Published in final edited form as:

Nat Rev Immunol. ; 12(1): 49–60. doi:10.1038/nri3132.

The bone marrow at the crossroads of blood and immunity

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Abstract

Progenitor cells that are the basis for all blood cell production share the bone marrow with more mature elements of the adaptive immune system. Specialized niches within the bone marrow guide and, at times, constrain the development of haematopoietic stem and progenitor cells (HSPCs) and lineage-restricted immune progenitor cells. Specific niche components are organized into distinct domains to create a diversified landscape in which specialized cell differentiation or population expansion programmes proceed. Local cues that reflect the tissue and organismal state affect cellular interactions to alter the production of a range of cell types. Here, we review the organization of regulatory elements in the bone marrow and discuss how these elements provide a dynamic means for the host to modulate stem cell and adaptive immune cell responses to physiological challenges.

The bone marrow provides a framework of microenvironmental domains or niches that support the function of immune cells and haematopoietic stem cells (HSCs). Cellular niches are functional compartments within tissues that control cell numbers by providing signals that regulate cell self-renewal, differentiation and quiescence¹. Such signals can be transmitted via direct cell contact, growth factors and cytokines, or components of the extracellular matrix. The concept of bone marrow cellular niches was first formulated as a hypothesis more than 30 years ago², and our understanding of these niches is based in part on the study of model organisms such as *Caenorhabditis elegans* and *Drosophila melanogaster*, as well as on the study of other mammalian tissues, such as the intestine and the epidermis³. Our goal in understanding how HSCs and immune cells process signals from their niche is to develop novel cellular therapies that enhance immune function or haematopoietic reconstitution after injury, or that assist in the treatment of haematological malignancies and autoimmune diseases.

Knowledge about niche components has been obtained through three main types of experiments. First, factors that modulate HSC and immune cell behaviour have been

isolated, both *in vitro* and *in vivo* (TABLE 1). Second, conventional or intravital microscopy has been used to define the relationship of HSCs and immune cells with their surrounding niche components. Third, genetic mouse models or drug treatments have been used to study the changes in HSC and immune cell function in response to specific alterations in different components of the niche (TABLE 2).

It is important to note that niches in higher organisms are unlikely to be created by a single cell type, but are a tissue — that is, a combinatorial interaction of cells, matrix, biophysical forces and metabolic substrate components. Studies that define a contributing cell type should not be interpreted as indicating that this cell type is the niche. In addition, because the haematopoietic and immune systems need to rapidly respond and adapt to the needs of the organism, their niche within the bone marrow should not be viewed as a static entity, but rather as a microenvironment that continually processes and conveys information. These aspects lead to challenges and controversies in understanding how various cell types generate the niche *in vivo*.

In this Review, we aim to dissect the individual components of the haematopoietic and immune cell niches in the bone marrow, and build on this knowledge to discuss how these elements collaborate to form a functional system. We begin by reviewing the various components of the haematopoietic and immune cell niches. Recent studies focusing on perfusion, oxygenation and innervation highlight how the bone marrow is divided into distinct domains. Against this background, we discuss how HSCs and immune cells respond to particular situations — such as inflammation or the depletion of mature progenitors — by integrating the signals provided by their niche.

Haematopoietic stem cell niches

HSCs arise in a sequential manner during embryonic development. They are first found in extraembryonic tissues and are subsequently present in the aorta–gonad–mesonephros area, then in the fetal liver and spleen, and finally in the bone marrow, which is the major haematopoietic organ in adults⁴. The HSCs can be enriched from the bone marrow cell pool on the basis of the expression of specific cell-surface markers. One such cell population is characterized as lineage marker-negative SCA1⁺KIT⁺CD41[−]CD48[−]CD150⁺, and these cells are referred to as LSK SLAM cells. One in two cells in this population are functional HSCs, and thus LSK SLAM cells are often used as a representative HSC population in flow cytometry or microscopy studies⁵. In the steady state, microscopy studies of the bone marrow have identified LSK SLAM cells in the periphery of sinusoids, near endosteal surfaces and in the poorly defined 'stroma' that is located between the sinusoids⁵, suggesting that HSCs can reside in multiple locations (FIG. 1). We describe below the known contributions of different bone marrow cell types to the regulation of HSCs.

It is important to note that, although most HSCs in adults are present in the bone marrow, this may not be an absolute requirement for their function, as haematopoiesis can also occur in extramedullary sites (for example, in the spleen, liver and lungs)⁶. The spleen in rodents is a well-defined site where HSCs can function. It is less clear whether the same is true in humans, although under stress conditions some degree of haematopoiesis is observed in the

spleen and other sites. Whether extramedullary sites provide comparable regulation, particularly of stem cell quiescence, is debated, but evidence suggests that the bone marrow may be distinct from the spleen in that regard⁷.

A defining feature of a niche is the ability to regulate the self-renewal of stem cells, but the precise links between HSC self-renewal and location have not yet been well defined. It has been shown that modulating osteoblastic cell function can increase haematopoietic stem and progenitor cell (HSPC) numbers, but whether it does so by direct or indirect action is not clear⁸. Therefore, our knowledge to date provides a picture of bone marrow niches with only a limited resolution.

Vascular contribution to the niche

Multiple indirect lines of evidence suggest that areas adjacent to blood vessels constitute an HSC niche. First, the generation of HSCs occurs in association with vascular areas and blood flow during embryonic development^{9–11}. Second, in humans, extramedullary haematopoiesis occurs in perivascular areas in the setting of haematopoietic stress⁶. Third, HSCs (as defined by their immunophenotype) have been observed in the vicinity of sinusoids⁵. Last, endothelial cells have been shown to express cell-surface molecules that facilitate the transit of HSCs and immune cells between the bone marrow and the periphery¹².

On the basis of *in vivo* and *ex vivo* studies, bone marrow endothelial cells were found to express factors that promote haematopoiesis, such as granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), stem cell factor (SCF; also known as KIT ligand), interleukin-6 (IL-6) and FMS-related tyrosine kinase 3 ligand (FLT3L; also known as FLK2 ligand)¹³. In addition, these cells were shown to express the adhesion molecules E-selectin, P-selectin, vascular cell adhesion molecule 1 (VCAM1) and intercellular adhesion molecule 1 (ICAM1)¹⁴. The bone marrow vasculature is heterogeneous in its expression of molecules that are thought to facilitate cell homing, such as E-selectin and CXC-chemokine ligand 12 (CXCL12). Such homing pathways can also be exploited by malignancies that can metastasize to the bone marrow^{12,15,16}. *In vitro*, immortalized endothelial cells can support HSC function in culture through the expression of Notch ligands¹⁷. Similarly, the expression of a constitutively active mutant form of AKT in endothelial cells (using a cell-specific inducible transgenic mouse model) leads to an increase in HSC numbers¹⁸. These characteristics support the idea that endothelial cells can provide niche functions *in vivo*, although conclusive evidence that they do so is not yet in hand.

Mesenchymal contribution to the niche

Endothelial cells are surrounded by perivascular mesenchymal stromal cells (MSCs), which provide structural support and harbour populations of cells that can regenerate the bone marrow stroma and interact directly with HSCs. A subpopulation of bone marrow adventitial cells that is characterized by the expression of the surface marker CD146 in humans¹⁹ or the cytoplasmic filament protein nestin in mice²⁰ can give rise to ectopic haematopoietic microenvironments following transplantation, suggesting that these cells are involved in the

regeneration of the bone marrow stroma. These cells wrap around blood vessels and adrenergic nerve fibres, both in the central and endosteal bone marrow regions, and they express several proteins that regulate HSC maintenance, including CXCL12, angiopoietin 1 and SCF. Deletion of nestin-expressing MSCs leads to a 50% reduction in bone marrow HSC numbers and a proportional increase in HSC numbers in the spleen, suggesting that nestin-expressing cells are involved in tethering HSCs in a perivascular location within the bone marrow²⁰.

CXCL12-abundant reticular (CAR) cells are a sub-population of mesenchymal cells that were identified in a genetic mouse model in which green fluorescent protein (GFP) was expressed under the control of the *Cxcl12* promoter²¹. CAR cells are scattered throughout the bone marrow, secrete factors that support haematopoiesis, and are located adjacent to a substantial proportion of immunophenotypically defined HSCs. The deletion of CAR cells in the adult mouse using a suicide-gene strategy leads to a decrease in HSC numbers and an increase in HSC quiescence²². As the aforementioned nestin-expressing MSCs also express high levels of CXCL12, nestin-expressing MSCs might represent a functional subtype of CAR cells that are found in the perivascular location. Distinct functions for the various types of mesenchymal cell populations are in the process of being defined. For example, recent data suggest that more-primitive mesenchymal cells, such as those expressing nestin, are participants in HSC regulation²⁰.

Adipocytes are another stromal component of the bone marrow microenvironment that is thought to regulate HSC function. In mice, the adipocyte-rich tail vertebrae have markedly fewer HSPCs and less cell cycling than the adipocyte-poor thoracic vertebrae²³. In a lipotrophic genetic mouse model, bone marrow transplantation led to accelerated haematopoietic recovery following irradiation compared with recovery times in control mice, although alterations in osteogenesis were also present in this model and a possible confounder of the adipocyte-specific effects²³.

Trabecular bone contribution to the niche

The isolation of different fractions of bone marrow cells according to their association with bone has shown that most HSCs are found within trabecular bone^{25,26}. In the bone marrow, the bone-forming osteoblasts secrete multiple haematopoietic cytokines, including G-CSF, M-CSF, GM-CSF, IL-1, IL-6, IL-7 and CXCL12 (ref. 27), and express molecules — such as Notch ligands^{8,28}, WNT ligands²⁹, thrombopoietin^{30,31} and angiopoietin 1 (ref. 32) — that may contribute to the maintenance of HSC function. Osteoblasts also express many types of adhesion molecules that may facilitate HSC–niche interactions; these molecules include VCAM1, ICAM1, annexin II, n-cadherin, CD44 and CD164 (ref. 33). Several, but not all, studies have demonstrated that a change in osteoblast numbers results in corresponding changes in HSC numbers (TABLE 2). Likewise, supraphysiological activation of osteoblastic cells using lineage-specific expression of a constitutively active parathyroid hormone receptor leads to expansion of the HSC pool⁸. Modification of the function of osteoprogenitors, which are defined by osterix expression, also appears to affect the integrity of haematopoiesis and the cycling of HSCs³⁴. Finally, conditional depletion of osteoblasts markedly compromises G-CSF-mediated mobilization of HSCs from the bone marrow³⁵.

Together, these data provide a strong case for the participation of cells of the osteolineage in the HSC niche.

Although osteolineage cells are participants in the niche, it is not true that all cells of the lineage have a role. The data indicate that it is more-primitive osteolineage cells rather than mature osteoblasts that provide niche functions. A study in which *Dicer1* was deleted in osteoprogenitors identified alterations in multiple parameters of haematopoiesis, including HSC cycling, whereas the same gene deletion in mature osteoblasts had no such effect³⁴. Others have also found that immature osteolineage cells have a more prominent role³⁶. Therefore, there appears to be a complex and hierarchical organization of niche-modulating functions among different mesenchymal cell types.

The products of osteolineage cells have also been shown to modulate HSC function *in vivo*. For example, osteoblasts secrete the extracellular matrix protein osteopontin, which regulates HSC numbers and migration to the endosteal surface^{37,38}. Furthermore, osteolineage cells lay down an extracellular matrix that becomes highly mineralized, and this creates a high Ca^{2+} concentration at the periendosteal surface. HSCs respond to Ca^{2+} via the calcium-sensing receptor, and this process is required for their engraftment in the bone marrow³⁹.

Osteoblasts are responsible for inducing the maturation of osteoclasts through the production of receptor activator of NF- κ B ligand (RANKL), and this molecule may participate in regulating HSCs. Acute activation of osteoclasts following RANKL-mediated stimulation leads to haematopoietic progenitor cell proliferation and mobilization⁴⁰, whereas the suppression of osteoclast activity by pharmacological methods decreases HSC numbers in the bone marrow⁴¹ and increases HSC mobilization⁴². However, genetic mouse models with altered osteoclast numbers have provided conflicting results regarding the effects on HSC mobilization^{42,40}. These results suggest that osteoclasts may have some role in maintaining the numbers of HSCs in the bone marrow, but their role in mobilization is still in question.

Trabecular and mesenchymal niche components include macrophages, as deletion of these cells *in vivo* leads to HSC mobilization through a decrease in the expression of HSC retention factors by nestin-expressing cells⁴³ and perhaps by osteoblasts^{44,45}. G-CSF-induced HSC mobilization appears to exert an inhibitory action on macrophages, and this in turn modulates HSC localization through effects on other niche populations^{43–45}. Therefore, macrophages are participants in the modulation of HSC localization.

Immune cell niches

Through cell-deletion and microscopy studies, several subsets of bone marrow cells have been shown to support immune cell function. Indeed, in addition to its role as a primary lymphoid organ through the support of lymphoid development, the bone marrow can act as a host for various mature lymphoid cell types.

HSC commitment to lymphopoiesis

Lymphoid development is a highly ordered process that is accompanied by the sequential expression of different growth factor receptors and cell-surface molecules. Deciphering the factors that instruct HSCs to adopt a lymphoid fate and the isolation of cellular intermediates in the process of lymphoid commitment are active areas of research. Several groups have demonstrated heterogeneity in the lymphoid reconstitution potential among HSCs, suggesting that some HSCs possess an intrinsically determined lymphoid bias^{46–48}. Whether this bias results from epigenetic changes programmed by interactions with the niche is unknown. The HSC bias is skewed towards myelopoiesis during ageing^{48,49}, but likewise it is unclear whether this is due to the accumulation of genetic changes in HSCs or an alteration in niche elements.

Niches for lymphopoiesis

Early in lymphoid development, multipotent progenitors with T cell potential migrate to the thymus, while progenitors with B cell potential remain in the bone marrow. Signalling through the Notch receptor has been shown to direct lineage specification towards a T cell fate (reviewed in ref. 50), but whether 'priming' of early thymic progenitors occurs in the bone marrow before migration to the thymus is still unknown. The bone marrow niches for B cell development are better understood. Through cell-deletion studies and microscopy, the sequential engagement of multiple niches has been shown to contribute to B cell development within the bone marrow. Osteoblasts, osteoclasts and CAR cells are required for the earliest stages of development, whereas IL-7-secreting stromal cells and sinusoidal endothelial cells promote further B cell maturation (FIG. 2a,b,c). Osteoblast ablation using expression of a suicide gene under the control of the type I collagen $\alpha 1$ promoter results in a rapid and dramatic decrease in the numbers of pre-pro-B cells and pro-B cells⁵¹. By contrast, inhibition of osteoclast function leads to relocalization of B cell progenitors to the spleen, presumably through a compensatory decrease in osteoblast function⁵², and this suggests that the endosteal surface is required for the initial steps in B cell lymphopoiesis.

CAR cells are found in close association with pre-pro-B cells⁵³, and their ablation leads to a decrease in the numbers of common lymphoid progenitors and pro-B cells, suggesting that CXCL12 expression is required for tethering early B cell progenitors in the bone marrow²². The transition from pre-pro-B cells to pro-B cells requires IL-7 signalling⁵⁴, and IL-7-secreting stromal cells (which do not express CXCL12) are found in proximity to pro-B cells, suggesting that they constitute a distinctive niche⁵³. In addition, as part of their maturation, most B cells migrate to peripheral organs; however, some B cells remain in contact with sinusoidal endothelial cells. This adhesion is mediated in part by VCAM1 and endocannabinoid receptor expression, and it contributes to the diversity of the immunoglobulin repertoire through an unknown mechanism⁵⁵. Finally, although most newly formed B cells continue their maturation in the spleen at this point, the bone marrow can also support the development of a small proportion of functional mature B cells⁵⁶. Collectively, these studies demonstrate that B cell lymphopoiesis is dependent on the successful engagement of multiple cellular niches in the bone marrow.

Niches for naive immune cells

The bone marrow perisinusoidal space is populated by clusters of dendritic cells (DCs) that colocalize with naive recirculating B cells and T cells^{57,58} (FIG. 2d). Deletion of these DCs using a suicide-gene strategy led to a decrease in the number of naive B cells in the bone marrow, as well as a decrease in IgM secretion following immunization, whereas B cell progenitors and T cells were not affected. These DCs are thought to provide naive B cells with specific survival signals, such as macrophage migration inhibitory factor (MIF)⁵⁷. Interestingly, perisinusoidal naive B cells can be activated by T cell-independent antigens, in a manner similar to that which occurs in the splenic marginal zone. Regarding T cells, the factors required for their maintenance in the bone marrow are less well understood, but perisinusoidal DCs can also cross-present blood-borne antigens to naive T cells⁵⁸. Collectively, this suggests that immune cells localized in perivascular regions in the bone marrow provide key functions that are important for protection against blood-borne pathogens.

Niches for plasma cells

Following antigen exposure, B cells can home back to the bone marrow, where they persist as antibody-secreting plasma cells⁵⁹. *In vitro* and *in vivo* studies have shown that plasma cells depend on survival signals from their microenvironment, and these signals include CXCL12, IL-6, CD44, B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL)^{60,61}. Plasma cells are found in close proximity to CAR cells⁵³, and mice genetically deficient in CXC-chemokine receptor 4 (CXCR4) show impaired homing of plasmablasts⁶², demonstrating that the engraftment of plasma cells requires CXCL12 signalling via CXCR4. Two other mature cell types, eosinophils and megakaryocytes, contribute to the plasma cell niche. Eosinophils secrete APRIL, a member of the tumour necrosis factor (TNF) superfamily, and their depletion leads to a decrease in the numbers of bone marrow plasma cells⁶³. Megakaryocytes also secrete APRIL and IL-6, and mice deficient in megakaryocytes have lower plasma cell numbers⁶⁴. Interestingly, CAR cells express low levels of APRIL but can recruit eosinophils and megakaryocytes through the secretion of CXCL12 (REFS 65,66). Thus, CAR cells might act as 'core organizers' of the plasma cell niche by coordinating the action of multiple cell components (FIG. 2f).

Niches for memory cells

Several studies have found that the bone marrow harbours a substantial population of memory immune cells. In mice, following short-term antigen challenge, the majority of long-lived CD4⁺ T cells localize to the bone marrow, where they reside in close contact with IL-7-secreting stromal cells⁶⁷ (FIG. 2e). Furthermore, these cells can be quickly activated following secondary challenge to provide efficient helper functions, qualifying them as bona fide memory CD4⁺ T cells. The majority of bone marrow CD4⁺ memory T cells are in the G0 phase of the cell cycle, and interaction with IL-7-secreting stromal cells has been proposed to maintain this quiescence in the absence of antigen receptor signalling^{67,68}. Experiments using adoptive transfer of central memory CD8⁺ T cells that were differentiated *in vitro* demonstrated that these cells can also home to the bone marrow and

respond to antigen stimulation⁶⁹. This suggests that the bone marrow has a role in the maintenance of both CD4⁺ and CD8⁺ memory T cells.

In addition, in a mouse transplantation model, allogeneic HSCs are protected against allojection by host regulatory T (T_{Reg}) cells that reside within the bone marrow; these two cell populations are found in close proximity at the endosteal surface, and deletion of T_{Reg} cells leads to allojection⁷⁰. This study raises the possibility that T_{Reg} cells share the same niche as HSCs and contribute to making this an immune-privileged site.

Niches for myeloid immune cells

The earliest recognizable myeloid precursors are found adjacent to the endosteal surface and small blood vessels⁷¹, suggesting that they reside in specific niches. Although these niches are not fully characterized, ablation of CAR cells leads to accelerated myeloid differentiation, suggesting that CAR cells maintain HSPCs in an undifferentiated state²². At baseline, the bone marrow constitutes a reservoir for mature myeloid elements; the release of these myeloid cells into the peripheral blood is mediated by specific ligands. Neutrophils and monocytes express chemokine receptors that antagonistically regulate their egress into the peripheral blood. In the steady state, both cell types express CXCR4, which promotes their retention into the bone marrow through CXCL12-induced signalling. In the setting of inflammation, CXCR2 mediates the release of neutrophils through interaction with CXCL1 and CXCL2 produced by megakaryocytes^{72,73}, whereas CC-chemokine receptor 2 (CCR2) mediates the release of monocytes through interaction with CC-chemokine ligand 2 (CCL2) produced by CAR cells, nestin-expressing MSCs and endothelial cells⁷⁴.

Collectively, these studies demonstrate that, in addition to its role as a primary lymphoid organ, the bone marrow has five other functions. First, it supports distinct populations of naive B cells and T cells in a perivascular DC network. Second, it supports antibody-secreting plasma cells. Third, it supports memory immune cells. Fourth, it serves as an immune-privileged site for HSCs. And, fifth, it serves as a reservoir of myeloid immune cells.

Functional organization of the bone marrow

At present, the quest to construct a model of HSC and immune cell niches is quite unidimensional, as it is based on the interpretation of experiments in which a single molecule or a single cell is manipulated, and this interpretation is limited by the lack of an interactive map. Mapping interactions might provide predictive algorithms for how alterations in each component can modulate changes throughout the system. Overcoming this limitation is possible by accumulating information about specific cell types and responses to individual changes. It can also be facilitated by adding another dimension to the model by including factors that inherently integrate inputs from the organism as a whole (such as innervation or vascular perfusion) or factors that influence cell state (such as metabolic substrates, including oxygen). Combining these parameters under different conditions may ultimately permit a less fragmented, more physiological and predictive portrait of bone marrow regulatory niches.

Perfusion

In mice, the systemic infusion of dyes that label cells to an extent proportional to their degree of perfusion allows the measurement of how blood flow relates to cell state (FIG. 3a). Two studies have described the relationship between the extent of perfusion and the presence of HSCs^{75,76}. In both studies, the perfusion dye Hoechst 33342 was injected intravenously and bone marrow cells were harvested shortly thereafter. Although the first study showed that the least-perfused fraction is enriched in HSPCs by 90- to 200-fold compared with the most-perfused fraction, it did not use specific cell-surface markers to clearly define whether stem cells were more abundant in the least-perfused region⁷⁵. The second study found that half of the HSCs defined by the LSK SLAM markers reside in the least-perfused area, a fraction substantially higher than that of other bone marrow cell types⁷⁶. These data provide an interesting and suggestive association between HSCs and low perfusion, but it is not clear whether the levels of oxygen or other substrates in these regions are different. Furthermore, it is not clear whether HSCs preferentially localize to low-perfusion zones or whether their primitive cell state is better preserved in that context.

The state of immune cell niches has not yet been studied with perfusion dyes. The observations that naive B and T cells are close to sinusoids does not provide sufficient evidence, as flow in these vascular beds may be slow and not associated with high nutrient and gas exchange. It is therefore unclear whether the localization of immune subsets corresponds to low or high perfusion regions.

Oxygenation

Cell residence in a hypoperfused environment might offer several advantages, including an increased concentration of locally secreted growth factors, protection from systemically distributed toxins and reduced generation of reactive oxygen species⁷⁷. The hypoxia-specific stain pimonidazole has been perfused to define the oxygenation of haematopoietic cells *in vivo*⁷⁸. A substantial proportion of LSK SLAM cells were observed in regions with low levels of oxygen (FIG. 3b). Several studies suggest that hypoxia is a negative regulator of the cell cycle in haematopoietic cells. *In vitro*, HSCs are better maintained in hypoxic culture conditions, possibly owing to lower cycling rates^{79,80}. *In vivo*, inducible deletion of hypoxia-inducible factor 1 α (*Hif1a*) in HSCs leads to decreased quiescence and a better reconstitution ability in primary recipients, but this potential is exhausted in secondary transplantations owing to cellular senescence⁷⁸. HIF1 α is known to regulate the expression levels of key metabolic enzymes that favour lactic acid fermentation over mitochondrial respiration⁸¹. In support of this model, HSCs have a distinct metabolic profile characterized by low mitochondrial activity⁸². As a result, HSC residence in a hypoxic niche coupled with low mitochondrial activity might serve as a protective mechanism against oxidative damage.

Hypoxia also modulates the expression of soluble mediators and cytokines, as well as that of their receptors. HIF1 α activity in haematopoietic progenitors leads to the secretion of vascular endothelial growth factor (VEGF)⁸³, a regulator of stem cell function⁸⁴. Additional pathways — such as those mediated by KIT, Notch 1 and β -catenin — have been associated with HIF1 α in other cell types^{85,86}, and it is possible that these pathways are similarly active in HSC maintenance. Finally, niche components are also responsive to hypoxia: endothelial

cells and MSCs react by expressing CXCL12, which facilitates the homing of HSCs⁸⁷; and osteoblasts react by secreting VEGF, which stimulates bone formation and angiogenesis⁸⁸. Thus, hypoxia might be an important step in the ontogeny of cellular niches within the bone marrow.

Factors other than hypoxia have also been shown to act upstream of HIF1 α . *In vitro*, the cytokines SCF⁸⁹ and thrombopoietin⁸³ can increase HIF1 α levels, possibly through protein stabilization. MEIS1, a crucial transcription factor for stem cell self-renewal, binds to the *Hif1a* promoter and increases its expression⁸². Finally, forkhead box O3A (FOXO3A), a regulator of stem cell activity, induces the expression of CBP/p300-interacting transactivator 2 (CITED2), a repressor of HIF1 α activity⁹⁰.

HIF1 α activity has been less well studied in the adaptive immune system, but HIF1 α has an important role in B cell lymphopoiesis. Ablation of HIF1 α in the immune system impairs the transition from pro-B cell to pre-B cell, owing to the downregulation of the glycolytic pathway in highly proliferating late pro-B cells⁹¹, and leads to autoantibody production through an unclear mechanism⁹². It remains to be shown whether HIF1 α is induced by a hypoxic niche or by other factors known to affect lymphopoiesis, such as insulin-like growth factor 1 (IGF1)⁹³.

Innervation

The bone marrow is innervated by autonomic and sensory nerves. Within the bone marrow, nerve fibres travel along the same path as the arterial blood supply and interact with perivascular mesenchymal cells and bone-lining osteoblasts and osteoclasts⁹⁴ (FIG. 3c). MSCs express β 2- and β 3-adrenergic receptors and mediate HSC egress in response to acute variations in sympathetic nervous system (SNS) tone⁹⁵. Osteoblasts express only the β 2 receptor and regulate their own growth, as well as osteoclast activity, in response to SNS signalling⁹⁶.

It has long been recognized that acute physiological stress leads to the rapid mobilization of bone marrow haematopoietic progenitors and splenic lymphocytes. In addition, circadian fluctuations in SNS tone correlate with the release of haematopoietic progenitors into the peripheral blood, as increased SNS tone induces the downregulation of CXCL12 expression by MSCs⁹⁵. *In vivo*, nestin-expressing MSCs are closely associated with nerve fibres and with HSCs, making them putative gatekeepers for the SNS-mediated release of progenitors²⁰. The existence of a gap junction signalling pathway in MSCs that leads to coordinated CXCL12 secretion also raises the possibility that SNS-derived signals can rapidly propagate in the bone marrow stroma⁹⁷. Physiologically, SNS tone variations may have a role in two aspects of progenitor egress. First, the SNS may regulate the release of progenitors during periods of acute stress; and, second, it may be involved in the homeostatic recycling of a small fraction of cellular niches on a daily basis, by inducing the periodic egress of HSCs.

Adrenergic stimulation of the cellular niches has an important role in the clinical use of supraphysiological doses of G-CSF for stem cell mobilization. Mice deficient in ceramide UDP-galactosyltransferase (CGT; also known as UGT8) display aberrant nerve conduction

and almost no stem cell mobilization⁹⁸. This phenotype is replicated in mice treated with the dopaminergic neuro toxin 6-hydroxydopamine and in mice that cannot synthesize noradrenaline owing to a lack of dopamine β -hydroxylase⁹⁸. Interestingly, this phenotype is rescued by co-administration of G-CSF and clenbuterol, a β 2-adrenergic agonist, highlighting the collaboration between these two signals⁹⁸. The nature of the cooperation between the two signals is still unclear and is an area of ongoing research (reviewed in detail in REF. 99).

In addition, HSCs can respond to sympathetic nervous stimulation in a cell-autonomous manner. Human CD34⁺CD38⁻ progenitor cells express dopaminergic receptors, and *in vitro* treatment with agonists for these receptors increases the chemotaxis, proliferation and engraftment capacity of these progenitor cells *in vivo*¹⁰⁰. These effects are only present when cells are pre-treated with the mobilizing cytokine G-CSF, and are abrogated by treatment with a dopamine antagonist. Cells of the innate and adaptive immune systems also respond to adrenergic stimulation¹⁰¹, suggesting that the SNS can modulate the behaviour of both HSCs and immune cells.

The responsiveness of cell niches

HSCs and immune cells need to rapidly adapt to the fluctuating requirements of the organism by generating progenitor cells and effector cells, respectively, while maintaining a reserve of cells for future challenges. In order to do so, these cells need to integrate signals from their environment. In this section, we discuss the various adaptations of the bone marrow microenvironment that allow it to 'sense' the numbers of progenitors and respond to particular states of injury and inflammation.

Feedback mechanisms

HSCs have the ability to alternate between quiescence and the cell cycle according to external cues. Indeed, treatment with cytotoxic agents (such as 5-fluorouracil) leads to a depletion of actively cycling cells, and this is followed by the entry of quiescent HSCs into the cell cycle in an effort to restore homeostasis¹⁰². How quiescent HSCs 'sense' the need to enter the cell cycle is still poorly understood, but this could be mediated by the loss of the inhibitory signals that were provided by the progenitors or by the concomitant injury of the niche and resulting alterations in the extrinsic cues that are detected by HSCs (FIG. 4). A better understanding of the mechanisms involved could yield considerable therapeutic benefit, as chemotherapy, radiation injury and invasion of the niche by malignancies are likely to alter HSC–niche interactions.

It is possible that HSCs and their progeny compete for soluble signals or niche occupancy. For example, depletion of DCs using a suicide-gene strategy leads to myeloproliferation associated with increased serum levels of the cytokine FLT3L¹⁰³; the receptor for FLT3L is present on both haematopoietic progenitors and DCs, and this suggests that mature DCs can regulate the proliferation of progenitors by controlling FLT3L levels. In aged mice, depletion of mature B cells enhances B cell lymphopoiesis¹⁰⁴, suggesting that the accumulation of mature B cells during ageing initiates a negative feedback loop. Furthermore, in this study, the recovery of B cell numbers was accelerated after each round

of depletion, indicating that HSCs and/or their niche might acquire reinforced programmes for B cell lymphopoiesis in this setting. Collectively, these studies suggest that extrinsic cues — including the relative abundance of other haematopoietic cell types — affect HSC proliferation and differentiation.

The role of niche occupancy and competition between HSCs for the niche remains a controversial area. It is a widely held notion that death or clearance of existing, niche-occupying HSCs is required for transplanted cells to engraft. However, parabiosis experiments suggest that HSCs leave the niche with sufficient frequency to enable a turnover of engrafted cells of approximately 1% over 3 weeks^{105,106}. This calculation is based on the non-physiological context of parabiosis, but it is validated by studies in which HSCs were intermittently infused into non-conditioned hosts¹⁰⁷. These data indicate that there is ongoing entry and egress of HSCs to and from the niche. It is not clear whether this is due to an HSC intrinsic 'off-rate' from the niche or to remodelling of the niche itself by processing mechanisms, such as bone turnover. However, the occurrence of this bone turnover supports the idea that HSCs are regularly challenged with the necessity of finding a new niche. This process is inherently one in which HSC fitness is tested and selection pressures may be exerted.

It has been observed by many researchers that increasing the number of HSCs infused in an experimental transplant context does not result in a saturation of engraftment. That is, there does not seem to be a point at which there is no longer additional engraftment, although the slope of the line reflecting the number of cells engrafted compared with the number infused may flatten. These intriguing observations have suggested that HSCs may have some capacity to induce their own niche, a phenomenon observed in other model systems¹⁰⁸. The notion that HSCs are not simply passive occupants of a preformed niche but participants in the construction of their own home is an appealing means of potentially explaining how extramedullary haematopoiesis can be established. This idea might also account for why leukaemic cells that are dependent on the bone marrow may have an expanded niche.

Effects of inflammation on cell niches

Information on tissue injury and infection is transmitted to the bone marrow through the systemic release of pro-inflammatory mediators. Within the bone marrow, the overall response to inflammation is geared towards the generation and egress of effector cells, although the type of response can vary according to the nature of the insult¹⁰⁹. In a mouse model of mycobacterial infection, increased levels of interferon- γ (IFN γ) in the bone marrow lead to increased cycling and mobilization of HSPCs, and these features are accompanied by a decreased repopulation capacity in secondary recipients¹¹⁰. HSPCs possess a functional IFN γ response pathway, and thus can respond directly to pro-inflammatory signals. Likewise, HSPCs have been shown to proliferate in response to IFN α in a cell-autonomous manner¹¹¹.

The bone marrow microenvironment also has the ability to respond to injury and infection. G-CSF, which is secreted in the context of inflammation, disrupts an important retention signal in the bone marrow: the CXCL12-CXCR4 axis¹¹². G-CSF also induces the expression of thymopoietin in the bone marrow, and this leads to the secretion of CXCR2

ligands by megakaryocytes, resulting in the mobilization of neutrophils⁷³. In addition, stimulation of Toll-like receptors on CAR cells, nestin-expressing MSCs and endothelial cells by microbial molecules leads to the secretion of CCL2, which induces the egress of monocytes into the peripheral blood¹¹³.

Additional data on the topic have been obtained mostly through *in vitro* studies that must be interpreted with caution. *Ex vivo*-purified MSCs have the ability to either suppress or stimulate immune responses. Once exposed to pro-inflammatory cytokines, MSCs can suppress lymphocyte proliferation through direct cell contact and secreted mediators¹¹⁴. In addition, *ex vivo* stimulation of MSCs with IFN- γ can allow these cells to present soluble antigens to effector T cells by upregulating the expression of MHC class I and MHC class II molecules¹¹⁵. However, whether these phenomena occur *in vivo* remains to be demonstrated. Endothelial cells can also respond to pro-inflammatory signals by modulating haemostasis, cell adhesion and vascular permeability¹¹⁶. Nevertheless, it is still unclear which signalling pathways are induced by inflammation in bone marrow endothelial cells, and whether they specifically modulate HSC or immune cell behaviour in this context. Collectively, these data support the hypothesis that the bone marrow stroma has a multi-faceted response to inflammation that modulates HSC and immune cell behaviour.

Perspectives

The ability to rationally manipulate the haematopoietic and immune systems therapeutically requires insight into the dialogue between haematopoietic cells and their niches. Several questions remain unanswered. What are the minimal requirements for HSC self-renewal? In which cellular compartment(s) does this self-renewal mainly occur? Do HSCs compete for the niche in a classical sense, whereby cell death is imposed on 'loser' cells? How do niches form and regenerate, are they generated in response to haematopoietic cells themselves and what determines the number of niches? How do memory immune cells compete for space within their niches? As experimentally induced niche dysfunction can pre-dispose an animal to myeloproliferative disease^{24,117} or myelodysplasia and cancer³⁴, are human haematological or immune diseases caused by acquired niche abnormalities? Are malignancies that localize to the bone marrow dependent on niche signals, and do those signals affect tumour responses to chemotherapy? Do HSCs compete with leukaemic cells for the same niche? Answers to these questions have clinical potential, as intervening at the interfaces of cells may be possible using biological or small-molecule drugs, thereby providing new therapeutic opportunities for stem cell transplantation, immune-mediated diseases and cancer (BOX 1).

Acknowledgments

The authors would like to thank D. B. Sykes, L. Silberstein and I. Droujinine for their comments on the manuscript. Thanks also go to J.-P. Lévesque and S. Méndez-Ferrer for generously contributing to FIG. 3. F.E.M. is a recipient of a Clinician Scientist Training Award from the Canadian Institutes of Health Research. C.R. was funded by Massachusetts General Hospital institutional funds. This work was supported by the Ellison Medical Foundation and the National Heart, Lung and Blood Institute (grants U01HL10042 and R01HL44851).

Glossary

Quiescence	A phase in the cell cycle in which the cell is not dividing or preparing to divide but still has the ability to do so in the presence of an appropriate signal.
Sinusoids	Specialized blood vessels that are similar to capillaries, but are lined by endothelial cells with fenestrations that allow the passage of cells and large molecules.
Adventitial cells	Connective tissue cells that surround small blood vessels and have a supportive structural role and possibly other functions, such as blood flow regulation.
Trabecular bone	A network of bony projections that is present at the ends of long bones and forms a highly vascularized, porous matrix that is the main site of haematopoiesis in adults.
Osterix	(Also known as SP7). A zinc finger-containing transcription factor that is required for the commitment of mesenchymal cells to the osteoblastic lineage.
Dicer1	This gene encodes Dicer, an endoribonuclease that cleaves double-stranded RNA and is important for processing pre-microRNAs.
Epigenetic changes	Modifications in the activation state of certain genes that persist following cell division. These alterations are not related to changes in DNA sequence, but are induced through other mechanisms, such as DNA methylation and histone modifications.
T cell-independent antigens	Antigens that do not require T cell help to induce specific antibody production. These antigens are commonly polymeric antigens, such as polysaccharides and lipids.
Splenic marginal zone	An area in the spleen with the major function of trapping circulating antigens. The marginal zone is populated by a distinct population of lymphocytes that can be readily activated following exposure to blood-borne pathogens.
CD34⁺CD38⁻ progenitor cells	A population of cells within the human haematopoietic system that is defined by the expression of cell-surface markers and is enriched in stem cells and progenitors.

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Box 1 | The therapeutic potential of niche modulations

- Enhancement of the haematological and immunological recovery following chemotherapy or stem cell transplantation
- Reversal of the age-associated decline in lymphopoiesis
- Enhancement of the chemotherapy-mediated killing of residual malignant cells
- Enhancement of immunological memory, or of the depletion of autoreactive immune memory cells

Figure 1. Haematopoietic stem cell niches

In the bone marrow, haematopoietic stem cells (HSCs) can be found near the endosteal surface (a); in association with CXCL12-abundant reticular (CAR) cells (b); and in the periphery of sinusoids and perivascular nestin-expressing cells (c). Each niche is thought to provide signals that support HSC behaviour, although the relationship between HSCs that are present in different niches is still unclear (dotted arrows). Likewise, blood vessels in the bone marrow are often in close association with bone, although their interaction is still poorly understood (d). At the endosteal surface, osteoblastic cells express factors that participate in HSC retention; osteoclasts regulate osteoblastic cell function by inducing bone remodelling; and macrophages regulate osteoblastic cell activity and the retention of HSCs. In the bone marrow stroma, HSCs are associated with CAR cells, which express factors that promote HSC retention. Adipocytes negatively regulate HSCs in the steady state. In the perivascular area, HSCs are associated with nestin-expressing cells, which promote HSC retention and are regulated by macrophages and the sympathetic nervous system (SNS).

Figure 2. Immune cell niches

During B cell differentiation, haematopoietic stem cells (HSCs) and pre-pro-B cells are found in close association with CXCL12-abundant reticular (CAR) cells (a), whereas pro-B cells are more often in contact with interleukin-7 (IL-7)-secreting stromal cells (b). Later in B cell development, a population of immature B cells can be found in close association with endothelial cells (c). Naive B and T cells, which can respond to blood-borne pathogens, are found within a perivascular niche that is constituted by a network of dendritic cells (DCs) (d). Bone marrow-resident memory CD4⁺ T cells reside next to IL-7-secreting stromal cells and are mostly found in a quiescent state (e). Plasma cells also reside in the bone marrow. CAR cells, eosinophils and megakaryocytes express factors that promote plasma cell engraftment and survival (f).

Figure 3. Functional organization of the bone marrow

The three panels show representative images obtained using techniques that can be applied to study perfusion, oxygenation and innervation of the bone marrow compartment and to highlight its heterogeneity. **a** | Study of the perfusion state of nucleated cells in the mouse bone marrow. Bone marrow was harvested 10 minutes after intravenous administration of Hoechst 33342 dye and fluorescence intensity was measured by flow cytometry. Different populations are defined according to the percentile of fluorescence intensity and can later be studied by immunophenotype or functional assays. **b** | Study of the oxygenation state in the mouse bone marrow. Hypoxic regions within femur sections were stained using pimonidazole and imaged 3 hours after intravenous pimonidazole injection. Pimonidazole is identified by a fluorescein isothiocyanate (FITC)-labelled pimonidazole-specific monoclonal IgG1 antibody (green), and the nuclei are visualized using 4',6-diamidino-2-phenylindole (DAPI) staining (blue). Images were acquired by confocal laser scanning microscopy using an X10 objective. The staining is heterogeneous and present both at the endosteal surface and at other locations, but the image is two-dimensional and therefore anatomical relationships are of limited precision. **c** | Study of the innervation of the mouse bone marrow. Whole-mount staining of the skull bone marrow revealed subendothelial nestin-expressing mesenchymal stromal cells that are innervated by sympathetic fibres. The projection stack (of depth ~100 µm) of fluorescent images shows the distribution of cells that express a nestin-GFP (green fluorescent protein) conjugate (green), as well as that of vascular endothelial cells that express CD31 (also known as PECAM1) (blue) and of sympathetic nerve fibres that express tyrosine hydroxylase (red).

Figure 4. Possible mechanisms by which bone marrow niches adapt to changes

a | The bone marrow cellular pool is divided between different populations of mature and immature cells. We propose here that mature cell types provide negative feedback to limit progenitor numbers through the secretion of negative regulators or by competing with variable efficiency for niche factors. **b** | Loss of inhibition by the dominant cell type allows the expansion of the progenitor populations. **c** | Alternatively, extrinsic factors that modify the composition of the niche alter the balance of the different populations.

Table 1

Soluble factors produced by bone marrow niches that contribute to HSC and immune cell maintenance

Soluble factor	Bone marrow source	Effects on HSCs or immune cells
<i>Factors that affect HSCs</i>		
Angiopoietin 1	Osteoblastic cells, nestin-expressing MSCs	Maintenance of long-term repopulating activity and quiescence ³²
CXCL12	CAR cells, nestin-expressing MSCs, endothelial cells, osteoblastic cells	Homing and retention ⁹⁹ ; maintenance of the HSC pool size ²¹
SCF	Endothelial cells, osteoblastic cells, nestin-expressing MSCs	Maintenance of long-term repopulating activity ¹¹⁸
Notch ligands	Endothelial cells, osteoblastic cells	Increased expression on osteoblastic cells after parathyroid hormone stimulation is associated with increased HSC numbers in the bone marrow; however, loss of this signalling pathway does not impair HSC function in the steady state ^{119,120}
Thrombopoietin	Osteoblastic cells	Maintenance of long-term repopulating activity and quiescence ^{30,31}
WNT ligands	Osteoblastic cells	Conflicting findings: enhanced self-renewal when used pharmacologically; however, loss of this signalling pathway does not impair HSC function in the steady state ¹²¹ ; inhibition by osteoblastic cell-specific expression of DKK1 increased HSC cycling and reduced HSC serial transplant capability ²⁹
<i>Factors that affect immune cells</i>		
APRIL	Granyocytes, lymphocytes, dendritic cells, megakaryocytes	Survival of plasma cell precursors ⁶¹
BAFF	Granulocytes, lymphocytes, dendritic cells	Survival of plasma cell precursors ⁶¹
CXCL12	CAR cells, nestin-expressing MSCs, endothelial cells, osteoblastic cells	Retention of B lymphoid progenitors in the bone marrow ²² ; homing and retention of plasma cell precursors ⁶⁸ ; homing and retention of HSCs ²¹
IL-6	Endothelial cells, osteoblastic cells, megakaryocytes	Survival of plasma cells ⁶⁰ ; pleiotropic effects on HSCs and myeloid progenitors
IL-7	IL-7-secreting stromal cells	Support of early B cell lymphopoiesis; survival of memory T cells ^{54,67}
MIF	Dendritic cells	Survival of plasma cell precursors ⁵⁷

APRIL, a proliferation-inducing ligand; BAFF, B cell activating factor; CAR, CXCL12-abundant reticular; CXCL12, CXC-chemokine ligand 12; DKK1, dickkopf-related protein 1; HSC, haematopoietic stem cell; IL, interleukin; MIF, macrophage inhibitory factor; MSC, mesenchymalstromalcell; SCF, stem cell factor.

Table 2

How alterations in cellular niche components affect the haematopoietic and immune systems

Niche component	Increase or decrease in niche component	Experimental model	Phenotype	Refs
Osteoblastic cells* (identified by activity of the <i>Colla1</i> 2.3kb promoter)	Increase	Constitutively active PTH receptor	• Increase in HSC numbers	8
Osteoblastic cells* (identified by activity of the <i>Colla1</i> 2.3kb promoter)	Decrease	Thymidine kinase suicide gene	• Decrease in bone marrow cellularity • Extra medullary haematopoiesis • Loss of pre-pro-B and pro-B cells	51,122
Bone marrow stroma (nonspecific)	Increase	Inducible knockout of <i>Bmpr1a</i>	• Increase in HSC numbers	123
Osteoblastic cells* (nonspecific)	Decrease	Biglycan knockout mouse strain	• No change in HSC numbers	124
Osteoprogenitors (osterix-expressing)	Decrease	Deficiency of <i>Gsa</i>	• Impaired B cell lymphopoiesis	125
Osteoclasts (nonspecific)	Decrease	Pharmacological inhibition (with zoledronic acid or alendronate); M-CSF-deficient mouse; FOS-deficient mouse; RANKL-deficient mouse	• Increased trabecular volume • Decreased HSC numbers • Decreased engraftment capacity • Decreased B cell precursor numbers • Increased HSC mobilization	41,42,52
Macrophages (M-CSFR ⁺)	Decrease	MAFIA suicide gene or drug treatment (with clodronate)	• HSC mobilization to peripheral blood and spleen	44
Macrophages(CD169 ⁺)	Decrease	DTR suicide gene	• HSC mobilization to peripheral blood	43
Stromal cells (nestin-expressing)	Decrease	DTR suicide gene	• Decrease in bone marrow HSC numbers • Extra medullary haematopoiesis • Decrease in the efficiency of HSC homing to the bone marrow	20
Reticular cells (CXCL12 ⁺)	Decrease	DTR suicide gene	• Decrease in bone marrow HSC numbers • Increase in HSC quiescence • Decrease in numbers of lymphoid progenitors	22

Niche component	Increase or decrease in niche component	Experimental model	Phenotype	Refs
Adipocytes	Decrease	A-ZIP/F1 knockout mouse strain or drug treatment (with BADGE)	<ul style="list-style-type: none"> • Increase in trabecular bone • Increase in HSC numbers • Increased HSC engraftment capacity 	23
Dendritic cells (CD11c ⁺)	Decrease	DTR suicide gene	<ul style="list-style-type: none"> • Decreased numbers of mature B cells 	57
Eosinophils	Decrease	dblGATA1 knockout mouse model	<ul style="list-style-type: none"> • Impaired long-term maintenance of plasma cells 	63
Megakaryocytes	Decrease or increase	<i>Tpo</i> knockout mouse strain or exogenous administration of TPO	<ul style="list-style-type: none"> • Proportional change in plasma cell numbers 	64

BADGE, bisphenol A diglycidyl ether; *Bmpr1a*, bone morphogenic protein receptor 1A; *Colla1*, type I collagen $\alpha 1$; CXCL12, CXC-chemokine ligand 12; DTR, diphtheria toxin receptor; HSC, haematopoietic stem cell; MAFIA, macrophage FAS-induced apoptosis; M-CSF, macrophage colony-stimulating factor; M-CSFR, M-CSF receptor; PTH, parathyroid hormone; RANKL, receptor activator of NF- κ B ligand; TPO, thrombopoietin.

* Osteoblastic cells are not synonymous with osteoblasts; these rows represent studies carried out using genetic constructs to evaluate a range of cells in the osteolineage.