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Dynamic responses of the haematopoietic stem cell niche to diverse stresses

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Adult haematopoietic stem cells (HSCs) mainly reside in the bone marrow, where stromal and haematopoietic cells regulate their function. The steady state HSC niche has been extensively studied. In this Review, we focus on how bone marrow microenvironment components respond to different insults including inflammation, malignant haematopoiesis and chemotherapy. We highlight common and unique patterns among multiple cell types and their environment and discuss current limitations in our understanding of this complex and dynamic tissue.

The bone marrow microenvironment and its effects on healthy, stressed and malignant haematopoiesis have received increasing attention over the past 15 years. Far from being a passive gateway for soluble factors, metabolites and signalling mediators, the bone marrow niche actively regulates haematopoiesis. Studying a tissue encased in bone is inherently challenging. However, phenotypic and functional characterisations of bone marrow stromal and immune subpopulations, together with advances in imaging techniques allowing the direct visualisation of these cells, have provided us with a deeper understanding of their supporting role in steady state haematopoiesis[1–18]. Insults can perturb the homeostatic balance of the bone marrow microenvironment in a variety of ways. They might induce changes in the cross-talk between HSCs and one or more components of the bone marrow microenvironment, often linked to shifts in the chemical makeup of intracellular and extracellular spaces. Several comprehensive Reviews covering multiple aspects of the niche are available[19–22].

In this Review, we focus on recent advances in our understanding of the cellular and molecular mechanisms through which a range of insults, such as infection and inflammation, haematological malignancies, radiation and drug treatments, change bone marrow stromal and immune cells and subsequently affect HSCs (Fig. 1). Several stromal cell subpopulations have been extensively studied, and the interest in immune cells is growing, as these cells mediate signals from sites of injury to bone marrow tissue components. Murine models are ideal systems for studying responses to all types of stress, and several findings have already been validated using human samples. We aim to elucidate commonalities and unique traits in the influence these perturbations have on the niche and further discuss challenges in the attempt to gain a more complete understanding of this complex tissue.

Bone marrow responses to infection and inflammation

Infections impair steady state haematopoiesis by inducing potent inflammatory responses that lead to drastic alterations in haematopoietic output to compensate for the increased demand for blood and immune cells[23]. HSCs directly sense pathogen-derived molecules[24] and display immune-surveillance properties[25], placing them among the primary responders to pathogens and inflammatory cytokines. In an effort to restore the balance, HSCs can alter their cycling properties[26,27], long-term function[28] and migration patterns within the affected bone marrow[29]. Although a number of studies have considered the consequences of infection and inflammation on haematopoiesis[16–19], more work remains to be done to address whether cellular and metabolic components of the bone marrow microenvironment mediate all or some of the haematopoietic phenotypes observed.

The bone marrow vasculature is lined by endothelial cells and functions as a protective barrier between sites of infection and inflammation and surrounding immune cells[30]. Recent work using interferon-α (IFN-α) to stimulate an acute inflammatory response in mice demonstrated that rapid activation and an increase in proliferation of endothelial cells leads to enhanced vessel remodelling[31]. The remodelling is mediated by an increase in production of vascular endothelial growth factor (VEGF) by bone marrow cells, including HSCs[32]. In addition, the increased vascular permeability is consistent with an enhanced release of immune cells in response to the stimulus. Endothelial cells also express a range of toll-like receptors (TLRs), sense pathogens and contribute to the haematopoietic response[31]. Upregulation of TLR4 and myeloid differentiation primary response 88 (MyD88) proteins by endothelial cells in response to lipopolysaccharide or *Escherichia coli* are required to recruit neutrophils, and subsequent secretion of granulocyte-colony stimulating factor (G-CSF) results in the initiation of emergency granulopoiesis[30,33]. All of these effects combined suggest that endothelial cells are key sentinels for detecting bacteria in the bone marrow. Interestingly, neutrophils regulate the rhythmic egression of haematopoietic stem and progenitor cells (HPSCs) from the bone marrow and may be an important link between infection and HSC function[34].

Multiple and often overlapping populations of mesenchymal stromal cells (MSC), depending on markers and genetic reporters used (see Table 1), are able to recognise surrounding inflammatory signals generated locally or systemically upon infection[35]. The activation of various pathogen-sensing receptors leads to the production of additional factors, modifying the cytokine and chemokine expression profile of the stroma. Two studies, using acute viral and parasitic infection models, suggested that MSCs are stimulated to secrete interleukin-6 (IL-6), leading to the expansion of myeloid

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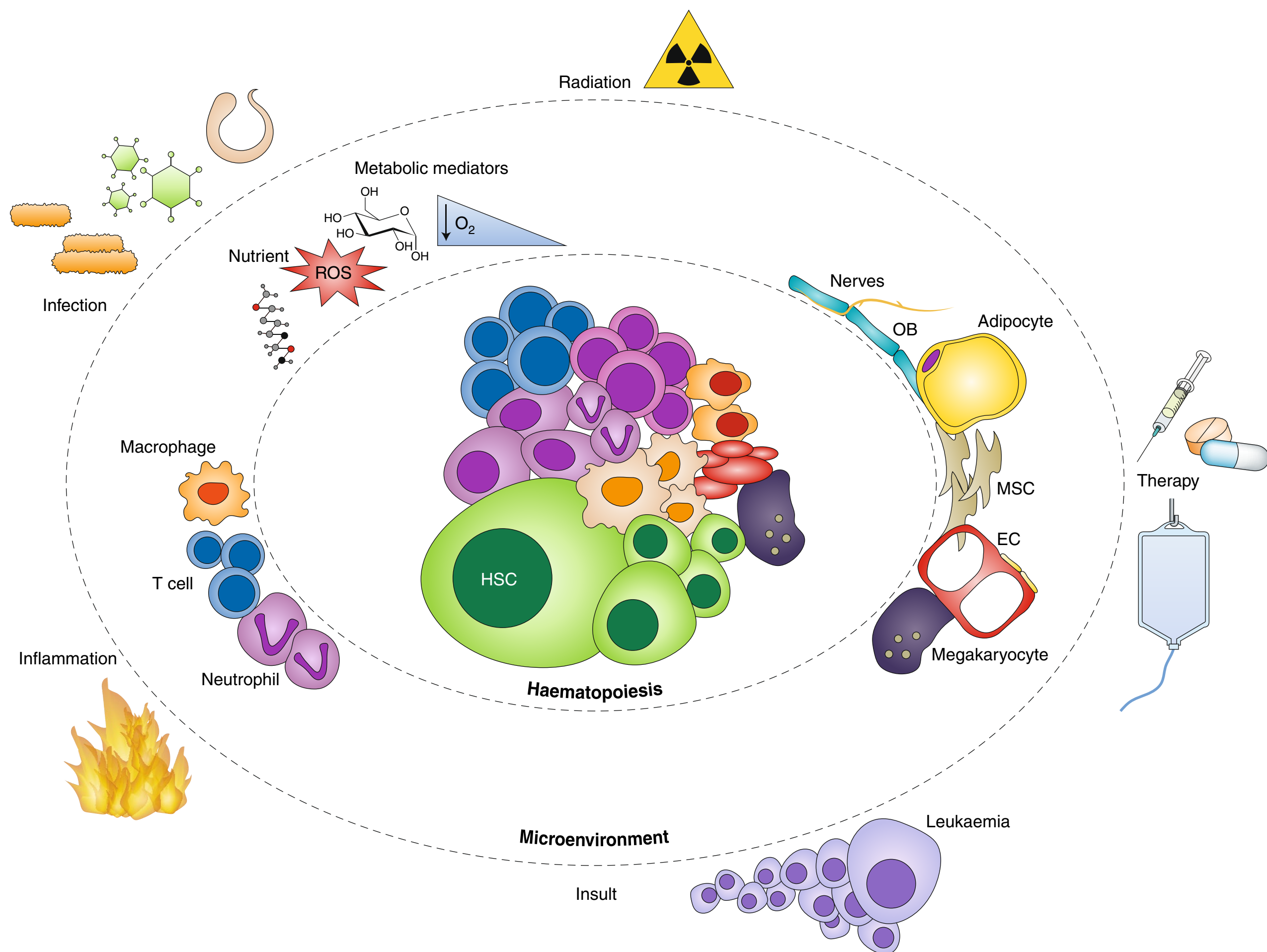

Fig. 1 | In the adult life, haematopoiesis happens in the bone marrow microenvironment, a tightly organized tissue in which cells of different origin and metabolic components act to orchestrate this process during homeostasis. The occurrence of insults such as inflammation, infection, therapy or leukaemia perturb the normal tissue balance and affect normal haematopoiesis in different manners. The bone marrow microenvironment has a central role in mediating, contrasting, balancing or endorsing these perturbations, representing an essential key to fully understand the physiopathology of these processes. OB, osteoblast; MSC, mesenchymal stromal cell; EC, endothelial cell; ROS, reactive oxygen species; HSC, haematopoietic stem cell.

progenitors and mature myeloid cells[36,37]. Furthermore, upregulation of G-CSF during infection induces enhanced myelopoiesis and suppresses the production of C-X-C motif chemokine 12 (CXCL12) in the bone marrow stroma[38], resulting in the mobilisation of HSCs into circulation[39,40]. CXCL12-abundant reticular (CAR) cells increase the number of circulating monocytes by upregulating expression of the pro-migratory chemokine C-C motif chemokine ligand 2 (CCL2), which is important for efficient clearance of *Listeria monocytogenes* infection[35].

Bone lining cells have long been studied in the context of the HSC niche, as proliferative and functional properties of HSCs often change when cells of the osteolineage are affected[41,42]. In the context of a murine sepsis model, for example, osteoblasts are rapidly ablated in a G-CSF-dependent manner[43], resulting in the reduction of osteoblast-derived interleukin-7 (IL-7) followed by that of common lymphoid progenitors. Additionally, evidence suggests that *Plasmodium* parasites reside within bone marrow cells in humans[44,45], and *Plasmodium* products in the bone marrow microenvironment generate MyD88-dependent inflammatory responses in osteoblast precursors, leading to a decline in osteoblasts[46].

Immune cells also contribute to HSC regulation[22,47]. However, few studies have directly addressed how infection and inflammation influence these cells in their role as niche regulators. Interferon-γ (IFN-γ), secreted by cytotoxic T cells in response to lymphocytic choriomeningitis virus (LCMV) infection, affects MSCs directly and subsequently mediates the production of myeloid cells[37]. Regulatory T cells (Tregs), enriched within the bone marrow compared to peripheral sites, contribute to the HSC niche by providing an immune-privileged environment[48] and protecting HSCs against oxidative stress[49]. Mice challenged with *Toxoplasma gondii* lose Tregs and plasma cells in the bone marrow, though the loss of plasma cells can be rescued by preventing a decrease of Tregs in the niche[50]. Whether a deficiency in Tregs has any consequences on HSC biology remains an open question.

Bone marrow macrophages indirectly regulate HSPC mobilisation and function[51]. Many bacterial and fungal infections elicit G-CSF secretion, promoting emergency granulopoiesis and HSPC mobilisation into the circulation[52,53]. The G-CSF-induced reduction in the number of bone marrow macrophages has been postulated to drive HSPC mobilisation[54–56]. Conversely, IFN-γ promotes the activation of macrophages[57]. In a model of intracellular *Ehrlichia muris* infection, IFN-γ was found to act on macrophages to drive a reduction in HSCs number and function[58], yet the mechanism underlying this observation remains unknown. During LCMV infection, IFN-γ causes anaemia by inducing macrophage-dependent phagocytosis of red blood cells[59]. IFN-γ might also induce macrophages to

Table 1 | Description of the transgenic mouse lines that have been used in the studies of the bone marrow microenvironment

Marker	Method of labelling	Overlap with endogenous markers	Overlap with genetically labelled populations	Spatial distribution	Contribution to HSC niche/biology	Reference
LepR	LepR-Cre	LepR, PDGFRβ, CD51, CD105, PDGFRα	CXCL12-DsRed; Nes-GFP^{dim}	Sinusoidal and arteriolar	HSC maintenance by expression of SCF, CXCL12	Ding et al., 2012[14]; Zhou et al., 2014[10]; Mizoguchi et al., 2014[13]
Osx	Osx-EGFPCre; Osx-Cre-ERt2	VCAM1, αSMA2, Neurofilament M	Not known	Pre-osteoblast, Growth plate	HSC maintenance by expression of CXCL12	Liu et al., 2013[15]; Strecker et al., 2013[18]
Nestin	Nes-GFP^{dim}		LepR-Cre	Sinusoidal		Kunisaki et al., 2013[16]
	Nes-GFP^{bright}	NG2, αSMA	NG2-CreER	Arteriolar	HSC quiescence	Kunisaki et al., 2013[16]
	Nes-GFP, Nes-CreERt2	PDGFRα	Not known	Perivascular	HSC homing	Mendez-Ferrer et al., 2010[106]
CXCL12	CXCL12-DsRed	MECA32; PDGFRα	SCF-GFP; Col1-GFP; LepR-Cre		HSC maintenance	Ding & Morrison, 2013[1]
Prx1	Prx1-Cre	PDGFRα; Sca1	Not known	Perivascular	HSC maintenance	Ding & Morrison, 2013[1]; Greenbaum et al., 2013[2]
Collagen-1a	Col2.3-GFP, Col2.3-Dtk	Osteopontin, CD44	Not known	Osteoblasts, osteocyte	Haematopoiesis, HSC and lymphoid progenitor maintenance	Visnjic et al., 2004[17]; Bowers et al., 2018[157]
Osteocalcin	OC-YFP	Alkaline phosphatase	Not known	Mature osteoblasts	HSC maintenance	Bilic-Curcic et al., 2005[3]; Karsenty et al., 2009[4]
GFAP	GFAP-GFP	Not known	Not known	Non-myelinating Schwann cells	HSC quiescence	Yamazaki et al., 2011[5]
Adiponectin	Adipoq-CreER, A-ZIP/F1 (depletion)	Perilipin	LepR	Adipocytes	HSC regeneration under stress, cycling of HSC	Naveiras et al., 2009[6]; Zhou et al., 2017[154]
Flk1	Flk1-GFP	CD31	Ve-Cadherin; PDGFβ-Cre; Tie2-Cre	Vascular	HSC maintenance, quiescence	Ishitobi et al., 2010[7]
PDGFβ	PDGFβ-Cre	CD31	Ve-Cadherin; Flk1-GFP; Tie2-Cre	Vascular	HSC maintenance, quiescence	Claxton et al., 2008[8]
Ve-Cadherin	VECad-Cre	CD31	Flk1-GFP; PDGFβ-Cre; Tie2-Cre	Vascular	HSC maintenance, quiescence	Alva et al., 2006[9]
Tie2	Tie2-Cre	CD31	Ve-Cadherin; PDGFβ-Cre; Flk1-GFP	Vascular	HSC maintenance, quiescence	Ding et al., 2012[14]

HSC, haematopoietic stem cell; LepR, leptin receptor; Osx, osterix; CXCL12, C-X-C motif chemokine 12; Prx1, paired-class homeobox 1; GFAP, glial fibrillary acidic protein; Flk1, fetal liver kinase 1; PDGFβ, platelet derived growth factor subunit β; VE-Cadherin, vascular endothelial cadherin; Tie2, tyrosine kinase receptor 2; αSMA: alpha smooth muscle actin; NG2, proteoglycan NG2; MECA32: panendothelial cell antigen antibody; Sca1, stem cell antigen-1; A-ZIP/F1, 'fatless' mice; Nes-GFP^{dim} or Nes-GFP^{bright}, Dim or Bright Nestin-Green Fluorescence protein positive expressing cells.

produce additional inflammatory factors, such as TNF-α, that might act directly on HSCs or the niche to drive HSC differentiation and/or apoptosis[57].

A known hallmark of acute inflammation includes the accumulation of large numbers of neutrophils and subsequent major shifts in tissue metabolism[60]. Metabolic factors, such as oxygenation levels, redox state, perfused nutrient availability, and glucose and fatty acid concentrations, can be affected by insults to the bone marrow and in turn impact haematopoiesis and tissue homeostasis[61]. In bacterial infections, the concentration of reactive oxygen species (ROS) increases in the bone marrow, depending on the expression of NADPH oxidase in myeloid cells, and is essential for myeloid progenitor expansion[62]. Sterile inflammation, initiated by non -infectious agents, such as chemicals or physical insults, triggers a similar response[63]. Evidently, both sterile and pathogen-induced inflammatory insults converge to a common point: NADPH-oxidase-dependent ROS production by bone marrow myeloid cells.

A number of studies have shed some light on the impact of infection and inflammatory stimuli on multiple components of the niche (Fig. 2 and Table 2). However, the effects on other stromal cells, for example adipocytes and sympathetic nerves, remain to be elucidated. Further insights into how the physiology of these cells changes under inflammatory stress will improve our understanding of infection-induced alterations in haematopoiesis. Long-term effects of these insults on the microenvironment will be particularly important to establish. Although most infections are eventually resolved, it remains unclear whether the bone marrow microenvironment ever truly returns to steady state. Epidemiological studies suggest that an accumulation of insults throughout one's lifetime may result in a higher likelihood of developing diseases linked to these environmental alterations, including bone marrow failure, clonal and malignant haematopoiesis[64,65].

Bone marrow changes elicited by malignant haematopoiesis

Historically, leukaemia has been considered a genetic disease[66]. However, the discovery that leukaemic stem cell (LSC) activity depends on microenvironmental cues has encouraged more research with a focus on extrinsic regulators of leukaemia, and HSC niche components are now well-studied in the context of haematological

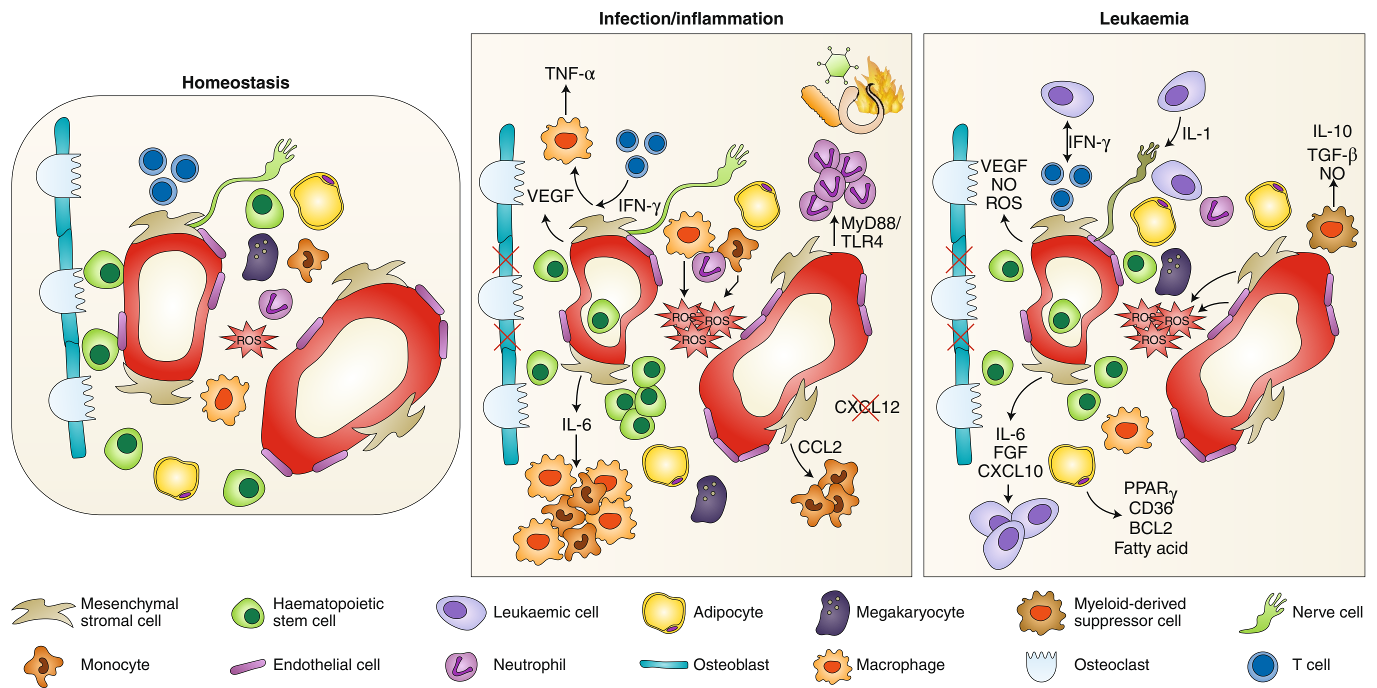

Fig. 2 | HSCs are localised within the bone marrow microenvironment where the various components orchestrate HSC maintenance, quiescence and differentiation. The occurrence of insults such as inflammation, infection or leukaemia perturb the niche components in terms of numbers and signalling. NO, nitric oxide; ROS, reactive oxygen species.

malignancies[67–71]. Abnormalities in the bone marrow vascular tree have been observed at both experimental and clinical levels. The detection of angiogenesis, first in myeloid and then in other haematological malignancies[72], has driven a strong clinical interest. Pro-angiogenic cytokines have been characterised in detail, including, most notably, members of the VEGF pathway[73]. Nonetheless, anti-VEGF trials have produced conflicting results, unveiling the need for a deeper understanding of the bone marrow vasculature. The characterisation of endothelial cell subpopulations and their regulation of haematopoiesis[74,75] and tissue homeostasis[76,77] is an important step towards this goal. Two recent studies highlight multiple abnormalities in the bone marrow vasculature in acute myeloid leukaemia (AML); notably, increased permeability with reduced perfusion and local remodelling in the endosteal region[78,79]. These alterations result in an exacerbated response to hypoxic stress in endothelial cells, with overproduction of ROS and nitric oxide, loss of barrier function and cell death[78]. Additionally, enhanced inflammatory signalling in endosteal leukaemic cells correlates with substantial loss of endosteal vessels, HSCs and HSC niches[79]. Preclinical approaches aiming to re-establish normal vascular function by either inhibiting nitric-oxide-dependent vascular permeability or boosting the growth of endosteal vessels were able to protect the pool of residual healthy HSCs[78,79]. In murine models of T-cell acute lymphoblastic leukaemia (T-ALL), endothelial-cell-derived CXCL12 was essential for disease growth[80]. CXCR4 inhibition successfully reduced T-ALL burden by inducing mobilization, reducing bone marrow homing and ablating T-ALL cells in the mesenchyme either alone or after chemotherapy[80–82].

The bone marrow stroma is profoundly perturbed in haematological malignancies[83]. The majority of studies so far have focused on how the remodelled microenvironment becomes supportive of malignant haematopoiesis, and a well-established hypothesis is that these same changes negatively affect healthy haematopoiesis and, specifically, HSCs[83]. For instance, human MSCs (defined based on the international society of cell therapy, ISCT[84]) extracted from patients with myelodysplastic syndrome (MDS) and AML show different methylation patterns compared to those from healthy donors[83]. Increased methylation of specific genes in MDS MSCs correlates with reported phenotypic changes and an inability to support healthy HSCs[83]. By contrast, a global hypomethylation pattern is evident in MSCs from patients with AML[85]. In addition, modifications in the expression of genes involved in adhesion, inflammation and signalling pathways, such as Notch and CXCL12, were reported in AML MSCs[85–87]. MSC differentiation data are heterogeneous across MDS, myeloproliferative neoplasia (MPN) and AML[83]. These differences could originate from the heterogeneity between or within these myeloid diseases, as well as from the methods used to isolate and expand MSCs ex vivo. MPN MSCs have a protective role for MPN stem cells through expression of IL-6, fibroblast growth factor (FGF) and CXCL10 (ref. [88]). Similarly, Pml expression by murine PDGFRα/Sca.1^{+} MSCs supports both chronic myelogenous leukaemia (CML) and AML through establishment of pro-inflammatory signals, whereas its deletion hinders disease progression[89]. Mutations in Ptpn11, a positive regulator of the Ras pathway, in murine MSCs (Nestin-Cre, Prx1-Cre, Lepr-Cre induced) and osteoprogenitors (Osx-Cre), but not osteoblasts (Osteocalcin-Cre) or endothelial cells (VE-Cad-Cre), promote MPN development through hyperproduction of CCL3 and hyperactivation of HSCs[90]. However, it is unlikely that mutations restricted to mesenchymal/osteo-lineage components will be sufficient to induce malignant haematopoiesis in human individuals. In patients with CML, MSCs had a protective function over leukemic cells through the CXCL12–CXCR4 signalling pathway[91]. Of note, CAR cells, which mainly surround sinusoidal endothelial cells, facilitate the retention of human AML leukaemic blasts within the bone marrow by producing CXCL12 (ref. [92]). Targeting CXCR4 increases murine and human AML blast mobilisation and their susceptibility to cytotoxic therapy[92] but does not affect migration and survival of murine AML blasts within the bone marrow parenchyma[82].

Table 2 | Comparison of the effects of various insults, such as infection, malignancy, radiation and chemotherapy, on the bone marrow microenvironment

Insult	BM microenvironment									Haematopoiesis
	Endothelial cells	MSC	Osteoblasts	Adipocytes	Nerve fibres	T cells	Myeloid cells	Megakaryocytes		
Infection/ inflammation	Increased[31] Activated (TLR4/ Myd88/ producing GM-CSF)[30,33]	Pro-inflammatory signals (IL-6, CCL2)[35,36]	Decreased[40]	---	---	Activated (Cytotoxic T cells)[36] Decreased (T-reg)[22]	Decreased (BM macrophages)[49,51] Increased (BM macrophages)[52]	---	Activated/ altered differentiation; Emergency granulopoiesis	
Haematopoietic malignancy	**MPN**	---	Pro-inflammatory signals[74]	Altered[86]	---	Decreased[107]	---	---	Decreased[163]	Promoted myeloid differentiation
	MDS	---	Altered potential/ Pro-inflammatory signals[74]	---	---	---	---	---	---	Increased immature precursors and anaemia
	AML	Increased[78]/ Altered[79]	Altered potential/ Pro-inflammatory signals[85]	Decreased[94,95]	Increased/ Decreased[97,98,100]	Decreased[106]	Tregs detected[113]	MDSC detected[115]	Decreased[163]	Impaired myeloid differentiation; Release of HSCs to the periphery
	ALL	---		Decreased[93]	Increased[86]	---	---	---	---	Increased immature lymphocytes
	CML	---	Pro-inflammatory signals[64]	---	---	---	Cytotoxic T-cells supporting LSC proliferation[110]	---	Decreased[163]	Increased mature granulocytes
	CLL	---	---	---	Increased[88]	---	Tregs detected[119]	MDSC detected[118]	---	Increased lymphocytes
	MM	---	---	---	Increased[89]	---	Increased (Treg)[121]	MDSC detected[117]	---	Decreased HSCs and MEPs
Irradiation/ therapy	Altered[155–157]	Altered potential[150,151]	Decreased[146,153]	Increased[153,154]	Decreased[143]	Radioresistant[169]	---	Increased[162,163]	Decreased	

MSC, mesenchymal stromal cell; MPN, myeloproliferative neoplasm; MDS, myelodysplastic syndrome; AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; CML, chronic myelogenous leukaemia; CLL, chronic lymphocytic leukaemia; MM, multiple myeloma.

The osteoblastic component of the niche is affected by haematological malignancies, and a loss of osteoblasts is documented in both murine and human AML and T-ALL[79,93]. Other reports have highlighted osteogenic differentiation priming of human AML MSCs in vitro[94], implying that an abnormal and likely inefficient expansion of this compartment occurs in these patients. Similarly, in a murine model of MPN, malignant cells transform osteoblasts into an inflammatory myelofibrotic unit, supporting malignant cells at the expense of normal haematopoiesis[95]. These observations obtained in both mouse and human settings, together with increasing evidence that genetic modifications of osteolineage cells in mice enhance leukaemogenesis[69,90,96], point to their supportive function for malignant cells, likely at the expense of healthy HSCs.

The adipocytic component of the niche expands during ageing and might also be involved in leukaemic progression. Human AML cell lines in an in vitro culture showed increased numbers of adipocytes, supporting AML survival via an increase in fatty acid β-oxidation (FAO), along with upregulation of PPARγ, CD36 and BCL2 genes[97]. Consistent with this finding, fatty acid binding protein-4 (FABP4) is upregulated in co-cultured adipocytes and AML cells, and FABP4 knockdown in human and murine AML prevents AML proliferation[98]. In T-ALL xenografts, distinct bone marrow niche components differentially regulate disease development, metabolism and cell cycle progression of leukemic cells owing to different abundancies of adipocytes[99]. In a mouse model of ALL maintained on high-fat diet, adipocytes contributed to chemoresistance by upregulating the survival kinase Pim-2 (ref. [100]), which has a protective function in multiple myeloma and chronic lymphocytic leukaemia (CLL)[101,102]. The overexpression of heparanase by multiple myeloma cells promotes adipogenesis at the expense of osteogenesis, enhancing bone lesions[103]. By contrast, analyses of samples from patients with AML in both in vitro co-cultures and in vivo xenotransplantation models revealed that human AML specifically disrupts the adipocytic component of the niche by inducing overexpression of connective tissue growth factors in MSCs[94], which results in impaired myeloerythroid maturation[104]. In support of this finding, administration of PPARγ agonists in vivo promoted bone marrow adipogenesis and rescued healthy haematopoiesis[104]. Future studies will resolve the controversial effects of adipocytes in different disease models.

The bone marrow is innervated by catecholaminergic nerve fibres, which mediate HSPC mobilisation and are in close contact with Nestin-GFP$^+$ cells[105]. Evidence suggests that neuropathy is essential for MPN development[106]. Specifically, in mice, loss of β3-adrenergic fibres induces the death of Nestin-GFP$^+$ cells[106]. Depletion of Nestin-GFP$^+$ cells accelerates MPN progression, whereas administration of β3-adrenergic agonists restores the sympathetic regulation of Nestin-GFP$^+$ cells and halts MPN development[106]. Further support for this theory comes from an AML mouse model in which quiescence of Nestin-GFP$^+$ cells was disrupted, resulting in increased osteogenic differentiation, adrenergic signalling via the β2 receptor and leukaemogenesis[13].

Immune cells are additional contributors to the regulation of normal haematopoiesis[22,58] and exhibit altered characteristics in various haematological malignancies. Increased Tregs have been recorded[107–110], and their depletion has been suggested as a potential immunotherapy treatment. However, a study indicates that bone marrow Tregs can protect HSC quiescence[49], raising the question of whether their expansion in cancer may not only favour malignant cells but also shield remaining healthy HSCs from oxidative stress and immune attack. A direct feedback loop between multiple myeloma cells and Tregs was recently uncovered, with multiple myeloma-derived type I IFN mediating Treg expansion and activation, thereby promoting disease progression[110]. The relationship between these two populations is consistent with the reported role of Tregs in regulating the number of bone marrow plasma cells in steady state and infection[50]. Whether Tregs may impact HSCs differently depending on local or systemic cues remains to be established.

Cytotoxic T cells with antileukaemic specificities have been detected in patients with leukaemia, and their role in disease development is under investigation[111,112]. Possible effects on the bone marrow microenvironment remain understudied. Intriguingly, in a mouse model of CML, the adoptive transfer of leukaemia-specific cytotoxic T cells increased the number of LSCs in vivo via secreted IFN-γ, which induced LSC proliferation[113]. Considering the well-documented negative effects of IFN-γ on HSC quiescence and function[114], it becomes clear that antitumour T cell responses, despite their unique ability to clear leukaemic cells, may create a microenvironment that favours malignant over healthy haematopoiesis.

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous immune population that only appear in the bone marrow under inflammatory conditions but are relevant to haematological malignancies[115]. MDSCs induce myelodysplasia, as they suppress haematopoiesis through the production of IL-10, TGF-β, nitric oxide and arginase[116]. These cells have been detected in patients with leukaemia[117–119], and in vitro studies indicate that cancer cells from multiple myeloma and CML, but not healthy MSCs, induce immunosuppressive granulocytic MDSCs[120,121]. MDSCs are also directly induced by leukaemic cells through the uptake of tumour-derived extracellular vesicles by myeloid progenitors[122]. In addition to suppressing antitumour specific T cells, MDSCs expand the pool of Tregs in haematological malignancies[123]. In summary, it is important to address whether the changes induced by haematological malignancies in immune cells known to regulate HSCs are helpful or detrimental to the HSCs, especially when restoring healthy haematopoiesis in patients receiving immunotherapy.

Classically, leukaemia is known as a metabolically demanding unit, requiring large energetic resources to sustain the elevated rate of cell growth and division[124]. These metabolic requirements have profound effects on the bone marrow microenvironment. Increased perivascular hypoxia, reported in AML xenografts, causes extensive remodelling of vascular endothelial cells and increases their oxidative stress, hypoxia inducible factor (HIF) pathway activation, ROS and nitric oxide production[78]. These changes result in poor retention of HSCs in the bone marrow niche, which also exhibits high levels of ROS[81]. In CLL, high expression of HIF-1α correlates with increased CXCR4 levels, which regulates the adhesion and migratory properties of leukaemic cells in both bone marrow and spleen[125]. Notably, the hypoxic state of the leukaemic microenvironment can also be exploited by therapeutic strategies that specifically target leukaemic areas of the bone marrow, as demonstrated in preclinical models of AML and multiple myeloma[126,127].

The oxidative stress that occurs during development of leukaemia in the bone marrow can be mediated by stromal components[128]. Strikingly, the same cells can help leukaemic cells to balance the redox homeostasis by providing metabolites used to produce antioxidants[128]. For instance, human bone-marrow-derived MSCs produce soluble factors that induce oxidative stress and mitochondrial calcium influx and increase ROS levels in ALL cells[129]. In response, leukaemic cells can activate antioxidants, leading to decreased mitochondrial membrane potential and ROS levels, favouring chemoresistance[129]. Human MSCs in the bone marrow convert cystine into cysteine, which can easily be metabolised by CLL cells into glutathione. In absence of this stromal effect, drug sensitivity increases[130].

Leukaemic cells require oxidative phosphorylation (OXPHOS) to survive[131,132]. However, in AML, the LSC compartment might adopt a metabolically dormant state with low ROS levels and low energetic demand[131]. Mitochondria are crucial regulators of energy metabolism, survival and cell fate. An intriguing process of mitochondrial exchange between cells in the bone marrow was recently reported[133]. In the context of AML, mitochondrial transfer from the stromal to the leukaemic compartment has been highlighted as a

consequence of oxidative stress[134]. This process of mitochondria transfer seems to be enhanced by chemotherapy, which promotes an increase of mitochondrial mass in the leukaemic compartment and leukaemia survival[128]. In addition to supporting OXPHOS, mitochondrial function may be essential for leukaemia survival and energetic demands through FAO. Two reports on AML have demonstrated that FAO inhibitors reduce the proliferation of leukaemic cells and sensitise them to apoptosis[2,135]. The source of fatty acid in the bone marrow might be the adipocytic component of the niche, which has been examined for its metabolic regulation of leukaemogenesis[97]. In CML, LSCs can adapt to survive and resist chemotherapy in an adipocytic component of the niche using FAO[136]. In a different setting, adipocytes have been shown to support ALL cells via glutamine production, especially after chemotherapy[137]. This protective effect is even more specific in the context of L-asparaginase treatment, which reduces the availability of asparagine and glutamine for leukaemic cells[137]. The high metabolic rates and energetic demands make leukaemic cells more sensitive to metabolite starvation compared to healthy HSCs. For instance, HSCs are resilient to amino acid and nutritional stress[138,139]. By contrast, dietary restriction is efficient in both B-ALL and T-ALL mouse models, though less in AML[140]. However, amino acid starvation has shown promising results in AML when achieved with mTOR inhibitors[141]. Specific nutrients can affect HSC epigenetic regulators and protect HSCs from the development of leukaemia. Two studies report that ascorbate (vitamin C) promotes Tet function in HSCs, suppressing leukaemia development and even leukaemia growth if given at a super-physiological dosage[142,143].

Extensive remodelling of the bone marrow environment is a hallmark of leukaemia development, with some changes shared across different haematological diseases and others restricted to specific subtypes (Fig. 2 and Table 2). Further work will elucidate whether the remodelling results from specific responses of each bone marrow component or, more likely, from a more complex cross-talk between different environmental components.

Effects of radiation, chemotherapy and immunotherapy

Radiation therapy is commonly used in solid cancers and haematological malignancies. Radiation exposure leads to a drastic reduction of haematopoietic populations and damages bone tissue. It also increases the mutation rate in the HSC compartment, potentially leading to secondary leukaemias[144]. However, the effect of radiation on the bone marrow microenvironment is largely unknown. As radiation therapy is used in patients with cancer, it is imperative to understand its effects and define a means to rescue bone marrow architecture[145], which is expected to improve haematopoietic regeneration post-treatment. After radiation, a severe decline in bone volume is evident, particularly in the trabecular region, as detected by micro-computed tomography analysis of mice[146]. An increase in osteoclasts, responsible for bone resorption, could be the reason for this morphological change[146–148]. In addition, the extracellular matrix within the bone is similarly destroyed (specifically collagen)[149]. Contrasting literature has been published on the survival of stroma cells after radiation therapy, which may have resulted from the variety of models and protocols used or from potential differences between murine models and human samples[150–152]. Of note, the clonogenic and differentiation capacity of mouse MSCs is perturbed, with a decrease in osteogenic and increase in adipogenic output[153]. Adipocytes become abundant and constitute an important source of stem cell factor, which positively regulates HSC regeneration in the irradiated animals[154]. Alternatively, co-transplantation of HSC with sorted CD73$^+$ CD105$^+$ Sca-1$^+$ stroma cells rescues niche damage and improves HSC reconstitution in mice[152]. The bone marrow vasculature and particularly sinusoidal endothelial cells are also severely affected by irradiation[154–157]. The mechanism of endothelial cell regeneration after irradiation is at least partially linked to VEGFR2 signalling[155,156]. Moreover, the transfer of granulocytes to irradiated mouse recipients promotes endothelial cell regeneration via TNF-α signalling[157], and bone marrow resident macrophages are essential for optimal outcomes of HSC transplantation. These macrophages should be considered in the development of pre-transplant conditioning therapies[158].

Chemotherapy, such as 5-fluorouracil (5-FU) or cisplatin treatment, causes myelo-ablation and has similar effects on the bone marrow niche as radiation therapy, namely loss of osteoblasts, increase in adipocytes and damage of the vascular network[154,159,160]. In addition, 5-FU leads to the activation of quiescent HSCs, concomitant with an increase in megakaryocyte numbers, which migrate into the bone marrow vessels and promote HSC expansion via FGF1 (refs. [161,162]). 5-FU treatment has been associated with massive vascular damage and leakiness, which is linked to G-CSF and IL-1 production. Megakaryocytes, by producing CXCL4 and TGF-β1, re-establish HSC quiescence following 5-FU treatment[163]. Chemotherapy also induces sympathetic nerve injury, which compromises haematopoietic recovery because of a loss of survival signals from Nestin$^+$ and endothelial cells as well as impairment of HSC mobilisation[164].

Cytotoxic T cells persist in the bone marrow of patients with leukaemia after chemotherapy and can be ex vivo expanded to be re-infused as tumour immunotherapy[165]. Interestingly, chemotherapy also induces antitumour immune responses[166–168] through autophagy-dependent ATP release from dying tumour cells[58]. However, ATP release post-chemotherapy can have a tolerogenic effect by increasing suppressive Tregs and dendritic cells. In fact, an increase in immunosuppressive immune cells following chemotherapy was observed in multiple leukaemia models[169,170]. The impact of these immune cells on the bone marrow microenvironment has not been specifically addressed, and more studies are needed to elucidate the immune cell composition of the bone marrow post-chemotherapy, especially in view of its influence on HSCs.

Immunotherapies target malignant cells more specifically than chemotherapy or radiation therapy and, in doing so, are supposed to have fewer side effects. However, their administration essentially unleashes an army of killer cells that produce high levels of pro-inflammatory cytokines as they clear tumour cells and can result in a potentially lethal cytokine response syndrome (CRS)[171]. Though CRS has been recognised as a major limitation of otherwise impressively effective immunotherapies[172,173], potential long-term consequences of immunotherapy and CRS on HSCs are likely, but still overlooked. Considering that curative responses in haematological malignancies depend on the restoration of healthy haematopoiesis, the greatly elevated serum levels of IFN-γ, IL-10 and IL-6 seen in CRS can have a direct effect not only on HSPC regeneration, but also on the bone marrow microenvironment, as discussed earlier in this Review. Interestingly, in a study utilising a mouse model of CRS, IL-6 was produced by myeloid cells recruited by the tumour-activated therapeutic CAR-T cells[174]. This finding demonstrates the need to consider possible interactions of cellular therapies not only with their malignant target, but also with healthy cells to achieve safe and curative protocols[174].

Concluding remarks

In this Review, we have summarised the literature on stress-mediated changes to the bone marrow microenvironment and their consequences for HSCs. A number of parallels are emerging when looking at the cellular responses to inflammation, infection, leukaemia and chemical insults with similarly disrupted components and pathological features (Table 2 and Fig. 2). Yet, other responses are highly specific and depend on the nature of the insult or type of malignant transformation. Notably, not all niche components have been assessed in the context of multiple stresses, and it may be essential to differentiate between systemic and local effects of bone marrow resident immune cells. Despite remarkable advances made

in this field, several pressing questions remain about the extent of bone marrow recovery and the possibility that a memory, or scar, from previous stresses is retained. It will be interesting to determine whether a microenvironment that has experienced repeated or severe infection might age more rapidly or become more susceptible to malignancy development. An unprecedented wealth of knowledge is now available about the complexity of cellular and molecular mechanisms co-developing in such scenarios, but understanding them deeply remains challenging. Standardising nomenclature, assays and flow cytometry gating strategies would be first steps towards tackling many current controversies. Several technical developments would aid future studies. Improved experimental models should provide more specific reporters to characterise stem cells, stromal and vascular subpopulations. Equally helpful will be the availability of improved imaging approaches that allow the tracking of a higher assortment of cellular components and active molecular signals using three-dimensional bone marrow sections[175], as well as intravital imaging using photoconversion[176–178], osteotomy[178] and longer monitoring windows[179] to capture complex, dynamic events. Single-cell transcriptomic approaches have revolutionised our understanding of the bone marrow stroma[87,160] and the ability to combine high-resolution microscopy with genome-wide analyses of the same cells at high throughput will help to uncover the underlying mechanisms. Refined systems biology and mathematical approaches and machine learning techniques will be essential to integrate all these data in a comprehensive manner. Ultimately, understanding the concerted changes in multiple components of the bone marrow microenvironment that may regulate HSCs directly or indirectly will lead to a more complete picture of how the HSC niche functions. Several other cell types beyond HSCs rely on the bone marrow microenvironment to survive and function, and their niches remain understudied. We hope that the body of work on HSC niches will also provide an excellent resource to draw on when developing these studies further.

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Competing interests

The authors declare no competing interests.

Additional information

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