Osteoimmunology — The hidden immune regulation of bone

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ABSTRACT

Osteoimmunology is an emerging field of research dedicated to the investigation of the interactions between the immune and skeletal systems. These interactions are not only mediated by the release of cytokines and chemokines but also by direct cell–cell contact. Recently, it was proposed that immunoreceptors found in the immune cells are also an essential signal for osteoclasts activation, along with receptor activator NF-κB (RANKL) and macrophage-colony stimulating factor (M-CSF). In addition, adipose tissue also produces several factors (adipokines) that are known to interfere with the immune system and bone homeostasis. Chronic inflammation strongly influences osteoimmunology determining profound metabolic, structural and functional changes in bone.

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1. Introduction

The term osteoimmunology was used for the first time in 2000 [1] to describe the interaction of cells from the immune and skeletal systems. In fact, one year before, receptor activator

NF-κB (RANKL) ligand (RANKL) was found in T lymphocytes and described as a regulator of dendritic cell and osteoclast function, having an important role in promoting osteoclastogenesis [2]. However this connection between bone and immune systems should not be surprising, once precursors of immune cells reside in the bone marrow and, thus, are in the same environment as differentiated bone cells.

2. Crosstalk between osteoblasts and osteoclasts

Two key factors produced by osteoblasts play a crucial role in regulating bone resorption by osteoclasts. RANKL is a member of the tumor necrosis factor (TNF) superfamily of proteins and...
osteoblasts synthesize it as a transmembrane protein. In bone, expression of RANK by osteoblasts allows the maturation, differentiation and activation of osteoclasts by binding to its receptor, RANK, present on pre-osteoclasts surface. On the other hand, osteoblasts also secrete a factor that exerts a protective effect on bone, osteoprotegerin (OPG). OPG is a member of the TNF receptor superfamily and, although expressed ubiquitously in several tissues, has a very important role in the skeletal system, acting as a decoy receptor for RANK–RANKL binding. OPG binds to RANKL with high specificity and, thus, prevents osteoclasts differentiation and activation and promotes osteoclast apoptosis [3]. Therefore, the balance between RANKL and OPG determines bone resorption.

Signalling through RANK–RANKL is the key factor for osteoclast proliferation and differentiation. However, RANK by itself does not have intrinsic enzymatic activity and needs to recruit adaptor proteins, such as the TNF-receptor-associated factor (TRAF) family of proteins [4], especially TRAF6. The cytoplasmic tail of RANK contains three TRAF6-binding domains and the binding of this protein to RANK induces trimerization of TRAF6 leading to the activation of nuclear factor kappa-B (NF-kB) and mitogen-activated kinases (MAPK), including Jun N-terminal kinase (JNK) and p38 [5]. Therefore, TRAF6 acts downstream of RANK inducing, in pre-osteoclasts, the expression of the target genes activator protein-1 (AP1) and nuclear factor of activated T cells, and cytoplasmic calcineurin-dependent 1 (NFATc1), leading to pre-osteoclasts fusion and to osteoclasts differentiation [4,6]. TRAF6 also plays an important role in osteoclastic bone resorption by inducing membrane ruffling and actin ring formation through the activation of c-Src signalling cascade [7].

Although RANK–RANKL signalling plays a central role, osteoclast proliferation also depends on the presence of macrophage-colony stimulating factor (M-CSF), produced by several cell types including osteoblasts, that upon binding to its receptor, c-fms, at the surface of pre-osteoclast cells, activates an intracellular cascade that ultimately leads to proliferation and survival of osteoclasts [4]. In this way, osteoblasts produce the key factors RANKL and M-CSF that promote osteoclasts proliferation and differentiation.

Bone remodelling is a multifaceted process, requiring several cross talk mechanisms. In addition to the RANK–RANKL system, the bi-directional signalling mediated by the transmembrane receptor EphB4 in osteoblasts and its ligand ephrinB2 in osteoclasts, further increases the complexity of the intricate communication between these two cells [8]. In fact, signalling through ephrinB2–EphB4 leads to the inhibition of osteoclast differentiation by blockage of c-fos and NFATc1 transcriptional cascade and stimulate osteoblast differentiation by inducing osteogenic regulatory genes like Distal-less homeobox 5 (Dlx5), Osterix (Osx) and Runx-related transcription factor 2 (Runx2) [8,9]. Thus, osteoblast–osteoclast communication through ephrinB2–EphB4 favours the coupling between bone formation and bone resorption [9]. Ephrin receptors (Eph) are the largest family of receptor tyrosine kinases and are activated only by membrane-bound proteins, not by soluble ligands. Therefore, direct cell-to-cell contact is required for receptor activation and a unique feature of ephrin–Eph interaction is the ability for bidirectional signalling; that is, upon binding of the ligand with the receptor, signals are transduced in both receptor-expressing cell (forward signalling) and ligand-expressing cell (reverse signalling) [10]. This bidirectional signalling has been shown to be important in angiogenesis, epithelial movement in the intestine, axon guidance, cell sorting, boundary formation, stem cell differentiation, immune response and skeletal patterning [9]. Furthermore, ephrin–Eph uses common signalling pathways such as Src family kinases and Ras/Rho family GTases that often have opposite effects in the interacting cells [10].

3. Immunoregulation of bone

Bone is constantly being resorbed and formed in a very dynamic process whose imbalance leads to bone metabolic diseases, such as osteoporosis. In fact, about 10% of the adult human skeleton is remodelled every year. This balance is tightly regulated by two systems: the immune and the endocrine systems.

4. Direct influence of the immune system on bone

Immune and skeletal systems have several regulatory factors in common, such as cytokines, transcription factors and receptors. Consequently, these two systems interact with each other in physiological or in pathological conditions. Pathological activation of one system affects the other, such as in the case of rheumatoid arthritis where abnormal activation of the immune system affects bone remodelling leading to pathological bone erosions. During chronic inflammation, the balance between bone formation and resorption is skewed towards osteoclast-mediated bone resorption. Moreover, in inflamed joints, osteoclasts are located in the interface between the inflamed synovium and the bone and, as in physiological conditions, the major player in bone resorption is the RANKL/RANK/OPG system [11].

RANKL is produced by several cell types besides osteoblasts, including monocytes, neutrophils, dendritic cells, B and T lymphocytes (Fig. 1). In this way, immune cells have the ability to induce osteoclast differentiation and, consequently, bone resorption. Also, these cells are known for producing a variety of pro-inflammatory cytokines that also contribute to bone damage by potentiating the effects of the RANK–RANKL signalling [11]. The cytokines TNF-α and interleukin (IL)-1, IL-3, IL-6, IL-7, IL-11, IL-15 and IL-17 potentiate bone loss either by increasing osteoclast generation and activation or by inducing RANKL expression by the osteoblasts. On the other hand, IL-4, IL-5, IL-10, IL-12, IL-13, IL-18 and interferon (IFN)-γ are inhibitors of osteoclastogenesis by blocking RANKL signalling, either directly or indirectly. Interestingly, IL-1 is a stimulator of TRAF6 expression on the osteoclast, thereby potentiating RANK–RANKL signalling cascade, whereas IFN-γ is known to downregulate TRAF6 by proteosomal degradation aborting osteoclast formation [12].

In addition, other factors contribute to the complex regulation of osteoclast differentiation. For instance granulocyte–macrophage colony-stimulating factor (GM-CSF) super-227 presses the transcription factors c-fos and Fra-1, which are key factors for osteoclast development, while transforming growth factor (TGF)-β can have both an inhibitory or stimulatory action over osteoclastogenesis, as on one hand TGF-β3 was described to down modulate RANKL expression in [177]
osteoblasts and on the other hand this cytokine potentiates RANKL expression in T cells [12].

Although T cells express RANKL, they are classical anti-osteoclastogenic cells as they produce cytokines that inhibit bone resorption (especially IFN-γ, the most potent inhibitor). However, the T-helper (Th) cell subset involved in the production of IL-17 (Th17 cells) is considered to be the typical osteoclastogenic Th subset due to the fact that expresses RANKL at higher levels than Th1 or Th2, does not produce high amounts of IFN-γ and produces pro-inflammatory cytokines (like TNF-α) that potentiate RANKL expression, in fact, Th1 and Th2 subpopulations suppress Th17 subset through the secretion of cytokines [13,14]. In addition, IL-17 induces the synthesis of matrix-degrading enzymes, such as matrix metalloproteinases, inducing bone and cartilage degradation [15]. These effects are balanced by regulatory T (Treg) cells which inhibit bone destruction through suppression of osteoclast formation by a cell contact-dependent manner, that might be mediated by the expression of cytotoxic T lymphocyte-associated (CTLA)-4, which binds to B7-1 and B7-2 in the pre-osteoclasts [16]. Treg cells also express cytokines, like IL-4, IL-10 and TGF-β, which not only have anti-inflammatory properties but also suppress osteoclastogenesis [17–19]. Therefore, in pathophysiological conditions, the effects of T cells on osteoclastogenesis should depend on the balance between positive and negative factors that they express.

Although RANKL and M-CSF are essential factors and key players on osteoclast differentiation, it was hypothesized that costimulatory molecules like immunoglobulin-like receptors are the third essential factor required for osteoclastogenesis (Fig. 1). Among these molecules are osteoclast-associated receptor (OSCAR) and paired immunoglobulin-like receptor (PIR)-A, that signal through FcRγ and DAP12, that signal through DNAX-activation protein 12 (DAP12) [12], and both activate an intracellular calcium signalling cascade. These processes lead to the dephosphorylation of calcineurin, thereby activating the auto-amplification of NFATc1, which, consequently, promotes osteoclast differentiation. Therefore, FcRγ and DAP12 are adaptor molecules that associate with immunoglobulin-like receptors helping their expression and transducing signals through immunoreceptor tyrosine-based activation motif (ITAM) [4,20]. RANKL also interacts with ITAM by inducing its phosphorylation, thus increasing expression of immunoglobulin-like receptors and enhancing ITAM signal [12].

Further modulation of osteoclastogenesis is provided by Toll-like receptors (TLR). TLR expression was detected on bone...
cells and direct signalling through TLR activates a signalling cascade, mediated by TRAF6, leading to the activation of transcription factors, such as NF-κB and AP-1 family factors and to the synthesis and release of pro-inflammatory cytokines [21–23]. However, the outcome of TLR activation depends on the stage of differentiation of the osteoclast; in this way, in early precursor cells, TLR inhibit osteoclastogenesis, but in cells that have already started to develop into osteoclasts, TLR is a potent pro-osteoclastogenic factor. In mature osteoclasts, TLR signalling promotes cell survival. On the other hand, Osteoblasts were also found to express TLR, namely TLR-4, TLR-5 and TLR-9, and exposure of these cells to pathogen-associated molecular patterns (PAMP) induces the secretion of pro-inflammatory cytokines. Therefore, TLR contribute to the modulation of osteoclastogenesis by modulating the function of both osteoblasts and osteoclasts [21].

Finally, bone also interacts with B cell biology as RANKL is known to influence B lymphocyte development, increasing pro-B cells proliferation [22]. Moreover, it was reported that B cell progenitors have the ability of differentiating into osteoclasts in vitro, and peripheral blood B cells can support osteoclastogenesis [12]. As we have discussed, immune system has several cross-talk points with skeletal system, among which T and B lymphocyte interactions with bone cells, cytokine and chemokine dependent bone resorption, TLR signalling and costimulatory molecules. Together, these factors modulate bone remodelling.

5. Osteoimmunoendocrinology

White adipose tissue (WAT) is actively involved in body homeostasis, being responsible for the production of over fifty soluble factors that are involved both in physiological and pathological processes. These peptides are called adipokines and have autocrine, endocrine and/or paracrine mechanisms of action. Leptin, adiponectin, visfatin and resistin are adipokines produced by the WAT that have immunomodulatory effects and are involved in chronic inflammation.

Leptin is the best well-known adipokine and is classically involved in the regulation of body weight through appetite suppression and increased energy expending. But leptin also plays a role as a pro-inflammatory cytokine modulating several cells involved in the immune response and has an important role in bone homeostasis, reproduction, development, hematopoiesis and angiogenesis [24]. Leptin is not only produced by adipocytes but also by other cells, like immune cells and osteoblasts. In fact, this adipokine induces peripheral blood mononuclear cells to secrete pro-inflammatory cytokines such as IL-6, TNF-α and IFN-α, and also activates monocytes, modulates phagocytosis of macrophages and stimulates neutrophil production of reactive oxygen species; furthermore, leptin interferes in the T-cell balance inducing T-cell activation towards a Th1-type response. In addition, adipocytes also secrete IL-1 and TNF-α, which are positive stimuli for the expression of leptin [25].

Two opposing mechanisms have been suggested to explain the effect of leptin on bone metabolism (Fig. 1). On one hand, leptin can act locally on bone receptors promoting the development of osteoprogenitor cells and stimulate osteoblasts to form new bone; on the other hand, leptin can act through the central nervous system decreasing osteoblast activity [26]. Further increasing the complexity of the system, primary human osteoblasts transcribe, translate and secrete leptin and leptin expression fluctuates during the differentiation of these cells. In fact, in mesenchymal cells, leptin expression is present and in proliferating osteoblasts is suppressed, while it reappears in late stage osteoblasts. Accordingly, it can be argued that leptin increases bone formation by enhancing human osteoblast proliferation, collagen synthesis and mineralization and positively favouring OPG/RANKL ratio through downregulation of RANKL [27]. Moreover, leptin has an antiapoptotic role by reducing the pro-inflammatory cytokines. Therefore, TLR contribute to the modulation of osteoclastogenesis by modulating the function of both osteoblasts and osteoclasts [21].

Interactions between adipocytes and osteoblasts should not be surprising since these two cells derive from the same precursor by the action of the peroxisome proliferative activated receptor (PPAR)-γ that is the dominant regulator of adipogenesis, favouring differentiation of mesenchymal stem cells (MSC) into adipocytes at the expense of osteoblasts [28]. By contrast, expression of Runx2 and Osx inevitably shifts the balance towards osteoblast formation [29]; recently, it was demonstrated that Wnt5a, a component of the non-canonical Wnt pathway, represses PPAR-γ transcription and induces expression of osteoblast-specific genes promoting osteoblastogenesis [29,30]. PPARs are nuclear receptors that have a wide range of effects on metabolism, cell proliferation, differentiation and immune response and are activated by ligands like unsaturated fatty acids, eicosanoids and metabolites of linoleic acid. PPARs are important regulators of the immune system and, in fact, are expressed by T and B lymphocytes, eosinophils, dendritic cells and macrophages and activation of PPAR downregulates several components of the inflammatory response, like cytokine [31] and chemokine secretion and expression of costimulatory molecules, exerting an anti-inflammatory activity [32].

Recently it was proposed that PPAR-γ, an essential regulator of lipid, glucose and insulin metabolism, has a dual role in promoting osteoclast lineage commitment and osteo-clast maturation at the transcriptional level by controlling the c-fos gene both in osteoclasts and their precursors [33,34]. In vitro experiments revealed that PPAR-γ activation on pre-osteoclast cells promote their differentiation, while activation of this receptor in cells of the mesenchymal lineage inhibits their support to osteoclastogenesis by blocking its differentiation in osteoblasts. Moreover, leptin and adiponectin, although with opposite effects, are under PPAR-γ control [35].

Therefore, PPAR not only have a direct effect on osteoblast and osteoclast differentiation but also have indirect actions upon bone remodelling through regulation of cytokines and chemokines secretion.

6. Effects of immune activation and inflammation on bone structure

Rheumatoid arthritis (RA) is the prototype of chronic inflammatory joint diseases and is characterized by persistent inflammation and progressive bone erosions, leading to functional disability and increased morbidity and mortality. In this disease it is clear that the pro-inflammatory cytokines TNF-α, IL-1 and IL-6 are involved in the perpetuation of the
inflammatory condition. The RANK/RANKL/OPG pathway is also strongly involved in RA pathology and it was observed that the RANKL/OPG ratio is increased, leading to bone erosions [36]. This effect is mainly dependent on osteoclastic activity and is expressed by two main forms, destruction of the organic matrix (type 1 collagen) by cathepsin K, and dissolution of the mineralized component (hydroxyapatite crystals) by the acid microenvironment generated by the osteoclastic proton pump. Chronic inflammation skews the bone remodelling balance towards osteolast-mediated bone resorption through the mechanisms previously described leading to derangement of mineral and organic components. We have shown, in an animal model of chronic arthritis, that after chronic arthritis is installed, there is a reduction in bone density and a change in the pattern of bone organization, which was reflected in the impairment of bone mechanical properties, namely stiffness, ductility and strength, reinforcing the hypothesis that deregulation of the immune system strongly affects bone metabolism, structure and function. [37]

7. Conclusion

Osteoblasts and osteoclasts are the central players of bone remodelling and every factor that affects these cells, ultimately, affects the entire process. Immune and bone cells are actively engaged in the maintenance of bone homeostasis. In fact, regulation of osteoclasts has some parallel points with the regulation of an immune response reinforcing the hypothesis that osteoclasts act like “immune cells”. Actually, osteoclasts attack bone when osteoblasts uncover the bone surface, as is the case of micro damage and inflammation, just like immune cells attack exposed foreign bodies. This bare bone is then recognised as “foreign body” and triggers an “immune response” leading to osteoclast differentiation and activation [38]. On the other hand, adipose tissue is also tightly involved in regulation of bone cells mainly by the production of adipokines, which not only act on osteoblasts and osteoclasts but also on immune cells. Therefore, these crosstalk mechanisms between skeletal, immune and adipose systems create several points of contact that can be used as potential therapeutic targets for controlling bone remodelling.

References


