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Heterogeneity of the Bone Marrow Niche

Vionnie W. C. Yu^{1,2,3,4}, David T. Scadden^{1,2,3}

¹Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA, USA.

²Harvard Stem Cell Institute, Cambridge, MA, USA.

³Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA

⁴Novartis Institutes for BioMedical Research, Cambridge, MA, USA.

Abstract

Purpose of review—The bone marrow niche is increasingly recognized as heterogeneous with specific subtypes of mesenchymal niche cells governing the development or homeostasis of selective parenchymal hematopoietic subsets. This review outlines recent efforts in dissecting these micro-niches regulated by unique cell pairings within the bone marrow and provides an overview of how the bone marrow orchestrates multiple facets of hematopoiesis.

Recent Findings—Recent advancement in technologies has significantly improved our understanding of the cellular and molecular constituents that contribute to regulate hematopoiesis and to maintenance of the hematopoietic stem cells (HSCs). Transgenic mouse models that enable endogenous cell deletion or lineage tracing, coupled with advanced intravital microscopy has identified several mesenchymal cell types, including the osteolineage cells, megakaryocytes, macrophages, perivascular cells, and Schwann cells, to be indispensable regulators of hematopoiesis. These niche cells, when perturbed, each caused very specific hematopoietic consequences including impairment in B cell maturation, T lineage development, erythropoiesis, and impact different aspects of HSC behavior such as quiescence, mobilization, and response to acute stress signals.

Summary—The emerging concept is that the bone marrow environment is comprised of multiple micro-niches, each consisting of unique pairing of distinct supportive stromal cells with distinct hematopoietic subtypes to regulate a particular branch of hematopoietic cell process. The bone marrow can be viewed as a carrier with sub-compartments tailored to support different hematopoietic activities.

Keywords

hematopoietic stem cell; bone marrow niche; osteolineage cell; mesenchymal stem cell; megakaryocyte; perivascular cells; mobilization; quiescence

Corresponding Author: David T. Scadden, 185 Cambridge Street, Boston, Massachusetts 02115, USA. david_scadden@harvard.edu. Phone: 617-724-3167. Fax: 617-724-2662.

Conflicts of Interest

The authors declare no relevant conflicts of interest.

Introduction

The bone marrow is the sanctuary where hematopoietic stem cells (HSCs) self-renew, differentiate into all cell types of the hematopoietic system, and sustain a lifelong production of blood for the organism. The niche concept was first articulated by Schofield in 1978¹ when he noticed that the colony unit forming cells obtained from the spleen (CFU-Ss), despite their multi-lineage differentiation capacity, were different from the cells obtained from the bone marrow that they could not reconstitute irradiated mice and had limited passage capacity. He hypothesized that stem cells needed to reside in the bone marrow to maintain their stemness. Once they left the niche, they could become less plastic progenitor cells at the expense of their immortality. Schofield's concept was relatively untested for decades and the critical cellular and molecular components of the specialized microenvironment he proposed have just begun to be unveiled over the last 15 years. Advancements in lineage-tracing, endogenous cell-deletion animal models, and high-resolution microscopy have significantly improved our understanding of the molecular and cellular intricacies that maintain and integrate many activities required to sustain the hematopoietic system. The importance of the bone marrow microenvironment is further highlighted by us² and others^{3–5} showing that mutations in non-hematopoietic niche cells can cause cell-autonomous hematopoietic neoplasia. Therefore, defining the interdependent functional relationship between the mesenchymal support cells and the parenchymal hematopoietic cells is imperative in understanding bone marrow diseases and may offer new opportunities for niche-based therapeutic intervention. In this review, we will summarize recent discoveries of niche participants essential for hematopoiesis, describe specialized micro functional units formed by specific niche cells that govern a particular hematopoietic cell process, and provide molecular insights for niche-based selective targeting of hematopoietic regeneration.

Osteolineage Subsets Create Specialized Lymphoid Niches

Osteolineage cells are particularly important for lymphoid cell development. Here we describe that distinct osteolineage cells create unique micro-niches that support the development of different immune cell types.

Osteoprogenitors Support B Cell Maturation

The bone marrow cavity in which hematopoiesis occurs contains a range of osteolineage cells at different developmental stages, including the most primitive mesenchymal stem cells, the osteoprogenitors, the bone-forming osteoblasts, the lining cells that are adjacent to osteoids, and the terminally differentiated osteocytes. Leveraging well-characterized osteolineage cell stage-specific promoters, we could achieve in vivo deletion of different subtypes of osteolineage cells in individual mouse models by driving cell type-specific expression of a suicide enabling protein. The protein was the diphtheria toxin receptor so that apoptosis of targeted cells only occurs upon diphtheria toxin administration to the animals, and will only occur in a selected cell population in which the receptor is expressed. Using this selective cell deletion strategy, we demonstrated that loss of a subset of bone lineage cells expressing osterix (Osx) led to a reduction of mature B cells and impaired

adaptive immune response⁶. In addition to providing interleukin 7 (IL-7), which is a key cytokine required for B cell differentiation, *Osx*⁺ cells also secrete insulin growth factor 1 (IGF1) to govern B cell maturation, as mice with *Osx*-specific deletion of IGF1 failed to transition from pro-B to pre-B cells while administration of recombinant IGF1 rescued the B cell defect in an *Osx*⁺ cell deleted mouse model. Therefore, *Osx*⁺ osteolineage cells serve to regulate the maturation of early B lymphoid precursors by promoting pro-B to pre-B cell transition through IGF1 production⁶. Prior studies by other laboratories reported similar defects in B progenitor cell maturation and reduced B cell number when early osteolineage cells were perturbed, including the *Osx*⁺ cells and the cells driven by a 2.3kb collagen type 1 alpha 1 promoter (Col(I)a2.3kb) that encompass a wider range of osteolineage subpopulations^{7–9}, or disrupted C-X-C motif chemokine 12 (CXCL12) expression from these cells¹⁰.

Mature Osteolineage Cells are Required for T Lymphopoiesis

Interestingly, using the same cell deletion strategy but targeting a mature osteolineage population, showed a completely different lymphopoietic phenotype. In an independent study, we found that in vivo depletion of mature osteocalcin-expressing (*Ocn*⁺) osteolineage cells in the bone marrow resulted in a loss of T lineage cells in the animals, demonstrating their absolute requirement for T immune cell development¹¹. These animals have decreased T competent common lymphoid progenitors (CLP) with a minimal effect on B cell biased CLP (*Ly6D*⁺). In addition, the *Ocn*⁺ cell deleted mice failed to generate C-C chemokine receptor type 7 (CCR7)-expressing thymic-seeding progenitor cells, resulting in a lack of progenitor T cells capable of homing to a functional thymus, and thereby impacting downstream T cell production. *Ocn*⁺ osteolineage cell appears to regulate T lineage development by communicating with the CLPs through Notch signaling, because the T lymphopenic effect seen in the *Ocn*⁺ cell depleted animals could be recapitulated in several mouse models by selective deletion of the delta-like protein 4 (DLL4) expression in *Ocn*⁺ cells, or of its receptor and downstream signaling molecules in primitive hematopoietic cells. Another laboratory deleted terminally differentiated osteolineage cells, the osteocytes, in an independent mouse model and reported systemic disruption of metabolism and similar loss of T cells,¹² albeit by a different mechanism including marked metabolic and thymic abnormalities. Therefore, mature *Ocn*⁺ osteolineage cells in the bone marrow are indispensable for T cell regeneration by ensuring the production of early T competent progenitor cells. In addition to supporting lymphoid cell development, *Ocn*⁺ cells also play important roles in other hematopoietic settings. For instance, in studies where *Ocn*-expressing cells were depleted, granulocyte colony-stimulating factor (G-CSF) induced hematopoietic stem and progenitor cell (HSPC) mobilization was markedly compromised¹³. Therefore, *Ocn*⁺ cell also controls the traffic of hematopoietic stem and progenitor cells in a G-CSF dependent manner.

Together, these studies show that early and late stage osteolineage cells play very different roles in supporting B and T lymphopoiesis. While *Osx*⁺ osteolineage cells produce IGF1 that support the maturation of B cell progenitors, mature *Ocn*⁺ cells distinctly affect T cell specification in the bone marrow (Figure 1). These studies redefine bone cells as important immune participants that regulate specific production of cells of the adaptive immune

system. The emerging model is one of a highly interrelated system with pairings of mesenchymal support cells with parenchymal lineage committed hematopoietic progenitors to create distinctive functional units to regulate lymphopoiesis. Of particular interest is whether the perturbation of these lymphoid niches is contributing to impaired immune response in pathological settings such as osteoporosis, multiple myeloma, or the lack of T cell generation post-allogeneic bone marrow transplantation. Future directions will involve exploring these heterologous cell interactions in malignant processes such as lymphoma, leukemia, and bone metastatic processes.

Erythroblastic Islands are Micro-factories for Red Blood Cells

Erythroblastic islands are highly specialized subcompartments within the bone marrow where intercellular interactions, in concert with stimulatory and inhibitory cytokines, play critical roles regulating erythropoiesis. Erythroid islands were first described by Marcel Bessis in 1958¹⁴. These islands consisted of one or more synchronously maturing cohort of erythroid cells undergoing four or five divisions between proerythroblast and normoblast. Each island was centered on a macrophage, with large areas of closely opposed erythroblast and macrophage membranes. At the conclusion of terminal differentiation, expelled nuclei were phagocytosed by the central macrophage (Figure 2). Despite this prophetic description of erythroid niches, these findings did not elicit further insights until recently.

A key component of the erythroid islands is the central macrophage. Island macrophages, derived from monocyte precursors and likely to be a specialized subset of resident macrophages. The bone marrow contains a heterogeneous population of macrophages at various differentiation stages with diverse phenotypes. Despite prior studies that perturbed macrophage differentiation and observed impaired red blood cell production, the specific identification of the island macrophage has remained elusive. A recent study revealed that specific depletion of CD169⁺ macrophages in mice reduced the number of erythroblasts in the bone marrow but did not lead to overt anemia under homeostatic conditions. However, in the case of hemolytic anemia, CD169⁺ macrophage depletion led to myeloablation, impaired erythropoietic recovery, and eventually acute blood loss¹⁵. Interestingly, in a JAK2(V617F)-driven mouse model of polycythemia vera and β -thalassemia, macrophage deletion normalized the erythroid compartment and reduced disease pathology¹⁶. As can be seen, macrophages can play opposite roles in governing erythropoiesis depending on the physiological conditions. Additional research is needed to understand how macrophages maintain red blood cell production under normal and pathological settings.

A Guardian of Hematopoietic Stem and Progenitor Cell Trafficking – the Macrophage

Another role of the macrophage is to mediate HSC mobilization through signaling the mesenchymal stromal cells. In studying the mobilization of HSCs into the bloodstream in response to G-CSF, investigators found that there was concomitant loss of macrophages and reduced bone formation¹⁷. In vivo depletion of macrophages, in either macrophage Fas-induced apoptosis (Mafia) transgenic mice or by administration of clodronate-loaded liposomes into wild type mice, led to marked reduction of endosteal osteoblasts and HSC

mobilization into blood, mimicking the phenotype observed during G-CSF administration. Specifically, it is the CD169⁺ macrophage but not the Gr-1^{hi} monocyte that promotes the retention of HSCs in the endosteal niche. CD169⁺ macrophage secretes oncostatin M (OSM), which in turn induces nestin⁺ cells and possibly other mesenchymal stromal cells to express CXCL12 via a mitogen-activated protein kinase kinase-p38-signal transducer and activator of a transcription 3-dependent pathway^{18,19}. CXCL12 engagement with CXCR4 receptor on HSC cell surface is crucial for HSC retention in the BM niche. In mice and patients with diabetes, there were increased CD169⁺ macrophages and elevated OSM in the bone marrow, and reduced HSC mobilization. OSM neutralization¹⁹ and CXCR4 antagonist¹⁸ in diabetic mice have been shown to improve G-CSF induced HSC mobilization, suggesting that targeting CD169⁺ macrophages or antagonizing OSM may be a strategy to restore niche function, particularly in diabetic settings.

Overall, these studies show that macrophages are an indispensable cellular participant needed for HSC retention in the BM, through signaling other bone marrow stromal cells, and also serve as a niche cell population that support erythropoiesis. The macrophage studies are excellent examples illustrating the concept of one cell type playing multiple roles in supporting different hematopoietic cell processes in the bone marrow, through very different mechanisms.

A Quiescent Gatekeeper for Hematopoietic Stem Cell – the Megakaryocyte

Prior studies have indirectly eluded that megakaryocyte, originated from hematopoietic lineage and responsible for plate production, regulates HSC activities. These evidence include transplanted HSCs preferentially lodge within two cell diameters from megakaryocytes near the endosteum²⁰, and pharmacological inhibition of megakaryocytic function impairs HSC engraftment²¹. Megakaryocytes appear to participate in hematopoietic recovery post-transplantation through stimulating the proliferation of osteolineage cells^{21,22} and producing thrombospondin, PDGF, FGF2, which are important factors for the regulation of vasculature following bone marrow damage²³. However, two recent studies provide strong mechanistic insights demonstrating how megakaryocytes directly regulate HSC quiescence and proliferation under normal and injury conditions. One group selectively depleted megakaryocytes in vivo using Cxcl4-Cre driving inducible diphtheria toxin receptor expression in megakaryocytes in transgenic mice²⁴. Both megakaryocyte-depleted mouse models and Cxcl4 complete knockout mice have an increased number of proliferating HSCs, marked by BrdU staining. However, administration of recombinant Cxcl4 into the megakaryocyte-depleted animals only partially rescued HSC numbers and proliferation²⁴, suggesting that additional factors likely contribute to megakaryocyte-mediated HSC quiescence.

In an independent study, Zhao et al. endogenously ablated megakaryocytes using a different promoter, Pf4-Cre, to drive inducible diphtheria toxin receptor expression on megakaryocytes (Pf4-Cre;iDTR). This version of a megakaryocyte-ablated mouse model also exhibited increased HSC activation and proliferation, and the authors attributed the effect to TGFβ1 because conditional deletion of TGFβ1 in megakaryocytes similarly enhanced HSC proliferation. In contrast, TGFβ1 injection into megakaryocyte-deleted

animals restored HSC quiescence²⁵. However, when the very same mouse model was challenged by 5-fluorouracil, a chemotherapeutic agent, HSCs from these mice were defective in expansion and failed to reconstitute recipients upon transplantation. The authors found that the loss of HSC number in stressed megakaryocyte-ablated mice could be rescued by another molecule, FGF1, but the engraftment capacity of these HSCs could not be restored. These interesting data support the hypothesis that under normal homeostatic condition, megakaryocytes maintain HSC quiescence by secreting TGF β 1, but upon chemotherapeutic challenge, megakaryocytes secrete FGF1 and additional molecules yet to be identified, to promote HSC expansion and to maintain a fully functional pool of HSCs.

As described, megakaryocytes constitute a specialized microenvironment that maintains the quiescence and proliferation of HSCs. These studies further illustrate that under stress conditions such as chemotherapy, the role of megakaryocytes may switch from a gatekeeper of quiescence to an advocate of HSC expansion.

A Stress Sensor for the Hematopoietic Cells – the Sympathetic Nervous System

Sensory and autonomic innervation of the bone marrow not only regulates physiological homeostatic trafficking of hematopoietic cells but also enables them to respond to acute stress signals. HSCs and progenitors circulate in the bloodstream under homeostatic conditions, exhibiting robust circadian fluctuations in antiphase with the expression of the chemokine CXCL12 by stromal cells in the bone marrow niche. Sympathetic tone delivered by nerves in the bone marrow is transmitted to stromal cells through the beta(3)-adrenergic receptor, leading to reduced Sp1 transcription factor and the rapid downregulation of CXCL12 expressed by these stromal cells²⁶. As such, the cyclical circadian release of HSCs into the bloodstream and the concomitant reduction of CXCL12 in the bone marrow microenvironment are maintained by core genes of the molecular clock through noradrenaline secretion by the sympathetic nervous system (SNS).

The concept that signals from the SNS are critical for HSPC egress from the bone marrow was illustrated by several studies using complementary mouse models targeting this pathway. In one study, nonmyelinating Schwann cells were proven to be responsible for maintaining HSC hibernation through inducing TGF- β production from stromal cells. Mice with autonomic nerve denervation, reduced number of TGF- β -producing cells, or TGF- β type II receptor-deficient HSCs all exhibited reduced levels of Smad activation in HSCs, loss of HSCs from the bone marrow, and impaired long-term repopulation activity²⁷. In a different study, UDP-galactose ceramide galactosyltransferase-deficient (Cgt^{-/-}) mice exhibited aberrant nerve conduction and displayed no HSPC egress from the bone marrow following G-CSF or fucoidan administration²⁸. Pharmacological or genetic ablation of adrenergic neurotransmission in mice indicates that norepinephrine (NE) signaling controls G-CSF-induced osteolineage cell suppression, CXCL12 downregulation from stromal cells, and HSPC mobilization. In contrast, administration of a β (2) adrenergic agonist enhances mobilization in both NE-deficient and control mice, suggesting that the sympathetic nervous system is responsible for the attraction of stem cells to their niche. It was found in later

studies that the cells that relay the adrenergic signals from the SNS to HSPCs appear to be nestin⁺ mesenchymal cells, as in vivo nestin⁺ cell depletion significantly reduces BM homing of HSPCs²⁹.

It is known that some diabetic patients fail to achieve sufficient CD34⁺ HSPCs upon G-CSF stimulation. Using mouse models of type 1 (streptozotocin-induced) and type 2 (db/db) diabetes, scientists found that HSPCs that were unable to egress upon G-CSF treatment and localized aberrantly in the bone marrow space with altered pattern of catecholaminergic nerve termini¹³. This altered sympathetic innervation was associated with decreased sensitization of beta-adrenergic receptor in nestin-GFP⁺ cells, which in turn unable to down-modulate CXCL12 in response to G-CSF treatment. The HPSC mobilization defect in diabetic mouse models can be rescued by pharmacological inhibition of the interaction of CXCL12 with its receptor CXCR4 using the receptor antagonist AMD3100¹³.

In essence, these studies described an unprecedented pathway of mesenchymal cell control of stem cell trafficking – through the SNS. Sympathetic nerve fibers were indispensable players in relaying signals from the niche cells to the HSCs and regulating stem cell trafficking during hibernation, circadian oscillation, and G-CSF induced mobilization. These data offer new intervention opportunities to overcome poor HPSC mobilization in clinical and disease settings including diabetes.

Conclusion

In summary, recent advancement in technologies enabled unprecedented precision in pinpointing stromal components that support specific aspects of hematopoietic development and maintenance. The common denominator of these discoveries is that each ‘stromal’ cell type affects a very specific parenchymal hematopoietic cell subset development or activities. The emerging picture of the bone marrow niche is a highly complex, integrated ensemble of numerous micro-niches each specialized in unique hematopoietic functions and responding differently to stress or injuries. The potential of unraveling niche contribution to normal and disease physiology is substantial with the new tools at hand. It is anticipated that with better understanding of what specific subsets of hematopoietic cells are governed by what stromal elements, the ability to engineer a particular hematopoietic outcome will become feasible. Further, how the niche contributes to hematologic malignancies or bone marrow disease remains largely unexplored. Is there competition of anatomical space or niche resources between normal and malignant cells? Is there a hierarchy of components in the niche that confers cancer cell survival? Can specific intervention of these niche interactions improve therapeutic outcome? Gaining a ‘systems’ level understanding of the hematopoietic bone marrow may provide useful paradigms for studying other tissue niches with the long term goal of ultimately manipulating better regenerative processes in contexts of disease.

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* Of special interest

** Of outstanding interest

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Key Points

- Osx^+ osteolineage cells create micro-niches for B cell development by producing IGF1 to enable pro-B to pre-B maturation
- Ocn^+ mature osteolineage cells affect T lineage development by specifying early T competent progenitor commitment in the bone marrow through Notch signaling.
- CD169^+ macrophages are an essential component of erythroblastic islands, a specialized subcompartment within the bone marrow where the erythroid lineage develops. CD169^+ macrophage promotes erythropoiesis under homeostasis, but antagonizes normal erythropoiesis in disease settings including Polycythemia vera and β -thalassemia.
- CD169^+ macrophages reduce HSPC mobilization by secreting factors which in turn induce nestin⁺ and other stromal cells to express the HSC-retaining chemokine, CXCL12.
- Megakaryocytes secrete $\text{TGF}\beta 1$ to maintain HSC quiescence under homeostatic conditions, but produce FGF1 and additional molecules to promote HSC expansion upon 5-fluorouracil challenge.
- Nonmyelinating Schwann cells maintain HSC quiescence through inducing $\text{TGF-}\beta$ production from stromal cells (possibly megakaryocytes).
- Sympathetic nervous system controls HSC mobilization. SNS relays adrenergic signals to nestin⁺ cells, which upregulate CXCL12 and retain HSPCs to the bone marrow niche through CXCL12-CXCR4 interaction.

Figure 1.

Illustration of Osx^+ cell regulation of B cell maturation and Ocn^+ cell control of T lymphopoiesis. Osx^+ osteoprogenitors produce IL7 to mediate early pre-pro B to pro B development and secrete IGF1 to promote later stage of pro B to pre B transition. Deletion of Osx^+ cells in transgenic mice led to a failure of B cell maturation at the pro B to pre B transition stage. Administration of IGF1, but not IL7, was able to fully rescue B cell deficiency, suggesting that IGF1 production by Osx^+ cells is necessary for late stage B cell differentiation while IL7, which mediate early B cell development, is supplemented by Osx^+ cells but also provided by additional stromal subtypes in the bone marrow. On the other hand, Ocn^+ mature osteoblasts and osteocytes regulate T lineage cell development. Endogenous depletion of Ocn^+ cells or Ocn^+ cell-specific deletion of the Notch ligand DLL4, results in loss of T lineage cells. Ocn^+ cells express DLL4, which binds and activates Notch receptors on common lymphoid progenitors (CLPs). This cell-cell communication ensures adequate production of T-cell competent CLPs (Ly6D⁻ CLPs) and the expression of chemotactic molecules, CCR7 and PSGL1, on T progenitor cell surface necessary for subsequent thymic seeding and downstream T cell development. Abbreviations: hematopoietic stem cell (HSC), multipotent progenitor (MPP), common lymphoid progenitor (CLP), early T-cell precursor (ETP), double negative (DN), double positive (DP),

single positive (SP), delta-like 4 (DLL4), interleukin 7 (IL7), insulin-like growth factor 1 (IGF1), C-C chemokine receptor type 7 (CCR7), P-selectin glycoprotein ligand-1 (PSGL1).

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Figure 2.

Schematic representation of erythroblastic island. Erythroblastic island consists of a central CD169-expressing macrophage and adherent erythroblasts at varying stages of differentiation. The central macrophage regulates many steps of erythropoiesis through direct interactions with developing erythroblasts and secretion of factors such as IL6, TGF β , TRAIL, and TNF α to promote erythropoiesis. The macrophage not only provides iron for developing erythroblasts but also phagocytoses expelled nuclei. Young, multilobulated reticulocytes are initially attached to the macrophage surface but later detach. Abbreviations: interleukin 6 (IL6), transforming growth factor beta (TGF β), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), tumor necrosis factor alpha (TNF α).