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REVIEW

Bone marrow and the control of immunity

Ende Zhao[1,2,7], Huanbin Xu[3,7], Lin Wang[2], Ilona Kryczek[1], Ke Wu[2], Yu Hu[2], Guobin Wang[2] and Weiping Zou[1,4,5,6]

Bone marrow is thought to be a primary hematopoietic organ. However, accumulated evidences demonstrate that active function and trafficking of immune cells, including regulatory T cells, conventional T cells, B cells, dendritic cells, natural killer T (NKT) cells, neutrophils, myeloid-derived suppressor cells and mesenchymal stem cells, are observed in the bone marrow. Furthermore, bone marrow is a predetermined metastatic location for multiple human tumors. In this review, we discuss the immune network in the bone marrow. We suggest that bone marrow is an immune regulatory organ capable of fine tuning immunity and may be a potential therapeutic target for immunotherapy and immune vaccination.
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INTRODUCTION

Bone marrow is the tissue comprising the center and the epiphysis of bones, which is the place where new blood cells are produced. Bone marrow has been long thought to be a hematopoietic organ. However, it is well known that B cells are produced and matured in the bone marrow. Antigen-specific antibody producing, long-term lived plasma cells are largely found in the bone marrow. Thus, bone marrow contributes to humoral immune responses. Although normal bone marrow lacks the organized T- and B-cell areas, bone marrow is a nest for function, migration and selective retainment of innate and adaptive immune cells. In this review, we discuss the immune networks in the bone marrow. We suggest that bone marrow is an immune regulatory organ capable of fine tuning immunity and may be a potential therapeutic target for immunotherapy and immune vaccination.

BONE MARROW STRUCTURE

Bone is an organ composed of cortical and trabecular bone, cartilage, hemopoetic and connective tissues. Spongy or trabecular bone is composed of a lattice of fine bone plates filled with hematopoietic marrow, fat containing marrow or blood vessels. Arterial vessels enter the marrow through foramina nutricia and then divide into several arterioles. Small arterioles and capillaries from these vessels span throughout the bone marrow and supply sinusoids, which are interconnected by intersinusoidal capillaries (Figures 1 and 2).[1] The bone marrow cavity in trabecular bone is subdivided into four regions: endosteal, subendosteal, central and perisinusoidal. Bone marrow consists of a hematopoietic component (parenchyma) and a vascular component (stroma) (Figure 1). The parenchyma includes hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (Figure 1), which are not randomly distributed in the bone marrow but rather are localized close to the endosteum of the bone and more around blood vessels (Figure 2).

Bone marrow stroma contains multipotential non-hematopoietic progenitor cells (Figure 1c) capable of differentiating into various tissues of mesenchymal origin, including osteoblasts, endothelial cells, reticular cells, fibroblasts and adipocytes. The bone marrow's microvasculature with a single layer endothelium forms sinusoid (Figure 1b), which radically distributes around the draining central sinus. The vasculature provides the barrier between the bone marrow compartment as a functional and spatial entity from extralymphoid organ and the peripheral circulation.[1]

The stromal cells including endothelial cells provide signals for migration of individual leukocytes into and out of the bone marrow, involving in rolling/extravasations along the vascular endothelium. Compared with other organ-specific endothelial cells, bone marrow-derived endothelial cells constitutively express certain cytokines and adhesion molecules like vascular cell adhesion molecule 1 (VCAM-1 or CD106) and E-selectin (Figure 3). Additionally, organ regeneration relies on the presence of endothelial precursors among the grafted cell population, which improves vascularization of damaged tissues or by secretion of pro-angiopoietic factors by the infused cells.[2] Therefore, it not only implies a role of bone marrow microvasculature system in stem cell mobilization and development, but also indicates that they play key roles in migration and maintenance of leukocyte function in bone marrow environment.

IMMUNE CELLS IN THE BONE MARROW

Immune system is functionally compartmentalized into primary lymphoid organs and secondary lymphoid tissues where immune responses are initiated and maintained. T-cell areas of secondary

[1]Department of Surgery, University of Michigan School of Medicine, Ann Arbor, MI, USA; [2]Union Hospital, Huazhong University of Science and Technology School of Medicine, Wuhan, China; [3]Tulane National Primate Research Center, Tulane University School of Medicine, Covington, LA, USA; [4]Graduate Program in Immunology, University of Michigan School of Medicine, Ann Arbor, MI, USA; [5]Graduate Program in Biology, University of Michigan School of Medicine, Ann Arbor, MI, USA and [6]University of Michigan Comprehensive Cancer Center, University of Michigan School of Medicine, Ann Arbor, MI, USA
[7] These two authors contributed equally to this work.
Correspondence: Dr Weiping Zou, University of Michigan School of Medicine, C560B MSRB II, 1150 W. Medical Center Dr., Ann Arbor, MI 48109-0669, USA.
E-mail: wzou@med.umich.edu
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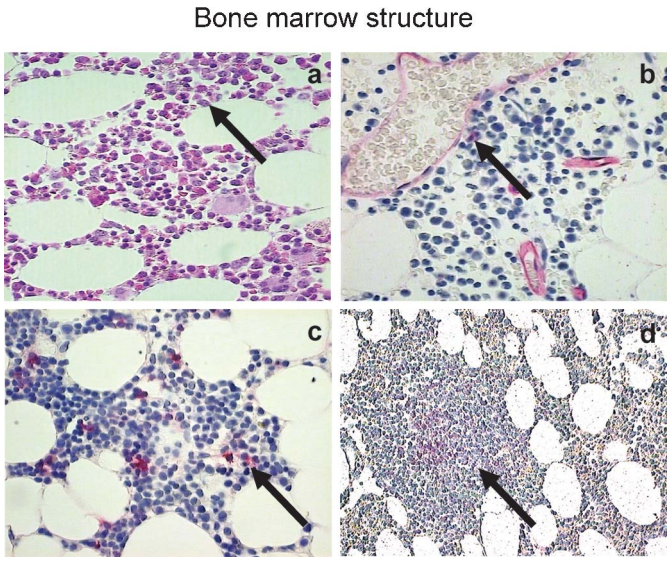

Figure 1 Bone marrow morphology and key cellular components: bone marrow core biopsy sections were subjected to HE staining (**a**), anti-CD34 staining (**b**), anti-CD38 staining (**c**) and anti-CD20 staining (**d**). The stained sections were revealed with fast red, analyzed by conventional microscope and images were shown with ×40 (**a**–**c**) and ×10 (**d**) magnification. The positive cells were judged by positive staining (red, **b**–**d**) as well as the morphology. The control mAb staining reveals no positive cells (not shown). HE, hematoxylin and eosin; mAb, monoclonal antibody.

lymphoid organs have a unique architecture and cellular composition which is thought to be a prerequisite for primary T-cell responses.[3] Bone marrow displays structural and functional features resembling a secondary lymphoid organ, and contains follicle-like structures similar to lymph nodes or spleen, although it lacks the organized T- and B-cell areas (Figure 1d). Bone marrow microenvironment provides

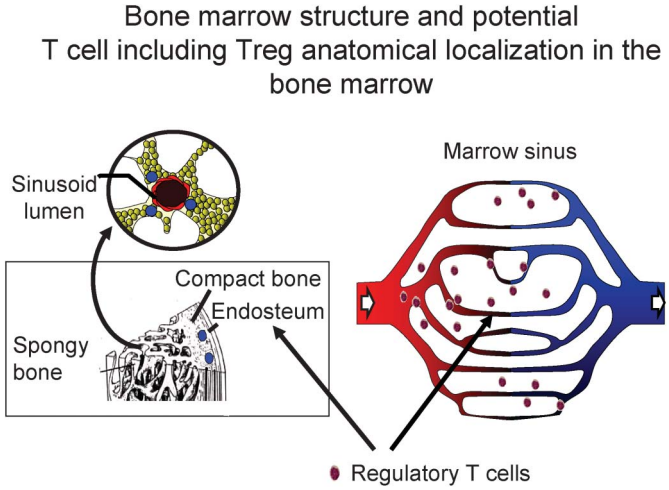

Figure 2 Bone marrow structure. The bone marrow is encased by cortical bone and traversed by trabecular bone. Bone marrow consists of a highly organized meshwork of thin-walled capillary-venous with extracellaur matrix that fills the space between the bony trabeculae. The artery and the periosteal capillary network are the two sources of arterial blood for the bone marrow. By successive bifurations, small branches of the artery ultimately form the capillary-venous sinus network. Murine and human bone marrow harbor immune cells including Treg cells. T cells including Treg cells may reside in the marrow sinus. Treg, regulatory T.

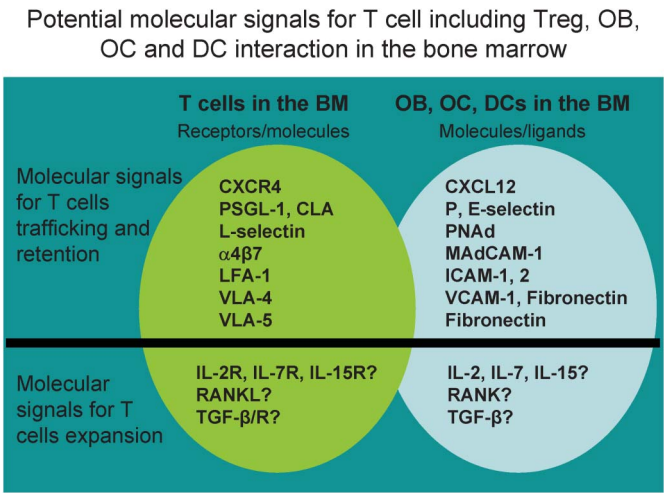

Figure 3 Molecular and cellular interaction between T cells and marrow sinus: T cells including Treg cells might be in the sinus areas or/and endosteum areas. The organization of molecular and cellular niches is known to have a key role in regulating T-cell immunity. Bone marrow contains a large amount of chemokines, adhesion and integrin molecules. These molecular signals may be important for immune cell bone marrow trafficking, retention and expansion. These structural and molecular niches may play a role in bone pathology in cancer patients with bone metastasis including prostate cancer and breast cancer. BM, bone marrow; CLA, conjugated linoleic acid; DC, dendritic cell; ICAM, intercellular adhesion molecule; LFA, leucocyte function antigen; OB, osteoblast; OC, osteoclast; PSGL, P-selectin glycoprotein ligand; RANKL, receptor activator of NF-kappaB ligand; TGF, transforming growth factor; Treg, regulatory T; VCAM, vascular cell adhesion molecule; VLA, very late antigen.

appropriate support for T cells to develop in the absence of the thymus.[4] Lymphoid follicles in the bone marrow are increased during infections, inflammation and autoimmunity.

Bone marrow is vascularized by blood (Figure 1b), not by lymphatic vessels, and could represent a major part of the lymphocyte recirculation network, with billions of lymphocytes recirculating through it per day. It has been shown that bone marrow contains various immune cells (Table 1). Approximately 8%–20% of bone marrow mononuclear cells are lymphocytes, with a T cell/B cell ratio of 5 : 1.[5,6] Bone marrow lymphocytes are distributed throughout stroma and parenchyma, and condensed in lymphoid follicles. Approximately 1% of the bone marrow mononuclear population represents plasma cells, which can produce antibodies. Mouse bone marrow contains 1%–5% $CD3^+$ T cells in mononuclear cells.[6,7] Among T cells, there are about 1.5% $CD4^+$ T cells and 2.0%–2.5% $CD8^+$ T cells.[8–11] Interestingly, approximately one-third of $CD4^+$ T cells are $CD4^+CD25^+$ regulatory T (Treg) cells,[12] and the CD4/CD8 ratio in the bone marrow is 1 : 2, which is inverted as compared to both peripheral lymph nodes and the

Table 1 Immune cells in the bone marrow

Immune cells	*Percent*	*Reference*
$CD4^+$ T cells	~1.5%	10, 11
$CD8^+$ T cells	2–2.5%	8, 9
Regulatory T cells (Treg)	~0.5%	10–12
$CD11c^+$ dendritic cells (DCs)	1–2%	6, 7, 13
B cells	~1%	63
Plasma cell	~0.5%	5, 66
Natural killer T (NKT) cells	0.4–4%	10, 14–16
Mesenchymal stem cells (MSCs)	0.01–0.1%	109, 117
Myeloid-derived suppressor cells (MDSCs)	20–30%	97, 100

blood.[8,10] Two-thirds of bone marrow T cells express surface markers indicative of antigen experience, such as $CD44^{hi}$ and $CD122^{+}$, whereas most T cells in spleen and peripheral lymph nodes exhibit naive phenotypes.[8] In addition to T cells, there are 1%–2% $CD11c^{+}$ dendritic cells[6,7,13] and 0.4%–4% natural killer T (NKT) cells in bone marrow.[14–16] Therefore, bone marrow contains substantial amount of immune cells. Altogether, the evidences suggest that bone marrow is a lymphoid organ which may play a key role in immunity.

$CD4^{+}$ T cell

Bone marrow contains a high proportion of $CD4^{+}$ T cells displaying a memory phenotype, which express high levels of CD44 in mice and low levels of CD45RA in humans (Figures 2 and 3).[8,11] Similar to the $CD8^{+}$ T cells, basal homeostatic proliferation and survival of $CD4^{+}$ memory T cells are regulated by IL-7, identified as the dominant cytokine, and IL-15, an accessory cytokine.[17] Although human $CD4^{+}$ memory T cells proliferate *in vitro* in response to IL-7 and IL-15,[18] studies in mice showed that acute homeostatic proliferation of 'memory-phenotype' $CD4^{+}$ T cells is independent of IL-7 and IL-15.[19] Both cytokines were also ruled out to participate in $CD4^{+}$ memory T-cell survival, because $CD4^{+}$ memory T cells deficient for CD132 (γc-chain, jointly used by IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 receptors) are effectively maintained *in vivo*, but other work demonstrated that IL-7 is actually required for survival of both memory phenotype and T-cell receptor transgenic $CD4^{+}$ memory T cells.[20]

Treg cell

Naturally occurring $CD4^{+}CD25^{+}$ Treg cells represent 5%–10% of the $CD4^{+}$ T cells. However, our group has reported that 30% of $CD4^{+}$ T cells are functional Treg cells in bone marrow.[12] In patients with prostate cancer bone marrow metastasis, bone marrow Treg cells are further increased (Zou *et al*., unpubl. data). It suggests that bone marrow is a preferential site for migration, or selective retainment and function of Treg cells.[12,21,22] Furthermore, we have demonstrated that CXCR4/CXCL12 (CXC chemokine ligand 12, stromal cell-derived factor-1) signaling mediates Treg cell trafficking to bone marrow (Figures 2 and 3).[12] Mouse Treg cells are known to reduce the severity of graft-versus-host disease (GVHD).[6,23] Granulocyte colony-stimulating factor (G-CSF) decreases bone marrow CXCL12, and in turn mobilizes bone marrow Treg cells. These findings may help explain why G-CSF administration reduces the severity and mortality in acute GVHD.[13,24]

Given that high levels of Treg cells are found in the bone marrow, bone marrow transplantation may result in increased numbers of Treg cells and in turn lead to a reduction of autoantibody production and protection from lethality caused by severe GVHD.[25] Additionally, antigen presentation by bone marrow dendritic cells can induce the expansion of $CD4^{+}CD25^{+}$ T cells while simultaneously activating their ability to suppress cytokine secretion by effector T cells. Therefore, agents that mobilize Treg cells from bone marrow would be therapeutically beneficial in some clinical settings.

It is well known that Treg cells are implicated in the pathogenesis of autoimmune diseases, tumors and organ transplantation.[26–29] Our unpublished data indicate that Treg cells may regulate bone biology. For example, Treg cells can suppress osteoclastogenesis and ameliorate osteolytic bone resorption and destruction. The levels of bone marrow Treg cells are much higher in patients with prostate cancer. Patients with prostate cancer often have bone metastases with bone precipitation as a pathological characteristic. It is assumed that bone marrow Treg cells may contribute to bone pathology in patients with prostate cancer. The studies in our laboratory will address this possibility. Nonetheless, bone marrow Treg cells might be a novel therapeutic strategy for clinical diseases and transplantation.[30,31]

$IL\text{-}17^{+}CD4^{+}$ T (Th17) cell

IL-17 (originally termed CTLA8, also known as IL-17A) belongs to a family of six members and has been of great interest recently owing to the discovery that the production of IL-17 characterizes a subset of $CD4^{+}$ helper T cells (Th17 cells).[32] The development of Th17 cells is coupled to signal transducer and activator of transcription 3 and the transcription factor RORγt and depends on IL-6 and transforming growth factor (TGF)-β, which is shared by regulatory T cells for development and function.[33] Th17 cells are characterized by the production of a distinct profile of effector cytokines, including IL-17 (or IL-17A), IL-17F, IL-21 and IL-22.[34–36] Th17 cells play an important role in inflammation, autoimmune disease and tumor.[37–39]

Th17 cells are induced and elevated in multiple myeloma by the elevated cytokine production of IL-6, IL-1β and TGF-β in bone marrow microenvironment.[33] IL-17 in turn promotes myeloma cell growth and suppresses immune function in multiple myeloma.[33] It is also reported that elevated Th17 cells mediate bone resorption and destruction by inducing osteoclast formation in multiple myeloma patients.[40] Bone marrow mesenchymal stem cells (MSCs) inhibit the differentiation and function of Th17 cells and ameliorate multiple sclerosis in an experimental autoimmune encephalomyelitis model.[41,42] Administration of bone marrow stromal cells ameliorates experimental autoimmune myasthenia gravis by reducing Th17 cells.[43] Several groups have shown that Th17 cells contribute to GVHD after bone marrow transplantation.[44,45] There is evidence showing increased Th17 cells in low risk myelodysplastic syndrome.[46] Thus, Th17 cells play an important role in bone marrow-mediated immunity and may serve as a therapeutic target for bone marrow-related diseases.

$CD8^{+}$ T cell

Bone marrow is the preferred site for proliferation of memory $CD8^{+}$ T cells.[47] Antigen specific memory $CD8^{+}$ T cells receive proliferative signals by IL-7 and/or IL-15 in the bone marrow. It suggests that the bone marrow is a 'niche' for the antigen-independent proliferation of memory $CD8^{+}$ T cells. Memory $CD8^{+}$ T lymphocyte populations in bone marrow display a CD44-positive phenotype with a higher percentage of HLA-DR molecules, which suggests that $CD8^{+}$ T cells in bone marrow are in an activated state. High numbers of tumor-associated antigen-specific $CD8^{+}$ T cells were shown to persist in the bone marrow for several months after acute infection or tumor development.[48,49] Adoptive transfer of bone marrow cells from lymphochoriomeningitis virus-immunized mice to immunodeficient recipients provides antiviral protection.[9] Thus, memory $CD8^{+}$ T cells in the bone marrow are able to mount an effective secondary response. A long time after priming, memory $CD8^{+}$ T cells proliferate more extensively in the bone marrow than they do in either secondary lymphoid or extra-lymphoid organs and undergo basal proliferation in the bone marrow.[47,50] Indeed, bone marrow-resident T cells are functionally distinct from those in other compartments.[10] Compared to their blood counterparts, bone marrow-derived $CD8^{+}$ T cells induce milder GVHD and possess higher anti-tumor activity.[6]

Bone marrow also functions as a site of recruitment and retention for central memory T cells by providing specific recruitment signals that mediate the recruitment of central memory T cells from the blood.[8] Central memory T cells constitute the largest endogenous

subset of $CD8^+$ T cells in murine bone marrow and are also prominent in human bone marrow. There are also naive $CD8^+$ T cells and effector memory $CD8^+$ T cells in bone marrow, but these populations are smaller (at least in mice) than naive T cells in secondary lymphoid organs or effector memory T cells in spleen, liver and lung.[51] Accordingly, adoptively transferred central memory T cells from immunized mice accumulated and colonized in recipient bone marrow more effectively than effector T cells and naive T cells. Overall, the bone marrow may be an important organ for $CD8^+$ T cell-mediated immunity.

NKT cell

NKT cells are defined as T cells bearing the common natural killer cell marker such as NK1.1 (NKR-P1C) in mice or CD161 (NKR-P1A) in humans, expressing IL-2Rβ (CD122), Ly49 family receptors and T-cell receptor.[52,53] In normal adult mice, NKT cells are found in thymus, bone marrow, liver and spleen at a level of 0.5–1.5 million cells per organ. NKT cells are rare in lymph nodes and virtually absent from gut intraepithelial lymphocytes. Besides thymus and liver, NKT cells can be generated in bone marrow of nude mice.[14,54] Reconstitution of adult thymectomized irradiated mice with syngeneic bone marrow cells gives rise to NKT cells in the recipient organs,[55,56] suggesting that NKT cells can develop extrathymically from the bone marrow. Peripheral NKT cells are rapidly deleted upon activation and replaced by NKT cells that have been generated by *de novo* proliferation in bone marrow. Thus, bone marrow plays a major role in restoring NKT cell homeostasis.

Bone marrow NKT cells may play immune stimulatory and inhibitory roles in the regulation of bone marrow transplantation immunity[15,57,58] and tumor immunity.[59,60] Adoptive transfer of donor NKT cells significantly ameliorates GVHD in a murine model of bone marrow transplantation.[58] The relative protection against GVHD was contributed to, to some extent, the population of NKT cells in bone marrow.[58,61] NKT cells are also believed to be among the most important anti-cancer cell populations in the mouse, causing rejection of malignant cells *in vivo*.[59,60] Thus, bone marrow NKT cells could differentially regulate immune responses in different settings.

B cell

Bone marrow is major organ for the development and maturation of B cells. B cells are generated from HSCs and developed in bone marrow before they egress into peripheral blood to reach peripheral lymphoid organs. Specific cellular niches for B-cell development include CXCL12-expressing cells and IL-7-expressing cells.[62] Immature B cells, like pro-B and pre-B stage cells, are regulated by extrinsic signals from the bone marrow during their development.[63] B-cell precursors and plasma cells reside in the specific niches and move between the niches as development proceeds.[62]

The majority of serum antibody is produced by terminally differentiated plasma cells. These non-dividing cells differ from memory B cells in typical B-cell markers, including major histocompatibility (MHC) class II and surface immunoglobulin. The main function of plasma cells is to continuously secrete large quantities of specific antibody. In contrast, memory B cells do not spontaneously secrete antibody. These cells proliferate and differentiate into antibody-secreting cells following appropriate stimulation. Interestingly, some plasma cells are long-lived. Most importantly, the long-lived plasma cells are found in the bone marrow. The bone marrow is a reservoir for long-lived plasma cells and is involved in the maintenance of long immunity.[64,65] Antigen-specific bone marrow plasma cells have been detected for more than 300 days post-viral infection.[64] The persistence of plasma cells within the bone marrow might be supported by soluble factors and/or cell–cell contact in the bone marrow microenvironment, in which one critical element is the bone marrow reticular stromal cells. Stromal cells provide growth factors as well as cell contact-dependent signals, such as IL-6 and cell contact-mediated signals (e.g., very late antigen-4) (Figure 2).[66] In addition to stromal elements, plasma cells could interact with various other bone marrow resident cells, including developing lymphoid and myeloid lineage cells. Therefore, bone marrow plasma cells are not intrinsically long-lived. Bone marrow stromal cells provide survival factors to them. The plasmablasts can migrate to the bone marrow[67–69] and differentiate into memory plasma cells after docking with CXCL12-expressing stromal cells.[62,69] The overall impact of these interactions supports the longevity of the plasma cells in bone marrow. Therefore, bone marrow contributes to humoral immune responses.

Neutrophil

Neutrophils are an essential component of innate immune system and may represent a critical link between the innate and adaptive immune system.[70] They are differentiated from stem cells in the bone marrow by a process termed granulopoiesis.[71] Neutrophils are generated at a rate of 1 to 2×10^{11} cells per day in a normal adult human under normal condition.[72] Several myeloid transcription factors are essential for granulopoiesis, including CCAAT-enhanced binding protein α PU.1 and growth factor independent-1 (GFI-1).[73] PU.1 is an ETS family transcription factor encoded by spleen focus forming virus proviral integration oncogene in human[74] and absolutely required for myeloid lineage commitment.[75,76] The balance between PU.1 and CCAAT-enhanced binding protein α determines the commitment of granulocytes and monocytes.[77–79] High expression of PU.1 drives monocytic differentiation and CCAAT-enhanced binding protein α promotes granulocytopoiesis.[72,80] GFI-1 is also necessary for neutrophil differentiation and it is upregulated during granulocytic lineage commitment.[81,82] G-CSF is critical in regulating granulopoiesis at several stages. G-CSF directs the commitment of multipotent progenitor cells down to the myeloid lineage, stimulates proliferation of granulocytic precursors and reduces the transit time of neutrophils through granulocytic compartment.[83,84]

Bone marrow is a large pool for the mature neutrophils.[85] There are 1%–2% of mature neutrophils in the circulation in mice.[86] The majority of neutrophils are reserved in the bone marrow. A large amount of neutrophils can be mobilized rapidly in response to infection and stress, which suggests that the bone marrow reserve is critical for host defense. CXCR4/CXCL12 signaling pathway plays a crucial role for maintaining neutrophils in bone marrow (Figure 3).[87,88] It is reported that administration of G-CSF reduces the expression of CXCR4 on bone marrow neutrophils and Treg cells, and the levels of bone marrow CXCL12.[12] This explains why G-CSF administration mobilizes neutrophils from bone marrow to peripheral circulation.[89,90]

Once released from the bone marrow, neutrophils circulate in the peripheral blood and have a relatively short half-life (about 6–8 h).[71,91] Bone marrow serves as an important site for neutrophil clearance under homeostatic conditions. Senescent neutrophils home back to bone marrow depending on Gαi subunit of the heterotrimeric G-protein.[92] Senescent neutrophils highly express CXCR4[93] and may home back to bone marrow *via* the CXCR4/CXCL12 chemokine axis.[88] Once senescent neutrophils return to bone marrow, they are phagocytosed and destroyed by resident stromal macrophages in bone marrow, which in turn stimulates the production of G-CSF by bone

marrow macrophages after uptake of apoptotic neutrophils and subsequently G-CSF acts as a positive feedback to promote granulopoiesis and regulate neutrophils release.[91,92] Thus, bone marrow plays an important role in the homeostasis of neutrophils.

Dendritic cell

Dendritic cells (DCs) play a key role in linking innate and adaptive immune responses.[13,94] Circulating DCs migrate to the bone marrow where they are retained better than in spleen, liver and lung tissues.[95] Homing of DCs to the bone marrow depends on constitutively expressed VCAM-1 and endothelial selectins in bone marrow microvessels (Figure 3). The migration of DCs to non-lymphoid organs might be advantageous for 'boosting' memory responses to previously antigen-experienced T cells.[96] Bone marrow DCs are able to trigger central memory T cells-mediated responses with antigen-dependent contacts. Bone marrow-derived dendritic cells are distinct from counterpart DCs in extralymphoid tissue, and are essential for innate immunity to intracellular infection. Bone marrow-derived dendritic cells are able to uptake the blood-derived cell-associated tumor-associated antigen, process them and induce antigen-specific systemic protective T cells-mediated immunity.[5] Thus, bone marrow DCs are functionally important in adaptive immunity.

Myeloid-derived cell

Myeloid-derived cells are a heterogeneous population of cells consisting of myeloid progenitors, immature myeloid cells and macrophages.[97] Immature myeloid cells that generated in bone marrow differentiate into mature myeloid cells under normal condition. But under pathological conditions (e.g., tumor), immature myeloid cells are expanded and activated, and become myeloid-derived suppressor cells (MDSCs), and acquire immunosuppressive activity by producing immune suppressive factors such as arginase I or inducible nitric oxidase synthase or TGF-β.[97,98] MDSCs express Gr-1 and CD11b, the surface marker for myeloid cell lineage in mice.[99] In healthy mice, 20%–30% of whole bone marrow cells express Gr-1$^+$CD11b$^+$ phenotype.[100] Based on the two different epitopes of Gr-1 antibodies, Ly6G and Ly6C, mouse MDSCs are subdivided into granulocytic MDSCs (CD11b$^+$Ly6G$^+$Ly6C^{low}) and monocytic MDSCs (CD11b$^+$Ly6G$^-$Ly6C^{hi}).[101,102] MDSCs may be defined as cells with CD14$^-$CD11b$^+$HLA-DR^{dim} phenotype in human.[103,104] MDSCs are shown to have the ability to induce the development and differentiation of Treg cells through cytokine production or cell–cell interaction,[105,106] which indicates that MDSCs and Treg cells might cooperate to regulate immune response.[97] Adoptive transfer of MDSCs inhibits T-cell alloresponses, prevents GVHD and prolongs the survival of mice.[107,108] MDSC–T cell interactions play an important role in protective anti-tumor immunity, infection, inflammation and GVHD.[107] Bone marrow MDSCs may be an important regulator of immune response and may serve as a potential therapeutic target for several clinical diseases.

MSC

In adult life, stem cells of mesenchymal lineage (MSCs), which compromise 0.01%–0.1% of total adult bone marrow cells, are mainly confined to the bone marrow,[109] where they are multipotential non-hematopoietic progenitor cells capable of differentiating into various tissues of mesenchymal origin. Human MSCs express human leukocyte antigen (HLA) class I but not HLA class II[110] or costimulatory molecules CD80 (B7.1), CD86 (B7.2) or CD40.[111] MSCs produce cytokines and growth factors for hematopoiesis and may attract infused HSCs to the bone marrow by inducing expression of homing receptors.[112,113]

In the bone marrow, MSCs display a great potential role in immunoregulatory activity, which comes from the observation that MSCs from various species can exert profound immunosuppression by inhibiting T-cell proliferation in responses to polyclonal stimuli and to their cognate peptide in a MHC-independent manner.[24,114–116] Although the conflicting results about MSC-mediated immunosuppression have been produced with different methods and species, overall data suggest that some soluble factors and mechanisms of cell–cell interaction might contribute to the inhibition of MSCs.[24,115,116] It is reported that MSCs could inhibit naive and memory T-cell responses to their cognate antigens, but it does not appear to be antigen-specific.[24] MSCs also induce T-cell anergy[117] or T-cell apoptosis,[118] suppress T-cell IFN-γ production and increase IL-4 secretion, and enhance Treg cell compartment.[119] MSCs selectively targets antigen-experienced T-cell responses in the contact with antigen-presenting cells in a non-cognate fashion, sparing those that have not been activated by T-cell receptor engagement.[116] Moreover, MSCs appear to discriminate between cellular responses to alloantigens and recall antigens.[120] The expression of early activation markers such as CD25 and CD69 on T cell is unaffected in the presence of MSCs, but IFN-γ production is reduced. The inhibitory effect of MSCs is directed mainly at the level of cell proliferation without interfering with early T-cell activation. MSCs do not preferentially target any T-cell subset. MSCs have a role in inhibiting proliferation and affecting differentiation, antibody production and chemotactic behavior of B cells.[117,121] MSCs may inhibit differentiation of hematopoietic progenitors into DCs and promote anti-inflammatory cytokine production of monocyte-derived DCs.[122,123] MSCs suppress cytokine-induced proliferation and prevent cytokine production and cytotoxic activity of natural killer cells.[124,125] In the context of limited understanding about MHC expression on MSCs, it is difficult to ascribe specific T-cell receptor/MHC/peptide interactions to their mechanism of immunoregulation. Notably, the inhibitory effect of MSCs does not require the presence of antigen-presenting cells and is not mediated through Treg cells.[116] MSCs have been reported to be used in clinical trials for autologous or allogeneic engraftment of bone marrow transplants,[126] osteogenesis imperfect,[127] stroke,[128] myocardial infarction[129] and GVHD.[130] Therefore, bone marrow MSCs have an important role in immune regulation and are potential candidates for clinical treatment.

Cytokines and chemokines

Bone marrow-derived cells including leukocytes, HSCs and stromal cells could secret lots of cytokines. Stroma cells and cells of hemopoietic lineage in the bone marrow produce both IL-7 and IL-15. IL-7 is a 'stromal cytokine' produced by a variety of stromal tissues including those in bone marrow. The production of IL-7 by bone marrow stromal cells is thought to be essential for early B lymphocyte development in mouse (but not in human),[131–133] and secreted IL-7 in bone marrow is postulated to play a critical role in post-thymic T-cell homeostasis.[54,134] IL-15 promotes basal homeostatic proliferation and survival of memory T cells in different experimental systems, whereas IL-7 performs an overlapping and complementary role during acute homeostatic proliferation in lymphopenic environments.[135,136] Recently, exogenous IL-15 was shown to replace exogenous IL-2 therapy during the treatment of established, non-manipulated poorly immunogenic tumors.[137] IL-21 plays a role in the proliferation and maturation of natural killer cell populations from bone marrow, and

contributes to the proliferation of T/B cell populations and anti-tumor effect.[138,139]

Chemokines could also be involved in the generation and regulation of bone marrow immune cells. Mobilization and interactions of stromal cell/hematopoietic precursors are thought to be controlled by cytokines, particularly, chemokines. Among chemokines, CXCL12 is particularly intriguing. CXCL12-expressing cells are a small population of bone marrow stromal cells scattered throughout bone marrow, such as osteoblasts, marrow fibroblasts and endothelial cells.[140] CXCL12-mediated interaction of progenitors with the bone marrow vascular niche allows the progenitors to relocate to a microenvironment that is permissive and instructive for megakaryocyte maturation and thrombopoiesis.[23] It has been recently determined that chemokine stimulation of HSCs and bone marrow-derived endothelial cells by CXCL12 leads to an enhancement in transendothelial and stromal migration *via* activation of adhesion molecules, in addition to its well-known ability to stimulate motility.[141,142] Signals for the translocation of HSCs from the fetal liver to bone marrow are provided by CXCL12. In response to CXCL12, HSCs or lymphocytes that express its specific seven transmembrane-span G protein-coupled CXCR4 receptor leave the fetal liver and colonize in the bone marrow, where they finally establish hematopoiesis.[143–145] As we discussed above, CXCR4/CXCL12 signaling also regulates the bone marrow trafficking of memory T cells, Treg cells and neutrophils.[12,67,68,87,88,93]

In addition to the role of chemokines in bone marrow, the adhesion molecules also regulate leukocytes migration to the bone marrow (Figure 3). Normal bone marrow sinusoids express VCAM-1 and E-selectin. The migration of B cells, $CD4^+$ and $CD8^+$ T cells to bone marrow is impaired in conditional VCAM-1-deficient mice, resulting in reduced B cells and T cells in bone marrow and mild leukocytosis in peripheral blood.[146] E-selectin and VCAM-1 are necessary for recruitment of hematopoietic progenitor cells to bone marrow.[147] Neutralizing antibody to E-selectin attenuates $CD8^+$ central memory T-cell rolling in bone marrow.[8] Thus, bone marrow plays an important role in immune cells homeostasis *via* the expression of cytokines, chemokines and adhesion molecules.

CONCLUDING REMARKS

Bone marrow is well-known as a primary hematopoietic organ. However, bone marrow contains high levels of multiple immune cell subsets with important and unique functionalities. It is evident that bone marrow can supplant the secondary lymphoid tissue either as a site of primary immune response or memory response. Immune regulation occurs in the bone marrow microenvironment in cell–cell contact manners or/and through soluble factors including cytokines. Thus, bone marrow is an immune regulatory organ, which may importantly affect systemic immunity and therapeutic efficacy of conventional and immune therapy/vaccination. Given that multiple human cancers including breast and prostate cancer preferentially metastasize to bone marrow, specific cellular and molecular niches in the bone marrow including high levels of Treg cells and MDSCs may impact tumor bone metastasis and contribute to bone pathology in cancer patients with bone marrow metastasis. Therefore, understanding the immune regulatory mechanisms in the bone marrow microenvironment will generate significant insight into human bone biology and immunology. Furthermore, given the unique functionalities of bone marrow memory T cells, one may expect that bone marrow serves as an excellent source of immune cells for adoptive immunotherapy for both malignancies and infectious diseases.

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