Annals of Anatomy 254 (2024) 152244

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.com/locate/aanat

Minireview

OsteoMac: A new player on the bone biology scene

Oscar Iglesias-Velazquez [1], Francisco GF Tresguerres [1], Isabel F. Tresguerres [*], Isabel Leco-Berrocal, Rosa Lopez-Pintor, Laura Baca, Jesus Torres

Department of Dental Clinical Specialties, Faculty of Dentistry, Complutense University of Madrid, Spain

ARTICLE INFO

Keywords:
OsteoMacs
tissue-resident macrophages
osteal macrophages

ABSTRACT

The knowledge of bone biology has undergone major advances in recent decades. In bone, resorbing osteoclasts have classically been described as tissue-resident macrophages, however, it is currently known that a new subtype of macrophages, called OsteoMacs, are specialised bone-resident macrophages, which, depending on certain conditions, may play an important role not only in bone homeostasis, but also in promoting pro-anabolic functions or in creating an inflammatory environment. There is growing evidence that these osteal macrophages may influence the development of bone-loss diseases. It is essential to understand the biological bases underlying bone physiological processes to search for new therapeutic targets for bone-loss diseases, such as osteoporosis, rheumatoid arthritis, or even periodontal disease. This narrative review provides an update on the origin, characterisation, and possible roles of osteoMacs in bone biology. Finally, the potential clinical applications of this new cell in bone-loss disorders are discussed.

1. Introduction

Bone is a fascinating tissue. Every time it is thought that great progress has been made in the knowledge of bone biology, a new protagonist appears.

Bone is a dynamic tissue that undergoes remodeling throughout life, in which damaged or old bone is replaced by the action of osteoclasts, and new bone is formed by the action of osteoblasts, to maintain the integrity and quality of bone over the years. Bone remodelling is a balanced process that takes place in the specialised structures known as BMUs (basic multicellular units), where osteoblasts, osteoclasts, osteocytes, capillary blood supply, lining cells, and macrophages are involved (Parfitt, 1995; Parffit, 2000; Kular et al., 2012; Batoon et al., 2017; Tresguerres et al., 2020).

However, with age or in certain pathologies, such as osteoporosis, bone remodelling becomes unbalanced towards bone resorption, leading to bone loss and increasing the risk of fractures (Riggs et al., 1982). In addition, under certain inflammatory conditions, such as rheumatoid arthritis, bone resorption becomes chronic, without subsequent formation, contributing to increase morbidity (Weivoda and Bradley, 2023).

The knowledge of bone biology has greatly advanced in the last few decades; among the highlights is the identification of a new subtype of tissue-resident macrophages into the bone, called OsteoMacs (OMs) (Hume et al., 1984; Chang et al., 2008) and the development of new area of research first called "Osteoimmunology" by Arron and Choi (2000), which focuses on the interactions between bone cells and the immune system (Nakashima and Takayanagi, 2009).

Macrophages are the first line of defence of the innate immune response; they are rapidly recruited to infectious and injury sites, where play a critical role in bone homeostasis that often results in bone destruction. This situation can be observed in different inflammatory

Abbreviations: BMUs, basic multicellular units; BMM, bone marrow-derived macrophages; BMP-2, Bone Morphogenetic Protein-2; CSF-1, Colony-Stimulating Factor-1; DAMPs, danger-associated molecular patterns; EMP, erythro-myeloid progenitor; HSC, Hematopoietic stem cells; IFN-γ, Interferon γ; IL-1β, interleukin 1β; IL-12, interleukin 12; IL-4, interleukin 4; IL-13, interleukin 13; IL-10, interleukin 10; IL-1, interleukin 1; LPS, Lipopolysaccharides; M-CSF, Macrophage Colony-Stimulating Factor; M-CSFR, Macrophage Colony-Stimulating Factor Receptor; MNGCs, Giant multinucleated cells; MFS, Mononuclear phagocytic system; MSC, mesenchymal stem cells; OSM, Oncostatin M; OMs, OsteoMacs; PS, phosphatidylserine; PTH, parathyroid hormone; RANKL, Receptor Activator of Nuclear Factor Kappa B ligand; TLR, toll-like receptor; TNF-α, Tumor Necrosis Factor-α; TGF-β, Tumor Growth Factor-β; TRAP, tartrate-resistant acid phosphatase; TRMs, Tissue-resident macrophages.

* Correspondence to: Department of Dental Clinical Specialties, Faculty of Dentistry, Complutense University, Plaza Ramón y Cajal s/n, Madrid 28040, Spain.
E-mail address: isabelfe@ucm.es (I. F. Tresguerres).

[1] Iglesias-Velazquez Oscar and Tresguerres Francisco GF have made the same contribution.

https://doi.org/10.1016/j.aanat.2024.152244
Received 5 December 2023; Received in revised form 21 February 2024; Accepted 5 March 2024
Available online 14 March 2024

bone diseases such as rheumatoid arthritis, and osteoarthritis (Gu et al., 2017), in which macrophages are activated and produce a great number of cytokines leading to bone resorption. However, macrophages also play a crucial role under physiological conditions in bone homeostasis, although they have also been implicated in tissue repair, specifically in bone formation and regeneration (Batoon et al., 2019; Rasheed, Rayner, 2021).

Macrophages are highly plastic and heterogenous myeloid lineage cells, present in all tissues of the body. Diversity and plasticity are two hallmarks of macrophages (Liu et al., 2014). Plasticity is the ability to modify the phenotype in response to the changes in the microenvironment (Stout and Suttles., 2004).

Macrophages can be classified into immune (or inflammatory) and tissue-resident macrophages (TRMs). Immune macrophages can be recruited as result of an infectious, inflammatory, or traumatic insult to the organism, and drive the innate immune response. In contrast, TRMs have been linked initially to homeostasis, but they have also been related to tissue repair (Hoeffel and Ginhoux, 2018). TRMs are derived from embryonic progenitors, while inflammatory macrophages are derived from bone marrow-derived monocytes (Hashimoto et al., 2013; Gordon, Plüddemann, 2017).

Macrophages can polarise, according to changes in their environment, towards two phenotypes with distinct functional abilities: M1 macrophages or classically activated, and M2 macrophages or alternatively activated. M1 macrophages present proinflammatory and antimicrobial characteristics, (also known as proinflammatory macrophages), while M2 macrophages have anti-inflammatory and regenerating capacities (or anti-inflammatory macrophages) (Mills et al., 2000; Bozec and Soulat, 2017). Thus, M1 macrophages are activated under pathological conditions, such as microbial antigens or IFN-γ (interferon- γ) and release proinflammatory cytokines, such as IL-1, IL-6, NO, superoxide anions, or TNF-α. While M2 macrophages are activated by IL-4 and IL-13 and are involved in tissue repair and wound healing, releasing anti-inflammatory cytokines such as BMP-2, TFG-β, or IL-10 (Wu et al., 2013; Gu et al., 2017). M1 and M2 phenotypes represent the two ends of a spectrum in macrophage activation, which depends on their microenvironment (Wu et al., 2013).

A new classification has been proposed, in which three types of macrophages with different origin, phenotype, and function can be involved (Gordon and Taylor., 2005; Murray, Wynn, 2011; Li et al., 2022), depending on the expression of the Ly6C antigen (Lymphocyte antigen 6 complex locus C), a glycoprotein expressed in murine circulating monocytes. Ly6C^{hi} macrophages are derived from Ly6C^{hi} monocytes recruited under inflammatory conditions. Ly6C^{hi} macrophages (further divided as Ly6C^{hi} and Ly6C^{middle} macrophages) show a potent capacity of phagocytosis and have pro-inflammatory capability, releasing IL-1, IL-6 or TNF-α. Ly6C^{hi} monocytes are more likely to mature to inflammatory M1 macrophages (Yang et al., 2014). Ly6C^{lo} macrophages are derived from Ly6C^{lo} monocytes and show protective and anti-inflammatory roles, secreting anti-inflammatory IL-10, and are involved in tissue repair (Yang et al., 2014; Li et al., 2022). Ly6C^{lo} monocytes are more likely to differentiate into M2 macrophages (Yang et al., 2014). A panel of experts on monocyte biology proposed a consensus nomenclature for human monocytes in 2010. Thus, they classified the monocytes as classical (CD14^{++}CD16^{-}), intermediate (CD14^{++}CD16^{+}) and non-classical monocytes (CD14^{+}CD16^{++}), that correlates with the murine classification (Ziegler-Heitbrock et al., 2010). (Table 1).

Macrophages present in different tissues are polarised according to changes in the microenvironment. The effect of the microenvironment on macrophage polarisation lies in the fact that IFN, TNF and LPS drive to M1 macrophages, showing antimicrobial and antitumoral activity, while M2 macrophages are induced by IL-4, IL-13, and IL-10, showing proangiogenic and profibrotic activity and promoting tissue repair (Wang et al., 2022).

In recent years, a new cell, belonging to bone-resident macrophages, has been discovered into the endosteal and periosteal bone-lining tissues (Hume et al., 1984; 2013). Depending on certain conditions, it may play a role in bone homeostasis or in inflammatory or repair processes. This cell is called osteoMac.

Table 1
Markers and functions of macrophages subsets in mice and human.

Species	Subsets	Surface markers	Functions
Mice	Ly6C^{high}	Ly6C^{high}	Phagocytosis
	Ly6C^{middle}	Ly6C^{middle}	Pro-inflammatory
	Ly6C^{low}	Ly6C^{low}	Tissue repair
Human	Classical	CD14^{++}CD16^{-}	Phagocytosis
	Intermediate	CD14^{++}CD16^{+}	Pro-inflammatory
	Non-classical	CD14^{+}CD16^{++}	Tissue repair

(Modified from Yang et al., 2014).

Osteal macrophages constituted a new area of interest for the scientific community, and efforts are being made to clarify the functions of this new subtype of bone-resident macrophages. Although many questions remain unanswered, this narrative review presents the current knowledge about osteoMacs, their origin, characterisation, and roles, and finally discusses the clinical relevance of this cell in bone-loss disorders.

1.1. Origin of Bone-Resident Macrophages – OsteoMacs

Macrophages are cellular components of the immune system, initially identified by Metchnikoff in 1892. He was a pioneer in the field of phagocytosis, since he discovered that macrophages were able to engulf not only pathogens during inflammation but also degenerated and dead cells (Metchnikoff, 1905; Schlundt et al., 2021). Van Furth and Cohn (Van Furth and Cohn, 1968) in 1968, introduced the term "mononuclear phagocyte system or MPS", originally described as a population of bone marrow-derived myeloid cells that circulate in the blood as monocytes and populate tissues as macrophages, where they mature into tissue-resident macrophages (Geissmann et al., 2010). The discovery of dendritic cells (DCs) as specialised antigen-presenting cell, by Steinmann in 1974, completed the third cell lineage involved in the MPS (Geissmann et al., 2010). In 1984, Hume and colleagues (Hume et al., 1984) described for the first time an additional population of bone-resident non-osteoclast macrophages in murine bone tissue, near the bone surface, which expressed the F4/80 marker. Later, Petit́s team called them osteoMacs (OMs) (Chang et al., 2008). In the 2000 s, an embryonic origin and self-renewal of tissue-resident macrophages was proposed (Gomez Perdiguero et al., 2015).

Although macrophages had been defined as mononuclear phagocytic cells derived from myeloid-lineage hematopoietic stem cells (HSC) (Davies, Taylor, 2015), their origin is more complex (Fig. 1). It is currently known that two different macrophage populations can be classified according to their ontogeny: immune or inflammatory macrophages derived from circulating monocytes originating from the HSC in the bone marrow, and tissue-resident macrophages, which originate from embryonic tissues, specifically from the yolk sac and foetal liver, and are seeded throughout the different tissues during organogenesis before birth, and persist in the adulthood as a resident population under steady state (Wu et al., 2013; Gordon et al., 2017; Bozec and Soulat, 2017; Schlundt et al., 2021). TRMs are capable of self-renewal and can remain independent from blood monocytes under homeostatic conditions (Davies et al., 2013).

Thus, TRMs are derived from embryonic cells, specifically from the yolk sac-derived erythro-myeloid progenitor (EMP), as first described Gomez Perdiguero et al. (Gomez Perdiguero et al., 2015). EMP generate macrophage precursors that colonise the whole embryo, transform into F4/80^{+} macrophages, and persist as TRMs in adult tissues. TRMs undergo tissue-specific adaptation, performing diverse functions in different tissues. Under homeostatic conditions, TRMs are maintained by self-renewal, without be replenished from monocytes (Cox et al.,

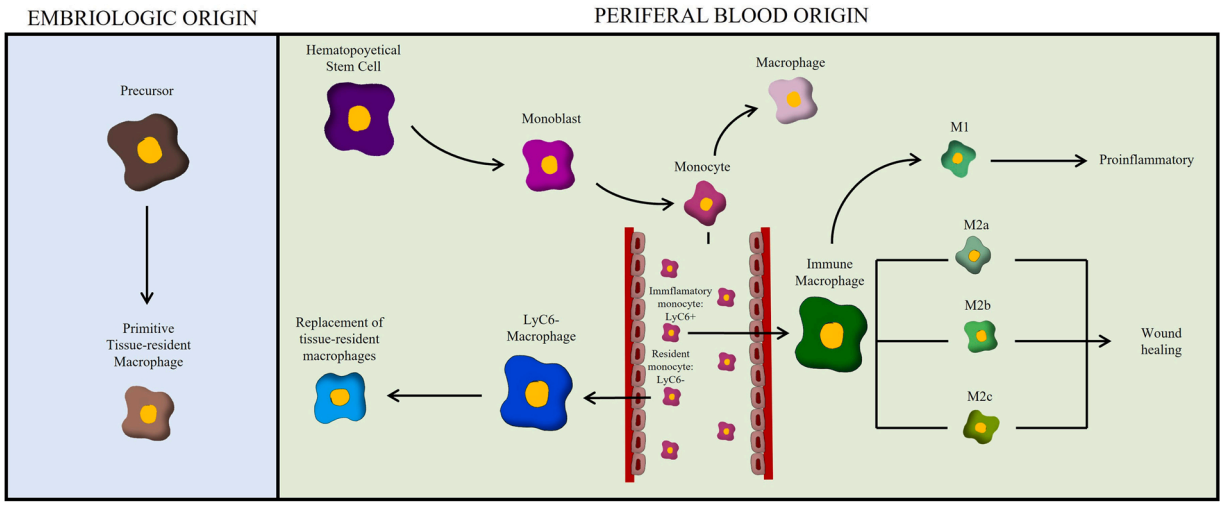

Fig. 1. Origin of inflammatory versus osteal macrophages. Inflammatory macrophages are derived from circulating Ly6C^{hi} monocytes. They can polarise to M1 (proinflammatory) macrophages that drive inflammatory responses, or M2 (M2a, M2b, M2c) macrophages, which regulate wound healing. OMs (F4/80^{+}Mac 2^{-}) are osteal macrophages, which are derived from the yolk sac and fetal liver and are seeded towards different tissues, regulating tissue homeostasis. Another source for OM replacement comes from circulating Ly6C^{lo} monocytes (F4/80^{+}Mac 2^{+}).

2021). However, under non-homeostatic conditions, TRMs in some tissues are also maintained by self-renewal (such as Kupffer cells in the liver, microglia in the central nervous system, Langerhans cells in dermis, etc.), except intestinal mucosa macrophages, which are replaced by blood monocytes (Cox et al., 2021) (Fig. 1). Bone osteoclasts represent a special case. They are multinucleated cells, and some of the nuclei are derived from EMP, but other nuclei are HSC-derived myeloid cells. Further studies will be needed to decipher this enigma (Lazarov et al., 2023).

With respect to the transcriptional control of macrophage differentiation, it is known that PU.1 transcription factor is critical for the differentiation of TRMs in almost all tissues, and it is considered the earliest transcription factor towards macrophages and osteoclasts (Cox et al., 2021). Another crucial factor in the differentiation of monocytes to macrophages and osteoclasts is CSF-1 (Colony-Stimulating Factor-1), also known as M-CSF (Macrophage Colony Stimulating Factor). CSF-1 regulates the number of TRMs and supports their self-renewal (Davies et al., 2013). CSF-1 can also promote macrophage differentiation and proliferation from bone marrow cells. Injections of CSF-1 into mice induce proliferation of most TRMs (Lazarov et al., 2023). Osteoclasts and macrophages share a common progenitor, and both express the CSF-1 receptor (CSF1R). CSF1R is expressed in all subtypes of macrophages, progenitors from HSC, and osteoclasts (Sehgal et al., 2021; Rasheed, Rayner, 2021).

1.2. *Differences among cells – OsteoMacs characterization*

Over the past decade, conceptual advances derived from the analysis of fate-mapping models and transcriptional studies have allowed researchers to decipher the functions of macrophages according to their origin and anatomical localisation.

Osteoclasts have been considered as specialised tissue-resident macrophages into the bone (Cox et al., 2021), although macrophages and osteoclasts are different mature myeloid cells within the bone microenvironment. They share not only progenitors of the myeloid lineage, but also growth factors and molecular markers (Batoon et al., 2017).

The osteoclast is the unique cell responsible for the resorption of calcified bone matrix. It has been known since the 1970 s that the osteoclast is derived from hematopoietic progenitors (Walker, 1973), specifically from monocytes and macrophages, as confirmed Udagawa et al., (1990). Two osteoblast-derived cytokines control osteoclast differentiation, CSF-1 and RANKL (Receptor Activator of Nuclear Factor Kappa B ligand), which activate their associated receptors c-fms and RANK (Receptor Activator of Nuclear Factor Kappa B), respectively. Osteoclast differentiation from monocytes and macrophages requires CSF-1, but it alone cannot complete the process of differentiation. RANKL is the additional cytokine that drives terminal osteoclast differentiation. RANKL- and CSF-1-stimulated macrophages fuse and give rise to osteoclasts (Pereira et al., 2018).

The osteoclast is a multinucleated cell, which express tartrate-resistant acid phosphatase (TRAP), cathepsin K, calcitonin receptor, and RANK, among other markers (Tresguerres et al., 2020).

OMs are a subtype of bone-resident macrophages, which express the F4/80 marker, and are located not only along the bone surface, at the endosteal and periosteal areas, but also at sites of active bone remodelling, immediately adjacent to mature osteoblasts. Furthermore, over 75% of osteoblasts of the endosteal surface were covered by a canopy-like cell structure F4/80^{+}, CD68^{+}, Mac-3^{+}, but TRAP^{-} macrophages (Chang et al., 2008; Wu et al., 2013). In normal mice, these F4/80^{+} cells of embryonic origin co-exist with F4/80^{-} macrophages of hematopoietic origin (Davies et al., 2013). Why an embryonic cell-derived population is maintained throughout life remains unexplained.

OsteoMacs are clearly not osteoclasts. OMs are bone-resident macrophages found in the endosteal bone surface. Several studies have showed that both OMs and inflammatory macrophages share many markers such as CD45, F4/80, CD115, CD68, CD11b, CD107b (Mac-3) and Gr-1, making it difficult to differentiate the two types of cells (Bozec and Soulat, 2017). OMs differ from osteoclast in the expression of CD169, which is also absent in osteoclast progenitors (Batoon et al., 2019), but it is also present in bone marrow macrophages (BMM) (Batoon et al., 2019; Mohamad et al., 2017). OMs and BMM share the CD45+F4/80+ marker (Mohamad et al., 2017). Moreover, Mohamad et al. demonstrated that CSF1R+CD166+ was the only specific marker for OMs, at least in vitro (Mohamad et al., 2017).

OMs also differ from osteoclasts in three basic facts: (1) morphologic structure: OMs are not multinucleate cells (Pettit et al., 2008; Wu et al., 2013). (2) markers expression: OMs are F4/80^{+} cells (Hume et al., 1984; Pettit et al., 2008), and, moreover, OMs do not express tartrate-resistant acid phosphatase (TRAP), cathepsin K, or RANK which are well-known osteoclast markers (Pettit et al., 2008). (3) OMs are involved in bone homeostasis by promoting bone deposition instead of bone resorption as osteoclasts (Wu et al., 2013).

1.3. Anatomical location of OsteoMacs

Chang et al. (Chang et al., 2008) carried out a study assessing the location and function of osteal macrophages in both human and mice bone tissue. Immunohistochemical analysis demonstrated the presence of F4/80^{+} CD68^{+} cells along endosteum and periosteum (Wu et al., 2013), but also intercalated among bone lining cells and around blood vessels (Chang et al., 2008). Thus, OMs are located immediately adjacent to osteoblasts, in active bone modelling sites, forming a canopy-like structure over mature osteoblasts, as first described by Chang and co-workers (Chang et al., 2008). Periosteal OMs, which are intercalated among osteoblast following a mosaic pattern, exhibit multiple ramifications differing from those OMs located in the endosteal layer, which are more elongated and are distributed along a relatively more continuous structure (Alexander et al., 2017) (Fig. 2).

OMs, extend the processes of its stellate shape towards the bone surfaces, leading to the connection among osteocytes (placed throughout the entire bone), osteoblasts, osteoclasts, and bone lining cells (localised in the periosteal and endosteal surfaces), establishing a crosstalk between cells and the microenvironment, providing a pro-anabolic support and inducing bone formation (Pettit et al., 2008; Sinder et al., 2015; Batoon et al., 2017). It seems that this location is closely related to the activity they carry out to facilitate the communication between cells and their environment.

1.4. Role of OsteoMacs

An interesting feature of macrophages is their plasticity, which allows them to adapt to their environment (Bozec and Soulat, 2017). Over the years, complex studies from basic research have shown that osteoMacs play an important role in bone homeostasis (Pettit et al., 2008), but also contribute to inflammation, a role related to destruction, and bone repair, a role related to formation. Depending on certain conditions, OMs can promote pro-anabolic functions on bone tissue or to create an inflammatory environment (Miron and Bosshardt, 2016). The following is a summary of the most relevant functions of OMs confirmed to date.

1.4.1. OMs as immune surveillance cells in the bone microenvironment

The presence of microbial pathogens drives to the differentiation of monocytes into macrophages or dendritic cells (DC), the major antigen-presenting cells.

Macrophages are phagocytic immune cells. Macrophages express receptors for recognition of PAMPs (pathogen-associated molecular patterns) and DAMPs (danger-associated molecular patterns), such as Toll-like receptors, NOD-like receptors, and scavenger receptors. DAMPs can be products of stressed or necrotic cells (Taylor et al., 2005).

Lipopolysaccharides (LPS) in bacterial walls are known to be one of the most reactive factors producing a local immune reaction. The interaction with LPS stimulates the releasing of macrophage cytokines, leading to an inflammatory response and then a destructive reaction. The cellular receptor that interacts with LPS is Toll-like R4 (TLR4); thus, TLR4 is one of the receptors that regulate the innate immune response. It is expressed in macrophages, neutrophils, and DC, all of which are phagocytic cells. LPS-TLR4 interaction provokes the secretion of IL-1, IL-6, and TNF-α. TNF-α is the mediator of many inflammatory diseases, such as rheumatoid arthritis, ankylosing spondylitis, Crohn disease, etc. It is released by the macrophages and monocytes in response to inflammation and lead to apoptosis and necrosis (Taylor et al., 2005). TNF-α and RANKL are members of TNF superfamily and share the same receptors. TNF-α is known to play a local role in osteoclast differentiation independently of RANKL signal (Kobayashi et al., 2000).

Chang and coworkers (Chang et al., 2008) observed for the first time in primary murine osteoblast culture that OMs were able to detect and respond to LPS. Mohamad et al., (2021) have recently demonstrated that OMs are functionally capable of phagocytosis, in fact, OMs performed equivalent phagocytosis in response to LPS or IL-4, which drive to M1 or M2 polarisation, respectively.

1.4.2. OMs as regulators of bone formation and mineralisation

The first role of the OMs was discovered by Chang (Chang et al., 2008), who removed them from primary osteoblast cultures via a magnetic technique, and a 23-fold reduction in mineral deposition was observed. Removal of osteal macrophages significantly reduced osteoblast mineralisation and gene expression of osteocalcin, a marker of bone formation. It was therefore concluded that OMs were required for optimal mineralisation at least in vitro (Chang et al., 2008). The OM ability to enhance osteoblast mineralisation was further confirmed by Mohamad et al., (2017).

OMs are closely adjacent to osteoblasts, establishing a communication network with the surrounding cells through their star-shaped, suggesting that OMs may provide pro-anabolic support to osteoblasts and promote bone formation (Chang et al., 2008; Sinder et al., 2015). These findings were further confirmed by Wu et al., (2013), Alexander et al., (2011), Raggatt et al., (2014), and Batoon et al., (2019).

Furthermore, the physiologic role of OMs was analysed after their depletion in vivo in several knockout systems, such as the Mafia (macrophage Fas-induced apoptosis) mice model, that is a transgenic mouse in which the macrophage progenitors have been eliminated

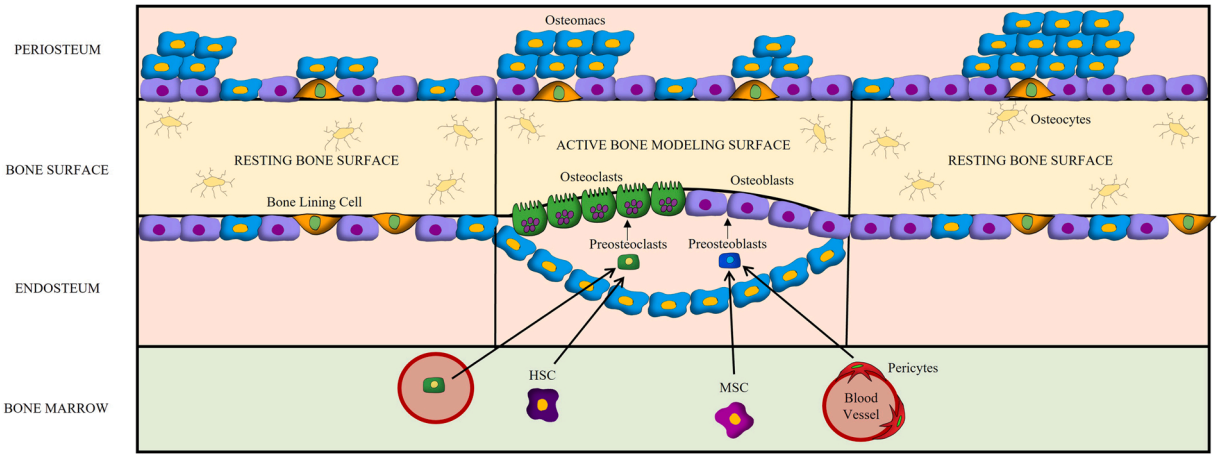

Fig. 2. Location of OMs in endosteal and periosteal tissues. Periosteal and endosteal OMs are located immediately adjacent to osteoblasts, forming a canopy-like structure over mature osteoblasts, but also intercalated among bone lining cells. Periosteal OMs are intercalated among osteoblast following a mosaic pattern and exhibit many layers, while OMs located in the endosteum are more elongated and are distributed along a relatively more continuous structure.

(Burnett et al., 2004; Bozec and Soulat, 2017). When macrophages were ablated, canopy-like structure was disrupted and bone formation was reduced (Chang et al., 2008); a reduction in bone mass was also observed six weeks after macrophage removal (Cho et al., 2014), demonstrating the role of OMs in bone anabolism.

Two molecules produced by OMs have been linked to mineralisation: Bone Morphogenetic Protein-2 (BMP-2) and Oncostatin M (OSM) (Bozec and Soulat, 2017). BMP-2 is an osteoinductive factor, which promote the Wnt/β-catenin pathway to induce bone formation. OSM is a member of the IL-6 family, which participates in bone mineralisation (Bozec and Soulat, 2017). Whether the effects of OMs on osteoblasts are direct or mediated by these molecules remains unexplained.

1.4.3. OMs as mediators of iPTH anabolism

When PTH is administered at intermittent and low dosages (iPTH), an anabolic response is produced in the skeleton, increasing not only the cortical thickness, but also the thickness and number of trabeculae. This is the unique anabolic therapy admitted by FDA and EMA for osteoporosis treatment (Canalis, 2018). For many years it was claimed that IGF-I was the mediator of the anabolic effect of PTH on trabecular bone (Canalis et al., 1989). However, it is known from the studies of Cho and colleagues (Cho et al., 2014), that OMs are involved in the anabolic effect of PTH, since the increase in bone formation after PTH administration coincides with expansion of osteal macrophages on the endosteal and periosteal surface of cortical bone in mice. Moreover, in Mafia mice, when PTH is administered, the expected anabolic response does not occur, suggesting that OMs are necessary to support PTH anabolism (Cho et al., 2014).

Therefore, OMs were necessary to support not only bone formation under homeostatic conditions, but also the anabolic effect of intermittent parathyroid hormone (iPTH).

1.4.4. OMs and its role in efferocytosis

Efferocytosis in bone biology is an area of growing interest. Efferocytosis is the removal of apoptotic cells (Martin et al., 2014). Cells can die from apoptosis (or programmed cellular death) or necrosis (in case of acute injury) (Sinder et al., 2015). To maintain homeostasis, dead cells can be effectively cleared.

It is known that a million cells become apoptotic every second in the organism (Sinder et al., 2015), and their clearance is crucial to prevent not only inflammatory but also autoimmune diseases (Michalski and McCauley, 2017). The removal of apoptotic cells is essential for proper organ functionality, since when a cell dies it is important that it is replaced by a new cell to maintain the function. The first step in efferocytosis is the recognition of apoptotic cells, which takes place through the exposition of signals 'find-me' and 'eat-me' by the dying cell itself (Cho et al., 2014; Sinder et al., 2015). During apoptosis, cells expose phosphatidylserine (PS) on their outer membrane, so that phagocytic cells can recognize and engulf them. It is believed that the removal of apoptotic cells by macrophages prevents the release of antigenic macromolecules into the tissues, such as nucleotides, which can be recycled by other cells, preventing an autoimmune reaction (Cox et al., 2021). After the engulfment of apoptotic osteoblasts, macrophages secrete the osteogenic factor TGF-β, involved in the chemoattraction of osteoblast progenitors, suggesting that, following osteoblast efferocytosis, the microenvironment profile changes to recruit progenitor cells in order to repopulate dead populations (Michalski et al., 2016; Michalski and McCauley, 2017).

Osteoblasts, within BMUs, can transform into osteocytes or bone lining cells, or suffer apoptosis (Manolagas, 2000). About 50% of osteoblasts at the remodelling sites are thought to undergo apoptosis. OMs are involved in the efferocytosis of osteoblasts, since OMs are located adjacent to osteoblasts, forming a canopy-like structure over them (Chang et al., 2008) and are specifically phagocytic cells (Sinder et al., 2015). The recognition and removal of apoptotic osteoblasts (efferocytosis) by OMs, has been confirmed by Cho et al., (2014), who observed that efferocytosis was linked to M2 macrophage polarization, promoting the secretion of specific factors, like TGF-β.

1.4.5. OMs and bone regeneration

Bone regeneration after trauma is a complex process involving sequential inflammatory, anabolic and remodelling phases, to remove damaged tissue and completely repair the injured tissue until full restoration is achieved. The injury is localised by certain cells, that initiate a cascade of cytokines to recruit the inflammatory immune cells to engulf the pathogens and cellular debris. It is now believed that TRMs are responsible for damage detection, specifically OMs, through the process of efferocytosis (Cho et al., 2014).

When the injury occurs in the bone, the blood vessels are disrupted and blood enters the damaged area, forming a hematoma. Different myeloid-lineage cells can be distinguished into the regenerated bone: bone-resident macrophages, immune macrophages, and osteoclasts. TRMs, are the first cells to reach the damaged area, where they perform the function of efferocytosis of the cellular debris (Batoon et al., 2017; Schlundt et al., 2021). This pro-inflammation environment drives to the activation of M1 macrophages, which secrete pro-inflammatory cytokines, such as TNF-α, IL-1 and IL-6. These cytokines stimulate the expression of RANKL (receptor activator of factor nuclear kappa B ligand) from osteoblasts and osteocytes. RANKL binds to RANK, located in the osteoclast membrane, and their union triggers an intracellular signal and through the TRAF (TNF receptor associated factor), activates NF-κB, c-Fos, and NFATc1 (nuclear factor of activated T cells c1); the latter has been considered as the master transcription factor required for terminal osteoclast differentiation from RANKL (Yao et al., 2021). Following M1 macrophages, a transition towards M2 can be observed, which coincides with the revascularisation process, reestablishing the vessel network (Schlundt et al., 2021), leading to the arrive of osteoblast precursors at the injury site. The switch from M1 to M2 phenotype is essential for successful healing, and it depends on the microenvironment (Batoon et al., 2017). OMs were found to be distributed throughout the regenerating tissue in the late anabolic and remodelling phases (Alexander et al., 2011; Raggatt et al., 2014).

1.4.6. OMs and endochondral bone healing

Fracture healing follows a sequence of inflammation, proliferation, and remodelling. The right sequence of events is crucial for normal healing (Marsell and Einhorn, 2011). The mechanism of bone healing depends on mechanical forces; when fractures are stabilised, direct intramembranous ossification occurs and when they are not stabilised, a fracture gap exists, and endochondral ossification takes place (Wu et al., 2013; Schlundt et al., 2021).

In endochondral healing, a periosteal cartilaginous callus is previously formed, which will be gradually resorbed and replaced by bone, as opposed to intramembranous ossification, in which the prior cartilaginous callus does not take place.

The process of repair via endochondral ossification can be simplified into 4 stages: inflammatory, soft callus formation, hard callus formation and remodelling (Wu et al., 2013). The first step after the trauma is the inflammatory phase, which consists of an initial hematoma, leading to the secretion of TNF-α, IL-1, IL-6, and IL-11. TNF-α acts as chemotactic, attracting mesenchymal stem cells (MSC) to the fracture site. IL-1 promotes the production of the primary cartilaginous callus, while IL-6 stimulates angiogenesis through VEGF (Vascular Endothelial Growth Factor) release (Marsell and Einhorn, 2011). Macrophages are one of the first cells infiltrating the area of fracture (Schlundt et al., 2018, 2021). The fracture repair is associated with an increase in both, resident and recruited macrophages through all phases of the healing process (Alexander et al., 2011). When macrophages were ablated in the Mafia mice model, bone union was impaired and a fibrotic response was observed in the callus instead of deposition of cartilage and bone (Vi et al., 2015).

Raggatt and colleagues (Raggatt et al., 2014) in a murine fracture

model observed by immunohistochemical analysis that F4/80$^+$ Mac-2$^+$ inflammatory macrophages were found in the granulation tissue and adjacent to the cartilage, in the transition from the soft to the hard callus, closely to osteoblasts and vessels. The authors have demonstrated the presence of OMs (F4/80$^+$ Mac-2$^-$) distributed by the callus tissue in the late anabolic and remodelling phases. When OMs were ablated in the Mafia mice at time of injury, the inflammatory phase was unable to take place, and a complete failure of fracture repair was seen (Raggatt et al., 2014). But, when the depletion of macrophages was delayed, a small and delayed hard callus was observed, in agree with Schlundt et al., (2018), because the mineralisation process of cartilage into woven bone was altered. It was concluded that macrophages in general, and OMs in particular, were indispensable for the initiation of the process and callus transformation during endochondral ossification.

Furthermore, Batoon and collaborators (Batoon et al., 2019), have considered that the CD169 marker is expressed in both endosteal and periosteal OMs. They observed that CD169$^+$ OMs depletion was associated with impaired bone healing via endochondral ossification, concluding that CD169$^+$OMs were crucial for normal periosteal callus healing.

1.4.7. OMs and intramembranous bone healing

Alexander and colleagues (Alexander et al., 2011) investigated the role of OMs in intramembranous bone healing, in a mouse tibia injury model. Immunohistochemical analysis revealed that at least two macrophage populations were present into the bone injury site: OMs, (which were identified by F4/80$^+$ Mac-2$^-$ markers) and inflammatory macrophages (F4/80$^+$ Mac-2$^+$), in smaller quantities, but both persisted throughout the healing period. OMs were observed in sites of bone deposition, forming a canopy-like structure over the matrix-forming osteoblasts. After OMs ablation at the time of injury, using the Mafia transgenic mice, a reduced woven deposition and mineralisation was observed, while if the ablation was delayed, a suppression of new bone formation could be seen, concluding for the first time that OMs were required throughout the mineralisation process in intramembranous bone healing.

Batoon and collaborators (Batoon et al., 2019) observed less woven bone formation and more fibrotic tissue in a murine model of CD169$^+$ OM depletion, concluding that CD169$^+$ OMs are required for normal bone repair via intramembranous ossification.

1.4.8. OMs as support of osteoclast-mediated resorption

Batoon and colleagues (Batoon et al., 2021), have recently developed an experimental osteoporosis model in mice, and observed that ovariectomy was able to induce bone loss, in both trabecular and cortical bone, included increased cortical porosity. This model was characterised by delayed fracture healing. OMs were found to be increased after ovariectomy on both trabecular and endocortical bone, confirming for the first time that OMs contribute to etiopathogenesis of osteoporosis itself (Batoon et al., 2021). Furthermore, OMs in contact with osteoclasts contained particles that were found to be TRAP+ and it was concluded, for the first time, that OMs were able to support osteoclastic bone resorption through the phagocytosis of resorption byproducts.

1.4.9. OMs as osteoclast precursors

Macrophages and osteoclasts are closely related, since monocytes, macrophages, and osteoclasts are derived from HSC-derived common myeloid progenitor located in the bone marrow (Weivoda and Bradley, 2023; Yahara et al., 2020) and the survival of macrophages and osteoclasts is CSF1-dependent (Miron et al., 2016; Hume and MacDonald, 2012). Moreover, osteoclast differentiation is stimulated by RANKL and CSF-1, (Kobayashi et al., 2000), cytokines required for osteoclastogenesis (Hume and MacDonald, 2012).

Both, bone marrow-derived and rheumatoid synovium-derived macrophages can differentiate into osteoclasts, as has been reported by Takeshita et al., (2000) and Haynes et al., (2001), respectively, supporting that macrophages can serve as osteoclast precursors.

Although Chang and collaborators (2008) had reported that OMs did not differentiate into TRAP$^+$ osteoclasts, Wu and coworkers demonstrated that TRAP$^+$ osteoclasts were localized near F4/80+ OMs in mouse endosteal bone (Wu et al., 2013). Moreover, Mohamad et al., (2017) have recently found that OMs act as osteoclast precursors at least in vitro and can form bone-resorbing cells in presence of CSF-1 and RANKL.

1.4.10. OMs as support of the hematopoietic niche

Bone and bone marrow contain multiple specialised TRM subpopulations that contribute to regulation of hematopoietic stem cells (HSC) (Winkler et al., 2010; McCabe and MacNamara, 2016; Kaur et al., 2017).

The concept of hematopoietic niche consists in a specific bone marrow microenvironment that controls the differentiation and migration of HSC and is regulated by complex multicellular mechanisms (Ghosh et al., 2021), mainly by osteoblasts, OMs, and megakaryocytes (Mohamad et al., 2017). Osteoblasts, located in the endosteum, were the first cellular lineage that was found in the endosteal niche, which were associated to hematopoiesis and play a role in regulating HSC function (Kaur et al., 2017).

Under physiological conditions, megakaryocytes have been shown to regulate HSC through the secretion of TGFβ1, a factor linked to HSC quiescence (Heino and Määttä, 2018).

Two types of TRMs can be found in the hematopoietic niche: OMs and BMM, which perform different functions. OMs and BMM share similar markers such as CD45+F4/80+, CD68, CD11b, Mac-2 and CD169 (Kaur et al., 2017). Moreover, CD166 marker is present in OMs, but not in BMM, and is required for promoting hematopoietic activity (Mohamad et al., 2017; Kaur et al., 2017).

Mohamad et al., observed for the first time that OMs were required for the hematopoiesis-enhancing activity of osteoblasts, which was increased by megakaryocytes (Mohamad et al., 2017). They concluded that it was the OM and not BMM that supported the hematopoietic activity of HSC. Moreover, OMs are likely to be important in bridging osteoblasts, megakaryocytes, and HSC together in the hematopoietic niche (Kaur at al., 2017).

Future studies will be needed to further develop these results.

1.5. Clinical application of bone biology: new strategies for bone-loss disorders

Bone-loss disorders, such as osteoporosis, periodontitis or bone metastatic cancer, or diseases with joint destruction, such as rheumatoid arthritis, show an increase in bone resorption, due to an increase in osteoclast differentiation and activity (Weivoda and Bradley, 2021).

Therapies against bone-loss disorders can be antiresorptive or anabolic (Langdahl et al., 2016). Antiresorptive therapies such as bisphosphonates and denosumab, inhibit bone resorption but end up inhibiting bone formation as well. Anabolic drugs, such as teriparatide or abaloparatide (only allowed by FDA), promote bone formation, but modulate the BMU response, so that the BMU machinery is balanced towards bone formation. This mechanism of action implicates that anabolic therapies depend, at least in part, on the osteoclast coupling factor (Alexander et al., 2011). It would be necessary to search for a therapy that would bypass the BMU and enhance bone formation by avoiding any link to resorption. OMs could be this target.

Furthermore, romozosumab is a new anti-osteoporotic agent recently approved by the EMA. It is a monoclonal antibody against sclerostin, which is a negative regulator of bone formation. It has a dual effect, early increasing bone formation, while persistently inhibiting bone resorption. However, it has undesirable side effects, such as myocardial infarction, or stroke, so it is advisable not to use more than 1 year (Yao et al., 2021).

In addition, bisphosphonates and denosumab have not been shown

to be effective in the prevention and treatment of join destruction in rheumatoid arthritis (Yao et al., 2021).

Moreover, denosumab induces a rebound effect when withdrawn. Anabolic agents promote bone formation in a transient manner, so that when they are withdrawn, bone density decreases abruptly, so it is advisable to continue with an antiresorptive treatment (Yao et al., 2021). Additionally, anabolic treatment should not exceed 1,5–2 years, as its use has been associated with the possibility of osteosarcoma, as it was shown in a rat model (Vahle et al., 2004). The above-mentioned considerations combined with the fact that bisphosphonates and denosumab have undesirable side effects, such as atypical femur fractures, hypocalcaemia, and osteonecrosis of the jaws, makes it necessary to search for new therapies against bone-loss disorders.

Since OMs have both destructive and constructive roles, new osteal macrophage-based therapies should be developed that target not only a specific macrophage subpopulation, but also a specific site. Macrophage polarisation could be a target of therapeutic interest in bone-loss disorders, chronic inflammatory diseases as well as in bone regeneration procedures (Gu et al., 2017).

2. Conclusions

OMs are a subtype of bone-resident macrophages which participates actively in bone homeostasis and are necessary for normal bone mineralisation and promote bone formation under homeostatic and iPTH-mediated conditions.

OMs are involved in bone regeneration, as well as in the ossification of both endochondral and membranous fractures.

OMs support the hematopoietic activity of HSC.

OMs are able to form osteoclast, at least in vitro.

3. Future research directions and challenges

Osteal macrophages constitute a new area of interest within the field of osteoimmunology. However, some considerations may be taken into account for the future.

New in vivo research models should be developed in the future to specifically study OMs functions, without altering the rest of the myeloid cells, to confirm the roles that have been indirectly attributed to them in the macrophage depletion models that have existed to date.

To unravel the complicated mechanisms that regulate the haematopoiesis in bone marrow and bone niches would be a major task for the future.

A better understanding of mechanisms underlying the interaction between OMs and bone cells would be helpful in the development of novel therapies against chronic inflammatory or compromised fracture healing diseases.

Further studies focusing on the roles of OMs and their behaviour in bone regeneration will provide more effective strategies for the treatment of bone-loss disorders.

Ethical approval

Not Applicable

Disclosures

The authors state that they have no conflict of interest.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Alexander, K.A., Chang, M.K., Maylin, E.R., Kohler, T., Müller, R., Wu, A.C., Van Rooijen, N., Sweet, M.J., Hume, D.A., Raggatt, L.-J., Pettit, A.R., 2011. Osteal macrophages promote in vivo intramembranous bone healing in a mouse tibial injury model. J. Bone Min. Res. 26, 1517–1532. https://doi.org/10.1002/jbmr.354.

Alexander, K.A., Raggatt, L.J., Millard, S., Batoon, L., Chiu-Ku Wu, A., Chang, M.K., Hume, D.A., Pettit, A.R., 2017. Resting and injury-induced inflamed periosteum contain multiple macrophage subsets that are located at sites of bone growth and regeneration. Immunol. Cell Biol. 95, 7–16. https://doi.org/10.1038/icb.2016.74.

Arron, J.R., Choi, Y., 2000. Bone versus immune system. Nature 408 (6812), 535–536. https://doi.org/10.1038/35046196.

Batoon, L., Millard, S.M., Raggatt, L.J., Pettit, A.R., 2017. Osteomacs and Bone Regeneration. Curr. Osteoporos. Rep. 15, 385–395. https://doi.org/10.1007/s11914-017-0384-x.

Batoon, L., Millard, S.M., Wullschleger, M.E., Preda, C., Wu, A.C., Kaur, S., Tseng, H.W., Hume, D.A., Levesque, J.P., Raggatt, L.J., Pettit, A.R., 2019. CD169+ macrophages are critical for osteoblast maintenance and promote intramembranous and endochondral ossification during bone repair. Biomaterials 196, 51–66. https://doi.org/10.1016/j.biomaterials.2017.10.033.

Batoon, L., Millard, S.M., Raggatt, L.J., Wu, A.C., Kaur, S., Sun, L.W.H., Williams, K., Sandrock, C., Ng, P.Y., Irvine, K.M., Bartnikowski, M., Glatt, V., Pavlos, N.J., Pettit, A.R., 2021. Osteal macrophages support osteoclast-mediated resorption and contribute to bone pathology in a postmenopausal osteoporosis mouse model. J. Bone Min. Res 36 (11), 2214–2228. https://doi.org/10.1002/jbmr.4413.

Bozec, A., Soulat, D., 2017. Latest perspectives on macrophages in bone homeostasis. Pflug. Arch. Eur. J. Physiol. 469, 517–525. https://doi.org/10.1007/s00424-017-1952-8.

Burnett, S.H., Kershen, E.J., Zhang, J., Zeng, L., Straley, S.C., Kaplan, A.M., Cohen, D.A., 2004. Conditional macrophage ablation in transgenic mice expressing a Fas-based suicide gene. J. Leukoc. Biol. 75 (4), 612–623. https://doi.org/10.1189/jlb.0903442.

Canalis, E., 2018. Management of endocrine disease: Novel anabolic treatments for osteoporosis. Eur. J. Endocrinol. 178 (2), R33–R44. https://doi.org/10.1530/EJE-17-0920.

Canalis, E., Centrella, M., Burch, W., Mc Carthy, T.L., 1989. IGF-I mediates selective anabolic ellefcts of PTH in bone culture. J. Clin. Invest 83 (1), 60–65.

Chang, M.K., Raggatt, L.-J., Alexander, K.A., Kuliwaba, J.S., Fazzalari, N.L., Schroder, K., Maylin, E.R., Ripoll, V.M., Hume, D.A., Pettit, A.R., 2008. Osteal Tissue Macrophages Are Intercalated throughout Human and Mouse Bone Lining Tissues and Regulate Osteoblast Function In Vitro and In Vivo. J. Immunol. 181, 1232–1244. https://doi.org/10.4049/jimmunol.181.2.1232.

Cho, S.W., Soki, F.N., Koh, A.J., Eber, M.R., Entezami, P., Park, S.I., Van Rooijen, N., McCauley, L.K., 2014. Osteal macrophages support physiologic skeletal remodeling and anabolic actions of parathyroid hormone in bone. Proc. Natl. Acad. Sci. U. S. A. 111 (4), 1545–1550. https://doi.org/10.1073/pnas.1315153111.

Cox, N., Pokrovskii, M., Vicario, R., Geissmann, F., 2021. Origins, biology, and diseases of tissue macrophages. Annu. Rev. Immunol. 39, 313–344. https://doi.org/10.1146/annurev-immunol-093019-111748.

Davies, L.C., Taylor, P.R., 2015. Tissue-resident macrophages: then and now. Immunology 144 (4), 541–548. https://doi.org/10.1111/imm.12451.

Davies, L.C., Jenkins, S.J., Allen, A.R., Taylor, P.R., 2013. Tissue-resident macrophages. Nat. Immunol. 14, 986–995.

Geissmann, F., Manz, M.G., Jung, S., Sieweke, M.H., Merad, M., Ley, K., 2010. Development of monocytes, macrophages, and dendritic cells. Science 327 (5966), 656–661. https://doi.org/10.1126/science.1178331.

Ghosh, J., Koussa, R.E., Mohamad, S.F., Liu, J., Kacena, M.A., Srour, E.F., 2021. Cellular components of the hematopoietic niche and their regulation of hematopoietic stem cell function. Curr. Opin. Hematol. 28 (4), 243–250. https://doi.org/10.1097/MOH.0000000000000656.

Gomez Perdiguero, E., Klapproth, K., Schulz, C., Busch, K., Azzoni, E., Crozet, L., Garner, H., Trouillet, C., de Bruijn, M.F., Geissmann, F., Rodewald, H.R., 2015. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. Nature 518 (7540), 547–551. https://doi.org/10.1038/nature13989.

Gordon, S., Plüddemann, A., 2017. Tissue macrophages: homogeneity and functions. BMC Biol. 15, 1–18. https://doi.org/10.1186/s12915-017-0392-4.

Gordon, S., Taylor, P.R., 2005. Monocyte and macrophage heterogeneity. Nat. Rev. Immunol. 5, 953–964. https://doi.org/10.1038/nri1733.

Gu, Q., Yang, H., Shi, Q., 2017. Macrophages and bone inflammation. J. Orthop. Transl. 10, 86–93. https://doi.org/10.1016/j.jot.2017.05.002.

Hashimoto, D.A., Chow, C., Noizat, P., Teo, M.B., Beasley, M., Leboeuf, C.D., et al., 2013. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. Immunity 38, 792–804. https://doi.org/10.1016/j.immuni.2013.04.004.

Haynes, D.R., Crotti, T.N., Loric, M., Bain, G.I., Atkins, G.J., Findlay, D.M., 2001. Osteoprotegerin and receptor activator of nuclear factor kappaB ligand (RANKL) regulate osteoclast formation by cells in the human rheumatoid arthritic joint. Rheuma (Oxf. 40, 623–630.

Heino, T.J., Määttä, J.A., 2018. Bone marrow niche: role of different cells in bone metastasis. Curr. Mol. Biol. Rep. 4, 80–87. https://doi.org/10.1007/s40610-018-0091-0.

Hoeffel, G., Ginhoux, F., 2018. Fetal monocytes and the origins of tissue-resident macrophages. Cell Immunol. 330, 5–15. https://doi.org/10.1016/j.cellimm.2018.01.001.

Hume, D., A., MacDonald, K.P., 2012. Therapeutic applications of macrophage colony-stimulating factor-1 (CSF-1) and antagonists of CSF-1 receptor (CSF-1R) signaling. Blood 119 (8), 1810–1820. https://doi.org/10.1182/blood-2011-09-379214.

Hume, D., A., Loutit, J., F., Gordon, S., 1984. The mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80: macrophages of bone and associated connective tissue, 1984 J. Cell Sci. 66, 189–194. https://doi.org/10.1242/jcs.66.1.189.

Kaur, S., Raggatt, L., J., Batoon, L., Hume, D., A., Levesque, J., P., Pettit, A., R., 2017. Role of bone marrow macrophages in controlling homeostasis and repair in bone and bone marrow niches. Semin Cell Dev. Biol. 61, 12–21. https://doi.org/10.1016/j.semcdb.2016.08.009. Epub 2016 Aug 10. PMID: 27521519.

Kobayashi, K., Takahashi, N., Jimi, E., Udagawa, N., Takami, M., Kotake, S., Nakagawa, N., Kinosaki, M., Yamaguchi, K., Shima, N., Yasuda, H., Morinaga, T., Higashio, K., Martin, T.J., Suda, T., 2000. Tumor necrosis factor alpha stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. J. Exp. Med 191 (2), 275–286. https://doi.org/10.1084/jem.191.2.275.

Kular, J., Tickner, J., Chim, S.M., Xu, J., 2012. An overview of the regulation of bone remodelling at the cellular level. Clin. Biochem. 45, 863–873. https://doi.org/10.1016/j.clinbiochem.2012.03.021.

Langdahl, B., Ferrari, S., Dempster, D.W., 2016. Bone modeling and remodeling: potential as therapeutic targets for the treatment of osteoporosis. Ther. Adv. Musculoskelet. Dis. 8, 225–235. https://doi.org/10.1177/1759720×16670154.

Lazarov, T., Juarez-Carreño, S., Cox, N., Geissmann, F., 2023. Physiology and diseases of tissue-resident macrophages. Nature 618 (7966), 698–707. https://doi.org/10.1038/s41586-023-06002-x.

Li, Y.H., Zhang, Y., Pan, G., Xiang, L.X., Luo, D.C., Shao, J.Z., 2022. Occurrences and functions of Ly6C^{hi} and Ly6C^{lo} macrophages in health and disease. Front. Immunol. 13, 901672 https://doi.org/10.3389/fimmu.2022.901672.

Liu, Y.C., Zou, X.B., Chai, Y.F., Yao, Y.M., 2014. Macrophage polarization in inflammatory diseases. Int J. Biol. Sci. 10 (5), 520–529. https://doi.org/10.7150/ijbs.8879.

Manolagas, S.C., 2000. Birth and dead of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr. Rev. 21, 115–137.

Marsell, R., Einhorn, T.A., 2011. The biology of fracture healing. Injury 42 (6), 551–555. https://doi.org/10.1016/j.injury.2011.03.031.

Martin, C.J., Peters, K.N., Behar, S.M., 2014. Macrophages clean up: efferocytosis and microbial control. Curr. Opin. Microbiol 17, 17–23.

McCabe, A., MacNamara, K.C., 2016. Macrophages: Key regulators of steady-state and demand-adapted hematopoiesis. Exp. Hematol. 44 (4), 213–222. https://doi.org/10.1016/j.exphem.2016.01.003.

Metchnikoff, E., 1905. Immunity and Infective Diseases. Cambridge Univ. Press, Cambridge, UK.

Michalski, M.N., McCauley, L.K., 2017. Macrophages and skeletal health. Pharm. Ther. 174, 43–54. https://doi.org/10.1016/j.pharmthera.2017.02.017.

Michalski, M.N., Koh, A.J., Weidner, S., Roca, H., McCauley, L.K., 2016. Modulation of Osteoblastic Cell Efferocytosis by Bone Marrow Macrophages. J. Cell Biochem 117 (12), 2697–2706. https://doi.org/10.1002/jcb.25567.

Mills, C.D., Kincaid, K., Alt, J.M., Heilman, M.J., Hill, A.M., 2000. M-1/M-2 macrophages and the Th1/Th2 paradigm. J. Immunol. 164 (12), 6166–6173. https://doi.org/10.4049/jimmunol.164.12.6166.

Miron, R.J., Bosshardt, D.D., 2016. OsteoMacs: Key players around bone biomaterials. Biomaterials 82, 1–19. https://doi.org/10.1016/j.biomaterials.2015.12.017.

Miron, R.J., Zohdi, H., Fujioka-Kobayashi, M., Bosshardt, D.D., 2016. Giant cells around bone biomaterials: Osteoclasts or multi-nucleated giant cells? Acta Biomater. 46, 15–28. https://doi.org/10.1016/j.actbio.2016.09.029.

Mohamad, S.F., Xu, L., Ghosh, J., Childress, P.J., Abeysekera, I., Himes, E.R., Wu, H., Alvarez, M.B., Davis, K.M., Aguilar-Perez, A., Hong, J.M., Bruzzaniti, A., Kacena, M. A., Srour, E.F., 2017. Osteomacs interact with megakaryocytes and osteoblasts to regulate murine hematopoietic stem cell function. Blood Adv. 1, 2520–2528. https://doi.org/10.1182/bloodadvances.2017011304.

Mohamad, S.F., Gunawan, A., Blosser, R., Childress, P., Aguilar-Perez, A., Ghosh, J., Hong, J.M., Liu, J., Kanagasabapathy, D., Kacena, M.A., Srour, E.F., Bruzzaniti, A., 2021. Neonatal Osteomacs and Bone Marrow Macrophages Differ in Phenotypic Marker Expression and Function. J. Bone Miner. Res. 00, 1–14. https://doi.org/10.1002/jbmr.4314.

Murray, P., Wynn, T., 2011. Protective and pathogenic functions of macrophage subsets. Nat. Rev. Immunol. 11, 723–737. https://doi.org/10.1038/nri3073.

Nakashima, T., Takayanagi, H., 2009. Osteoimmunology: crosstalk between the immune and bone systems. J. Clin. Immunol. 29, 555–567.

Parffit, A.M., 2000. The mechanism of coupling: a role for the vasculature. Bone 26 (4), 319–323.

Parfitt, A.M., 1995. Bone remodeling, normal and abnormal: a biological basis for the understanding of cancer-related bone disease and its treatment. Can. J. Oncol. 5 (Suppl 1), 1–10. PMID: 8853518.

Pereira, M., Petretto, E., Gordon, S., Bassett, J.H.D., Williams, G.R., Behmoaras, J., 2018. Common signalling pathways in macrophage and osteoclast multinucleation. J. Cell Sci. 131 (11), jcs216267 https://doi.org/10.1242/jcs.216267.

Pettit, A.R., Chang, M.K., Hume, D.A., Raggatt, L.J., 2008. Osteal macrophages: A new twist on coupling during bone dynamics. Bone 43, 976–982. https://doi.org/10.1016/j.bone.2008.08.128.

Raggatt, L.J., Wullschleger, M.E., Alexander, K.A., Wu, A.C., Millard, S.M., Kaur, S., Maugham, M.L., Gregory, L.S., Steck, R., Pettit, A.R., 2014. Fracture healing via periosteal callus formation requires macrophages for both initiation and progression of early endochondral ossification. Am. J. Pathol. 184 (12), 3192–3204. https://doi.org/10.1016/j.ajpath.2014.08.017.

Rasheed, A., Rayner, K.J., 2021. Macrophage Responses to Environmental Stimuli During Homeostasis and Disease. Endocr. Rev. 42 (4), 407–435. https://doi.org/10.1210/endrev/bnab004.

Riggs, B., L., Wahner, H., W., Seeman, E., Offord, K., P., Dunn, W., L., et al., 1982. Changes in bone mineral density of the proximal femur and spine with aging. Differences between the postmenopausal and senile osteoporosis syndromes. J. Clin. Invest 70 (4), 716–723. https://doi.org/10.1172/jci110667.

Schlundt, C., El Khassawna, T., Serra, A., Dienelt, A., Wendler, S., Schell, H., van Rooijen, N., Radbruch, A., Lucius, R., Hartmann, S., Duda, G.N., Schmidt-Bleek, K., 2018. Macrophages in bone fracture healing: Their essential role in endochondral ossification. Bone 106, 78–89. https://doi.org/10.1016/j.bone.2015.10.019.

Schlundt, C., Fischer, H., Bucher, C.H., Rendenbach, C., Duda, G.N., Schmidt-Bleek, K., 2021. The multifaceted roles of macrophages in bone regeneration: A story of polarization, activation, and time. Acta Biomater. 133, 46–57. https://doi.org/10.1016/j.actbio.2021.04.052.

Sehgal, A., Irvine, K.M., Hume, D.A., 2021. Functions of macrophage colony-stimulating factor (CSF1) in development, homeostasis, and tissue repair. Sem. Immunol. 54, 101509 https://doi.org/10.1016/j.smim.2021.101509.

Sinder, B.P., Pettit, A.R., McCauley, L.K., 2015. Macrophages: Their Emerging Roles in Bone. J. Bone Min. Res 30, 2140–2149. https://doi.org/10.1002/jbmr.2735.

Stout, R.D., Suttles, J., 2004. Functional plasticity of macrophages: reversible adaptation to changing microenvironments. J. Leukoc. Biol. 76 (3), 509–513. https://doi.org/10.1189/jlb.0504272.

Takeshita, S., Kaji, K., Kudo, A., 2000. Identification and characterization of the new osteoclast progenitor with macrophage phenotypes being able to differentiate into mature osteoclasts. J. Bone Min. Res 15, 1477–1488.

Taylor, P.R., Martinez-Pomares, L., Stacey, M., Lin, H.H., Brown, G.D., Gordon, S., 2005. Macrophage receptors and immune recognition. Annu Rev. Immunol. 23 (1), 901–944.

Tresguerres, F.G.F., Torres, J., López-Quiles, J., Hernández, G., Vega, J.A., Tresguerres, I. F., 2020. The osteocyte: A multifunctional cell within the bone. Ann. Anat. 227, 151422 https://doi.org/10.1016/j.aanat.2019.151422.

Udagawa, N., Takahashi, N., Akatsu, T., Tanaka, H., Sasaki, T., Nishihara, T., Koga, T., Martin, T.J., Suda, T., 1990. Origin of osteoclasts: mature monocytes and macrophages are capable of differentiating into osteoclasts under a suitable microenvironment prepared by bone marrow-derived stromal cells. Proc. Natl. Acad. Sci. USA 87 (18), 7260–7264. https://doi.org/10.1073/pnas.87.18.7260. PMID: 2169622; PMCID: PMC54723.

Vahle, J.L., Long, G.G., Sandusky, G., Westmore, M., Ma, Y.L., Sato, M., 2004. Bone neoplasms in F344 rats given teriparatide [rhPTH (1-34)] are dependent on duration of treatment and dose. Toxicol. Pathol. 32 (4), 426–438.

Van Furth, R., Cohn, Z.A., 1968. The origin and kinetics of mononuclear phagocytes. J. Exp. Med 128, 415–435. https://doi.org/10.1084/jem.128.3.415.

Vi, L., Baht, G.S., Whetstone, H., Ng, A., Wei, Q., Poon, R., Mylvaganam, S., Grynpas, M., Alman, B., A., 2015. Macrophages promote osteoblastic differentiation in-vivo: implications in fracture repair and bone homeostasis. J. Bone Miner. Res 30 (6), 1090–1102. https://doi.org/10.1002/jbmr.2422.

Walker, D., G., 1973. Osteopetrosis cured by temporary parabiosis. Science 180 (4088), 875. https://doi.org/10.1126/science.180.4088.875.

Wang, W., Liu, H., Liu, T., Yang, H., He, F., 2022. Insights into the Role of Macrophage Polarization in the Pathogenesis of Osteoporosis. Oxid. Med. Cell. Longev., 2485959 https://doi.org/10.1155/2022/2485959.

Weivoda, M., M., Bradley, E., W., 2023. Macrophages and Bone Remodeling. J. Bone Min. Res 38 (3), 359–369. https://doi.org/10.1002/jbmr.4773.

Wu, A.C., Raggatt, L.J., Alexander, K.A., Pettit, A.R., 2013. Unraveling macrophage contributions to bone repair. Bone Rep. 2, 1–7. https://doi.org/10.1038/bonekey.2013.107.

Yahara, Y., Barrientos, T., Tang, Y.J., et al., 2020. Erithromyeloid progenitors give rise to a population of osteoclasts that contribute to bone homeostasis and repair. Nat. Cell Biol. 22 (1), 49-45.

Yang, J., Zhang, L., Yu, C., Yang, X.F., Wang, H., 2014. Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases. Biomark. Res 2 (1), 1. https://doi.org/10.1186/2050-7771-2-1.

Yao, Z., Getting, S.J., Locke, I.C., 2021. Regulation of TNF-Induced Osteoclast Differentiation. Cells 11 (1), 132. https://doi.org/10.3390/cells11010132.

Ziegler-Heitbrock, L., Ancuta, P., Crowe, S., Dalod, M., Grau, V., Hart, D.N., Leenen, P.J., Liu, Y.J., MacPherson, G., Randolph, G.J., Scherberich, J., Schmitz, J., Shortman, K., Sozzani, S., Strobl, H., Zembala, M., Austyn, J.M., Lutz, M.B., 2010. Nomenclature of monocytes and dendritic cells in blood. Blood 116 (16), e74–e80. https://doi.org/10.1182/blood-2010-02-258558.