophages regulate HSPC also under stress; in a transplantation setting, radiation eliminates the majority of leukocytes but spares a population of resident macrophages that repopulate the spleen and marrow via autonomous cell division [41]. These CD169^{+} radiation-resistant macrophages are needed for optimal donor-derived HSC reconstitution [41].

MK, the precursors of platelets, are in close contact with sinusoidal vessels in the marrow, where they extend cytoplasmic protrusions into the vessel lumen to release newly produced platelets. A subset of HSC localizes near MK in the sinusoids, and this spatial relationship was shown by several studies to correlate with regulation of the HSC pool size [42,43]. Specifically, HSPC expanded dramatically after depletion of MK in *Cxcl4*-Cre; iDTR mice. These effects could be pinned down to the production of key regulators of HSPC proliferation by MK, including CXCL4 and TGFβ1, both of which promote HSC quiescence. Consequently, deletion of these factors from MKs resulted in increased HSC numbers in the steady state. In contrast to these results, a separate study showed that depletion of MK resulted in reduction of HSC numbers despite a similar loss of quiescence, an effect that was accounted for by the production of thrombopoietin [44]. Besides homeostasis, MK can promote HSPC recovery after ablation with irradiation by secretion of FGF1 [43], or indirectly through osteoblast expansion [45].

The bone marrow is a major reservoir of a population of CD4^{+}CD25^{+} T lymphocytes with immune-modulatory functions, or Tregs [46]. A subset of Tregs that expresses high levels of the stem marker CD150 was found in the endosteal region of the bone marrow, proximal to HSPC [47,48]. CD150^{high} Tregs control HSPC quiescence and engraftment through the production of adenosine generated via the CD39 ectoenzyme [48], and it has been proposed that Tregs confer immune-privilege to the HSPC niche [47].

In summary, ample evidence now shows that the hematopoietic niche is regulated, secured and nurtured by the very descendants of HSPC residing therein, perhaps providing a regulatory loop that feeds on output cells and benefits from the exquisite sensing properties of mature immune cells. Given the precedents described above, it is not surprising that other hematopoietic cell lineages can actively regulate the bone marrow niche. Below, we focus our discussion on neutrophils, the most abundant among HSPC descendants, whose extreme sensitivity to stress, tissue damage and even temporal cues may provide additional layers of regulation of the bone marrow niche.

Regulation of HSPC quiescence and proliferation by neutrophils

Besides perivascular cells and MK, myeloid cells have been shown to maintain HSPC quiescence through a negative feedback histaminergic circuit. Indeed, a myeloid population expressing the histidine decarboxylase (Hdc) produces histamine (Figure 1). This biogenic amine inhibits active cycling of a myeloid-biased Hdc^{high} HSC population (MB-HSC) through the histamine receptor 2 (H_2R), and promotes its self-renewal [49]. This pathway elicited by granulocytes and possibly other myeloid subsets was important for HSPC maintenance, because ablation of histamine producing cells caused MB-HSC and progenitors to exit dormancy and induced loss of serial transplantation capacity [49].

Along the same line, neutrophils stimulate emergency myelopoiesis via production of ROS, which oxidizes the PTEN phosphatase to directly activate HSPC proliferation upon acute infection or inflammation [50]. We expect that, as we continue to extend our knowledge on neutrophil biology in the bone marrow, new mechanisms by which these cells directly regulate HSPC fate will emerge. At present, however, the most prominent known roles of neutrophils on HSPC are mediated through regulation of their niche, as discussed below.

Role of neutrophils in regeneration of the bone marrow niche

Hematopoietic stem cell transplantation (HSCT) remains the only curative treatment for most malignant and non-malignant hematopoietic diseases. In this procedure, the diseased host hematopoietic cells are wiped out by high dose chemotherapy or radiotherapy. Healthy HSPC and more mature hematopoietic cells are then transferred into the recipient's circulation where they home to the bone marrow to engraft and regenerate a new hematopoietic system. Unfortunately, the treatments used to eliminate the host hematopoietic cells invariably cause an almost complete destruction of the vascular HSPC niche in the bone marrow. Specifically, they ablate the sinusoidal

vasculature and associated perivascular cells, while leaving arteries and arterioles mostly intact [51-53]. Although the transplanted HSPC can engraft for short periods near endosteal arterioles and MK [43,45], long-term restoration of normal hematopoiesis demands reestablishment of a healthy sinusoidal network [51-53], as initially demonstrated by Rafii and colleagues. Indeed, deletion of vascular-borne VEGFR2 does not affect baseline hematopoiesis, but strongly impairs regeneration of the vasculature and the hematopoietic compartment after injury [52]. In addition to the aforementioned functions of nutrient support and providing a niche for HSPC, the sinusoidal network also produces many molecules like Notch ligands and pleiotrophin that promote HSPC engraftment specifically after injury [51,54-57]. Thus, regeneration of the sinusoidal network is the rate-limiting step in restoring healthy hematopoiesis after HSCT, and a long-standing question has been which environmental cues instruct vascular regeneration of the damaged niche.

We recently discovered that bone marrow $Gr1^{+}$ $CD115^{NEG}$ neutrophils drive sinusoidal regeneration after transplantation [58]. We noticed that, in mice transplanted with total bone marrow mononuclear cells, regeneration of the host vascular niche correlated directly with the number of donor hematopoietic cells transplanted, and adoptive transfer experiments demonstrated that only bone marrow neutrophils were capable of driving sinusoidal regeneration. In agreement, depletion of mature neutrophils from the initial graft or genetic ablation of donor-derived neutrophils delayed regeneration of the vasculature. These experiments indicated that neutrophils are both necessary and sufficient to drive vascular regeneration after HSCT. Imaging experiments showed that bone marrow neutrophils are selectively recruited to the injured sinusoids, where they secrete TNFα, a cytokine that promoted endothelial cell survival and regeneration of the sinusoids[58] (Figure 2). After transplantation, donor HSPC initiate a pro-regenerative program that greatly increases their proliferation and their capacity to generate neutrophils and other myeloid cells [59]. These findings suggested that newly generated neutrophils can promote regeneration of the sinusoidal network, which in turn facilitates hematopoietic progenitor engraftment [58]. This positive feedback loop continues until the sinusoidal network is restored and the bone marrow returns to homeostasis. Surprisingly, the signals and mechanisms that sense regeneration of the sinusoidal niche, halt further vessel growth, and induce HSPC return to quiescence are almost completely unknown, although it is likely that TGFβ signaling plays a major role in this process [53,60]. Identification of these mechanisms may lead to the development of better therapies to promote faster myeloid cell recovery, with restoration of innate immunity and reduced infections after HSCT.

Although the role of neutrophils in bone marrow regeneration was previously unclear, it was well established that they contributed to tissue regeneration (reviewed in [61]). Neutrophils are recruited to injured tissues via DAMPs (damage-associated molecular patterns) [62], where they can exert both positive and negative effects in the regeneration program. This is dependent on cellular context and in the amount and type of neutrophils recruited to each tissue [61,63]. In the context of vascular development and repair, it is now clear that different neutrophils subsets crosstalk with endothelial cells to regulate their function.

As described above, embryonic neutrophils induce generation of definitive HSC by signaling via TNFα to the hemogenic endothelium [17]. The Phillipson group identified a VEGFR1^{+} neutrophil subset in the circulation (representing ~5% of blood neutrophils) that is selectively recruited to hypoxic tissues, where they induce vessel growth via MMP9 release [64-66]. Intriguingly, however, blood-borne neutrophils are unable to induce vascular regeneration in the marrow despite expressing high amounts of TNFα, a limitation that may reflect their inability to home to injured sinusoids after adoptive transfer [58]. An emerging concept is that neutrophils are a heterogeneous population both in tissues and in peripheral blood, and that they can adopt unique physiological functions [67,68]. In the particular case of medullary regeneration, we highlight that there are at least two subsets of angiogenic neutrophils, one in the bone marrow that acts on niche-associated sinusoids, and one in the periphery that acts on peripheral vessels. A recent study by the Ng group also showed that classically-defined bone marrow Gr1^{+}CD115^{NEG} neutrophils are in fact a heterogeneous population that comprises a proliferating neutrophil progenitor as well as immature and mature neutrophils with transcriptional signatures distinct from those of circulating neutrophils [8]. It will be interesting to dissect the behavior of each of these medullary neutrophil subsets after HSCT and their contribution to vascular niche regeneration.

Bone marrow neutrophils are recruited specifically to injured sinusoids. This direct interaction is clearly important for the sinusoids as areas of the bone marrow that have no neutrophils showed no sinusoidal regeneration [58]. In the steady state, neutrophil trafficking is regulated, almost exclusively, via CXCR2 and CXCR4 [69]. However, pharmacological blockade of both pathways does not affect neutrophil recruitment to injured vessels [58], thereby indicating the existence of an unidentified mechanism in the sinusoids, induced by damage to the vasculature that specifically recruits neutrophils to injured bone marrow vessels.

In addition to aiding regeneration of the vascular niche, neutrophils have been reported to support niche activity by enhancing the capacity of pre-osteoblastic cells to produce osteopontin, an important retention factor for HSPC in the marrow [70]. Interestingly, adrenergic stimulation of neutrophils through the β3 receptor induced production of prostaglandin E2, a well-known support factor for hematopoiesis [71], which in turn induced osteoblastic activity through the EP4 receptor [70]. Thus, neutrophils appear to counteract to some extent the inhibitory effects that catecholamines exert on the niche, thereby preventing excessive HSPC mobilization (Figure 2). The identification of neutrophils as intermediary and regulators of the mobilization process provide important mechanistic links between the various pathways that regulate hematopoietic niches.

Circadian regulation of the hematopoietic niche

In almost all life forms on Earth, the planet's rotation have led to the evolution of daily circadian cycles of 24h. In mammals, peripheral clocks are normally synchronized with the environment by entrainment from daily exposure to light-dark cycles. The central circadian pacemaker located in the suprachiasmatic nuclei receives photic information conducted from the retina. The synchrony between autonomous circadian clocks found in all major organs and tissues is maintained by a complex network, involving neuronal signaling, secretion of hormones, and metabolic cues (reviewed in [72]). As discussed above, the bone marrow is extensively innervated by autonomic nerve fibers, including sympathetic nerves, which play important physiological roles in the bone marrow. Sympathetic nerves have been shown to be responsible for cytokine-elicited mobilization of HSPC outside of the bone marrow into blood [32], although active signaling in monocytic cells has also been demonstrated [39]. G-CSF, a cytokine broadly used in the clinic to mobilize HSPC into circulation for transplantation therapies, promotes the release of noradrenaline by autonomic neurons located in the periphery. Released adrenaline mediates the suppression of osteoblasts located in the endosteal marrow, thereby reducing the synthesis of CXCL12 and causing HSPC mobilization [32]. Additionally, sympathetic nerves regulate perivascular Nestin-GFP^{+} stem cells by acting on β3 adrenergic receptors [35]. This neural-mesenchymal axis is responsible for the circadian expression of CXCL12 by bone marrow stromal cells, which causes the homeostatic release of HSPC into circulation [34]. In mice, the lowest levels of CXCL12 protein in the medullary space coincide with HSPC egress around zeitgeber time 5 (ZT5, or 5 hr after the onset of the light), and the highest CXCL12 levels occur at ZT13 and correlate with the lowest numbers of circulating HSPC [34]. In contrast to mice,

humans display inverted circadian oscillations with maximum levels of progenitors in blood in the evening [73].

Given the bidirectional flux between blood and marrow, it is not surprising that adrenergic nerves also control the expression of endothelial-adhesion molecules in the medullary vasculature, as these adhesion molecules are necessary for HSPC homing back to the marrow [74]. It is also likely that a cross talk exists between the levels of the chemokine CXCL12 and those of endothelial-adhesion molecules; for instance, in mice higher levels of CXCL12 at night correlate with a higher retention of hematopoietic stem cells in the bone marrow. The genuine circadian nature of this process was illustrated by jet-lag experiments showing that repeated shifts in light cycle was sufficient to ablate circadian HSPC recruitment into tissues [74].

Interestingly, much like HSPC, mature leukocytes infiltrate the bone marrow in a circadian manner [74], with peak homing to the marrow and other organs at ZT13 in mice. The circadian migration of mature leukocytes (and HSPC) may be beneficial to provide a readily available set of tissue-resident leukocytes that mediate immune defense during the animal's active phase, when the individual's probability of injury or encountering pathogens is highest. The circadian fluxes of mature leukocytes that return to the marrow also suggest potential regulation of bone marrow niches by cells that have "sampled" the extramedullary environment. For example, it is likely that various myeloid cell subsets regulate circadian oscillations in HSPC activity through TNFα. Indeed, this cytokine has been shown to regulate circadian migration, proliferation and differentiation through modulation of ROS and melatonin signaling in HSPC, and its medullary levels are controlled in part by neutrophils and monocytes [58,75].

Neutrophil aging and temporal control of the hematopoietic niche

Neutrophils are the most abundant myeloid population in the bone marrow. Because of the short lifespan, vast amounts of neutrophils must be released into the blood every day to maintain homeostatic numbers [10]. This implies that, even under homeostatic conditions, large numbers must also be eliminated every day, yet possible functions for these naturally-cleared neutrophils were enigmatic [76,77].

Only recently, we and others discovered that circulating neutrophils undergo circadian fluctuations that affect not only neutrophil numbers, but also their phenotype. This spontaneous change over time is referred to as neutrophil aging [78]. As neutrophils age in the circulation their repertoire of surface receptors change: they up-regulate markers like CXCR4 and VLA-4, both of which are important for the retention in and homing to

the marrow [76,77,79], and downregulate others including CD62L (L-selectin) and CXCR2 ([77] and Adrover, unpublished data October 2018) (Figure 1). This CXCR4^{hi} CD62L^{lo} population of aged neutrophils follows marked circadian oscillations throughout the day and are completely cleared out from circulation by night (ZT13), when the active behavioral phase of the mice begins [77]. Interestingly, recent studies in mice have proposed that aged neutrophils gain immune-competence by enhancing β2 integrin-dependent adhesion, as well as their capacity to phagocytose and to form DNA-based extracellular traps (NETs;[80,81]). In addition, microbiota-derived metabolites have been proposed to drive neutrophil aging through Toll-like receptor signaling [81], although our own data suggest that cell-intrinsic circadian programs can also drive aging (Adrover, unpublished data October 2018). Thus, while the evolutionary drive and physiological role of this diurnal aging process of neutrophils remains to be fully elucidated, it is tempting to speculate that diurnal "priming" of neutrophils is needed for these cells to fully mature and to fulfil additional functions after their lifetime in blood, once they have cleared into tissues.

Aged neutrophils that clear from the circulation into tissues are believed to be ultimately engulfed and eliminated by tissues macrophages [78,82]. The bone marrow is one of the tissues in which aged neutrophils are cleared in larger numbers. Clearance in this organ not only serves to control neutrophil numbers but, importantly, generates homeostatic signals that modulate the bone marrow niche [77]. When aged neutrophils infiltrate the mouse bone marrow between ZT5 and ZT13, they are engulfed by tissue-resident macrophages. This efferocytic process generates LXR-dependent, but otherwise undefined signals that down-regulate the number of niche cells and, consequently, the amount of CXCL12 in the marrow, thereby promoting HSPC egress into blood (Figure 2). Consistently, the number of CXCL12-producing reticular cells and osteoblasts in the bone marrow increase when neutrophils are experimentally depleted, indicating that neutrophils modulate the size of the niche stroma. More importantly, interruption of this natural niche-inhibitory pathway by depletion of circulating neutrophils or macrophages completely blunted the diurnal oscillations of HSPC in blood, indicating that circadian clearance of aged neutrophils drives rhythms in the hematopoietic niche [77]. These findings in mice reveal a coalescence of hematopoietic, neural and immune inputs in the bone marrow to provide multi-layered regulation of hematopoiesis. Importantly, alterations of this axis appear to powerfully influence disease, as shown in the context of cardiovascular disease or cancer [83-85].

Contrary to common belief, homeostatic clearance of aged neutrophils is not unique to the bone marrow, spleen or liver; it also takes place in many extramedullary tissues such as the lung, skin or muscle in which they can perform tissue-specific roles [2,78,86]. Surprisingly, infiltration of neutrophils in the intestinal mucosa enhances bone marrow niche activity remotely, by preventing *Il23* transcription in intestinal macrophages. Similar to the aforementioned "neutrostat" model [12], inhibition of this cytokine results in reduced systemic levels of G-CSF and preserved niche function, thereby preventing excessive mobilization of HSPC into blood. However, unlike the rhythmic inhibitory roles of marrow-infiltrating neutrophils [77], infiltration in the intestine does not follow circadian patterns, and consequently niche regulation from the intestine does not influence the diurnal oscillations of circulating HSPC [86] (Figure 2). These recent findings expand the regulatory mechanisms of hematopoietic niches to include distant anatomical sites, and are consistent with the reported effects of microbiota-derived signals emanating from the gut in regulating stem cell and niche activity [87-89].

Concluding remarks

It is becoming increasingly clear that dysregulation of hematopoiesis is an important underlying driver of disease. Therefore understanding the multiple regulatory mechanisms of this highly dynamic process becomes a question of major biomedical relevance. Disturbance of neural regulation of the niche occurs during organismal aging [90] and is also prominent in the context of ischemic disease [91,92], whereas dysregulated cytokine pathways appear to be more common in cancer [83]. We have discussed here emerging evidence that neutrophils provide additional layers of regulation in hematopoiesis. The realization that neutrophils influence multiple aspects of niche physiology, from maintenance of the mesenchymal niche to HSPC quiescence, demands for urgent evaluation of their contribution to inflammatory disease and hematological malignancies. Neutrophils are also instrumental in regenerating the injured vascular niche and may provide strategies to accelerate regeneration of patients undergoing HSCT. More generally, we propose that the unique temporal properties of neutrophils, their basal presence in multiple tissues, and exquisite capacity to sense danger make these cells ideal intermediaries for niche regulation and repair not only in the bone marrow, but also other in tissues that demand rapid responses to environmental challenges.

Authorship

I.C., D.L. and A.H. contributed equally to the writing of this review article.

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Figure legend

Figure 1. Functional and phenotypic diversity of neutrophils in the bone marrow. Neutrophils are produced inside the bone marrow through progressive maturation of hematopoietic progenitors (LT-HSC to GMPs). Proliferative precursors (NeP and preNeu) differentiate into immature neutrophils and finally into mature neutrophils that are released into blood. A fraction of aged neutrophils return into the marrow after several hours in the circulation. Top and bottom panels indicate specific phenotypes and functions, respectively, of neutrophils at each stage of their life cycle.

Figure 2. Regulation of the hematopoietic bone marrow niche. The sympathetic nervous system (SNS) exerts control on the HSC niche by the circadian release of catecholamine, which targets β3-adrenergic receptors on stroma cells. The same signals can act through neutrophils to produce prostaglandin E2 (PGE_2) and stimulate the osteoblastic niche. The stromal niche is also circadianally regulated by aged neutrophils that return to the bone marrow after only several hours in the circulation. Aged neutrophils that infiltrate the bone marrow are engulfed by macrophages and activation of the LXR receptors lead to inhibition of the hematopoietic niche. Excessive G-CSF production associated with several inflammatory processes or impaired neutrophil clearance in extramedullary tissues is also a potent inhibitor of the HSPC niche. All these regulatory mechanisms ultimately inhibit production of CXCL12, thereby promoting HSPC egress into blood. This has been shown in the intestine, where neutrophil infiltration in the mucosa and engulfment of neutrophils by tissue-resident macrophages inhibits the IL-23/IL-17/G-CSF axis and remotely supports niche activity in a circadian-independent manner. Boxes indicate the presence or absence of circadian oscillations in each tissue.

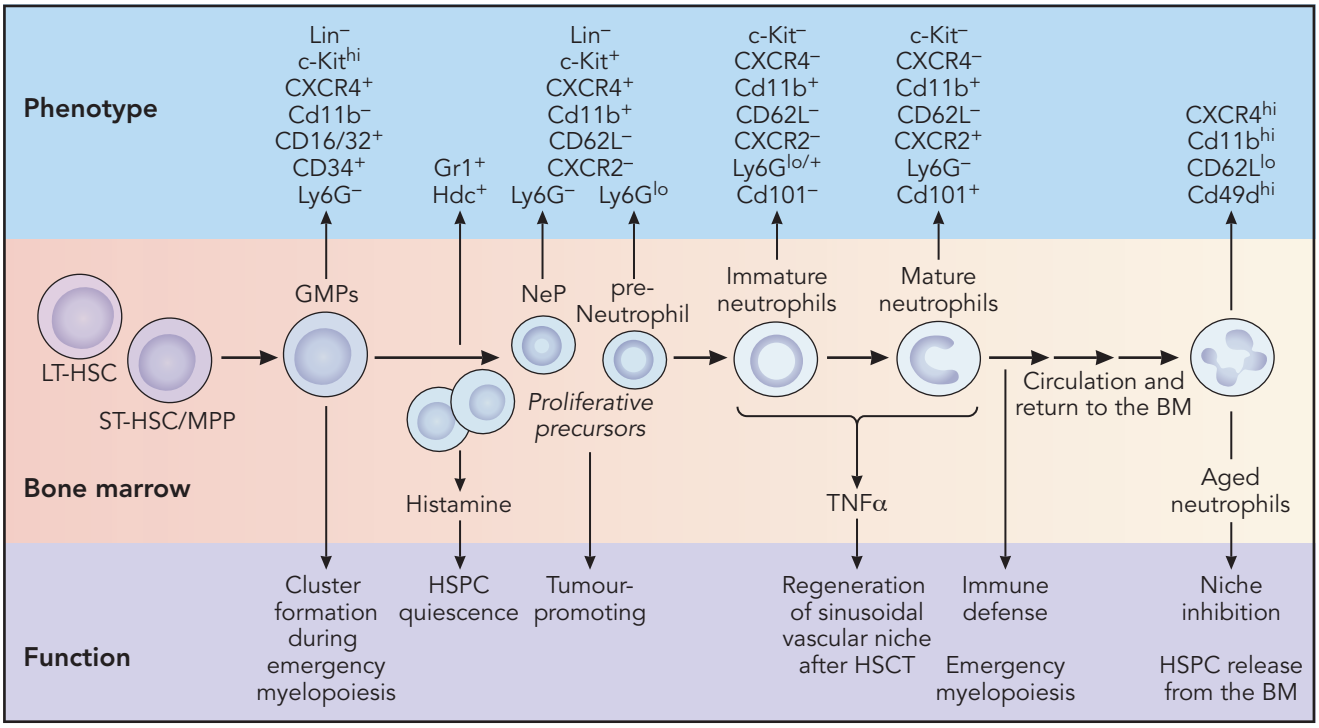
Phenotype
Lin⁻ c-Kit^hi CXCR4⁺ Cd11b⁻ CD16/32⁺ CD34⁺ Ly6G⁻
Gr1⁺ Hdc⁺
Lin⁻ c-Kit⁺ CXCR4⁺ Cd11b⁺ CD62L⁻ CXCR2⁻
Ly6G⁻
Ly6G^lo
c-Kit⁻ CXCR4⁻ Cd11b⁺ CD62L⁻ CXCR2⁻ Ly6G^lo/+ Cd101⁻
c-Kit⁻ CXCR4⁻ Cd11b⁺ CD62L⁻ CXCR2⁺ Ly6G⁻ Cd101⁺
CXCR4^hi Cd11b^hi CD62L^lo Cd49d^hi
LT-HSC
ST-HSC/MPP
GMPs
NeP
pre-Neutrophil
Immature neutrophils
Mature neutrophils
Circulation and return to the BM
Proliferative precursors
Bone marrow
Histamine
TNFα
Aged neutrophils
Function
Cluster formation during emergency myelopoiesis
HSPC quiescence
Tumour-promoting
Regeneration of sinusoidal vascular niche after HSCT
Immune defense
Emergency myelopoiesis
Niche inhibition
HSPC release from the BM

Figure 1

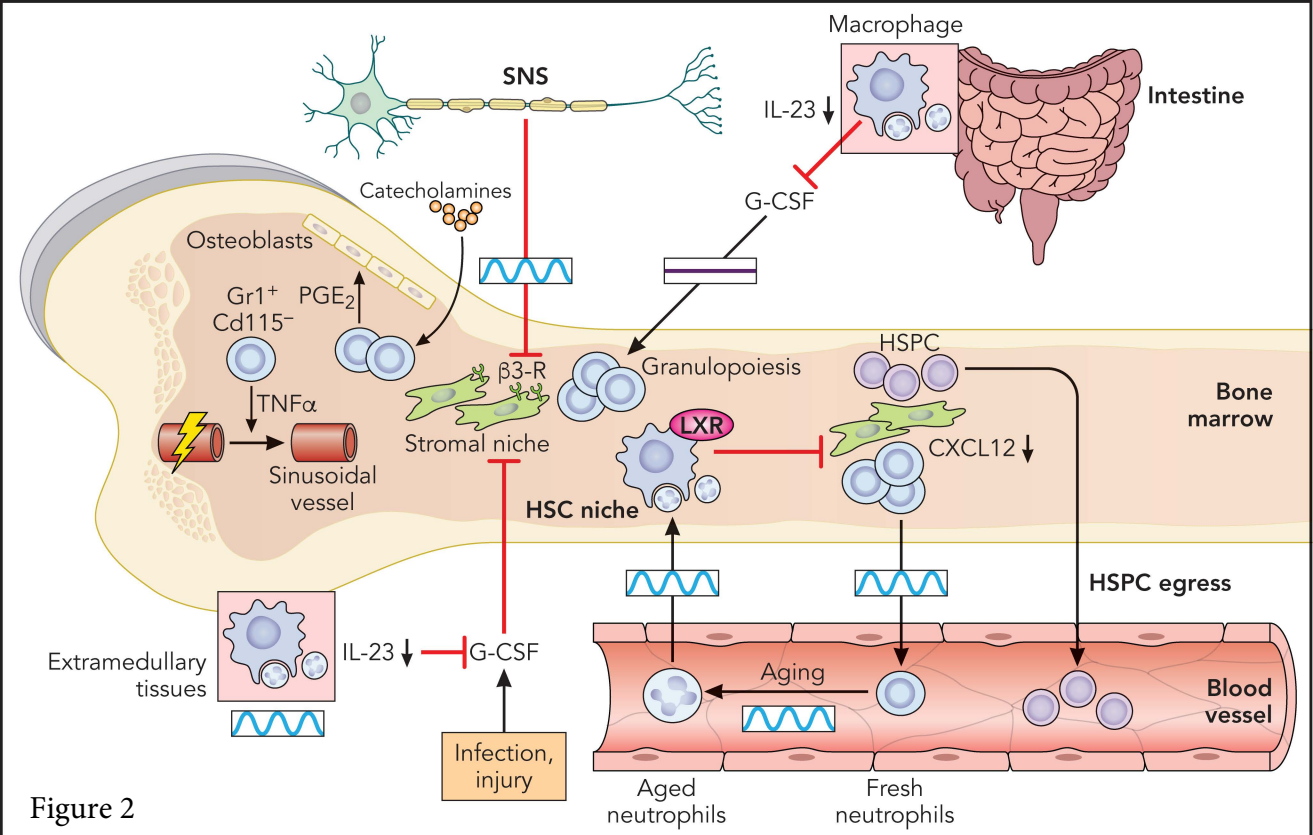
SNS
Macrophage
IL-23
Intestine
G-CSF
Catecholamines
Osteoblasts
Gr1+ Cd115−
PGE2
TNFα
Sinusoidal vessel
β3-R
Stromal niche
Granulopoiesis
HSPC
Bone marrow
LXR
CXCL12
HSC niche
HSPC egress
Extramedullary tissues
IL-23
G-CSF
Infection, injury
Aging
Blood vessel
Aged neutrophils
Fresh neutrophils

Figure 2