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Review

The immune system, bone and RANKL

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ABSTRACT

Bone and immune systems are tightly linked. In the past years, many molecules originally believed to belong to the immune system were found to function in bone cells. It is now evident that the two systems are coregulated by many shared cytokines and signaling molecules. Here we exemplify the complex interaction between bone metabolism and immune response focusing on the multifaceted role of receptor activator of NF-κB ligand (RANKL). RANKL is expressed by cells of both systems, is an essential regulator of bone degradation and exerts either pro or anti-inflammatory effects on the immune response. In the present review, we summarize the multiple functions of RANKL in bone and in the immune systems, aiming to provide an overview of the field of osteoimmunology.

Introduction

The skeletal system supports locomotion and protects the organs from traumatic injury, among its critical functional roles. Even though it has long been regarded as being comprised of hard and inert tissue, it is in fact constantly and dynamically being renewed under homeostatic conditions in order to maintain its mechanical properties. Osteoclasts are giant multinucleated cells specializing in bone degradation. They are able to firmly adhere to the bone surface and degrade both mineral and organic components of the bone matrix. Osteoclasts differentiate from myeloid precursors that originate in the bone marrow [1]. The myeloid ancestry of osteoclasts constitutes the primary intimate link between the bone and immune systems.

Receptor activator of NF-κB ligand (RANKL)[1], encoded by the *Tnfsf11* gene, was cloned from activated T cells in 1997 as a new member of the tumor necrosis factor (TNF) superfamily [2]. Its receptor RANK is expressed on the surface membrane of dendritic cells (DC). The fundamental role of RANKL as the critical osteoclastogenic factor was first uncovered in 1999, when RANKL knockout mice were found to develop severe osteopetrosis due to complete absence of these cells [3]. RANKL expressed by cells of mesenchymal origin in bone is a direct regulator of osteoclast formation and bone turnover. In addition, cells of the immune system, such as T and B cells, express RANKL upon activation [4,5]. Indeed, under various pathological conditions, activation of the immune system leads to abnormal bone loss. T cells play a major role in the pathogenesis of rheumatoid arthritis (RA) and it is now accepted that RANKL is responsible for bone loss in RA [6], as is discussed in detail below. Thus, RANKL directly links the bone to the immune system.

The bone marrow is the site of adult hematopoiesis [7,8]. Bone cells and bone marrow hematopoietic cells share the same microenvironment, suggesting that bone metabolism and hematopoiesis may be coregulated. Osteoblasts and osteoclasts both affect the maintenance and the mobilization of hematopoietic stem cells [9–13]. Moreover, osteoblasts control the proliferation of hematopoietic progenitors [11]. Thus, a body of evidence suggests the two systems regulate each other under physiological and pathological conditions.

A major effort has been put forth in recent years to elucidate the multiple functions of the molecules that are expressed in both bone and immune cells. Several molecules that were initially identified and studied in the immune system have been shown to have essential functions in the bone, too. A number of mouse strains deficient in various immune-related molecules, including cytokines, receptors, signaling molecules and transcription factors, were found to have abnormal bone homeostasis (for an extensive review, please refer to [14]). As our knowledge of biological systems has grown in molecular detail, a holistic connection

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[1] *Abbreviations used:* RANKL, receptor activator of NF-κB ligand; TNF, tumor necrosis factor; DC, dendritic cells; RA, rheumatoid arthritis; OSCAR, osteoclast associated receptor; PIR-A, paired immunoglobulin-like receptor; TREM2, triggering receptor expressed in myeloid cells-2; Gab2, Grb2-associated binding protein 2; EGFR, epidermal growth factor receptor; FHL2, four-and-a-half LIM domain 2.

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between apparently distinct physiological systems has come to be realized. In the first years of this century, an interdisciplinary field embracing immune and bone biology was brought together and named osteoimmunology.

In this review, we aim to provide an overview of the field, focusing on RANKL, a molecule that is crucial to our understanding of the complex interactions between immune system and bone.

Signaling downstream of RANK in osteoclast differentiation

Since the discovery of the RANKL/RANK axis, the signaling pathways stimulated by RANKL have been extensively studied (Fig. 1). RANK, encoded by the *Tnfrsf11a* gene, belongs to the TNF receptor superfamily and is the exclusive receptor for RANKL. M-CSF induces RANK on osteoclast precursor cells and supports their proliferation. Binding with RANKL promotes RANK trimerization and activates intracellular signaling [15]. RANKL/RANK signaling is inhibited by osteoprotegerin (OPG), encoded by the *Tnfrsf11b* gene, which acts as a decoy receptor: it binds to RANKL and prevents its interaction with RANK [16]. OPG is expressed by several cell types in both the bone (osteoblasts [17]) and immune systems (B cells and DCs [18]), and fine tunes the interaction between RANKL and RANK.

The binding of RANKL with RANK results in the trimerization of the receptor and the rapid recruitment of tumor necrosis factor associated factor 6 (TRAF6) to its intracellular tail. RANK interacts with various TRAF molecules (TRAF1, TRAF2, TRAF3, TRAF5 and TRAF6), but only TRAF6 is essential for osteoclast function [19]. TRAF6 activates the mitogen activated protein kinases (MAPKs), such as p38 and Jun N terminal kinase (JNK) [20], as well as the IκBα kinases (IKKs) that activate nuclear factor-κB (NF-κB). NF-κB and nuclear factor of activated T cells 2 (NFATc2) then cooperatively trigger the induction of NFATc1. NFATc1 was identified as the key transcription factor responsible for the specification to the osteoclast lineage [21]. In response to activation of the receptors of the TNF superfamily, NFATc1 is specifically activated downstream of RANK. c-Fos is induced by RANK activation in a manner dependent on NF-κB [22] and, at a later stage, on Ca^{2+}/calmodulin-dependent protein kinases (CaMK)s [23]. This in turn activates the AP-1 transcription factors together with c-Jun phosphorylation by JNK.

Activation of RANK immediately leads to the phosphorylation of the immunoreceptor tyrosine-based activation motif (ITAM) in DNAX activation protein of 12 kDa (DAP12) and Fc-receptor γ subunit (FcRγ). DAP12 and FcRγ are intracellular adaptor molecules that interact with immunoglobulin-like receptors, such as osteoclast associated receptor (OSCAR), paired immunoglobulin-like receptor (PIR-A) and triggering receptor expressed in myeloid cells-2 (TREM2), that are expressed on the cell membrane of osteoclast precursors [24]. They are known as RANK costimulatory receptors because they are essential for osteoclastogenesis, but their activation by itself does not induce osteoclast formation. These receptors lack an intra-cytoplasmic tail and rely on FcRγ and DAP12 for intracellular signaling. Once phosphorylated, ITAM recruits the protein kinase Syk [25,26], which activates phospholipase Cγ (PLCγ) together with the BTK and Tec kinases [27]. PLCγ stimulates calcium mobilization and the calcium-dependent phosphatase calcineurin becomes activated, leading to dephopshorylation of NFATc1 [24]. Dephosphorylated NFATc1 then translocates into the nucleus, where it interacts with its gene promoter and triggers its own auto-amplification [21].

At this stage, calcium/calmodulin dependent protein kinase IV (CaMKIV) promotes the activation of cyclic AMP responsive element binding protein (CREB), which results in further induction of the transcription factor c-Fos [23]. NFATc1, in cooperation with AP-1 and other transcription factors such as CREB, PU.1 and MITF, drives the expression of osteoclast-specific genes [20]. A sudden increase in the intracellular calcium by itself is not sufficient to activate NFATc1 signaling. Instead, oscillation of the cytoplasmic calcium concentration must occur. RGS10, which is activated

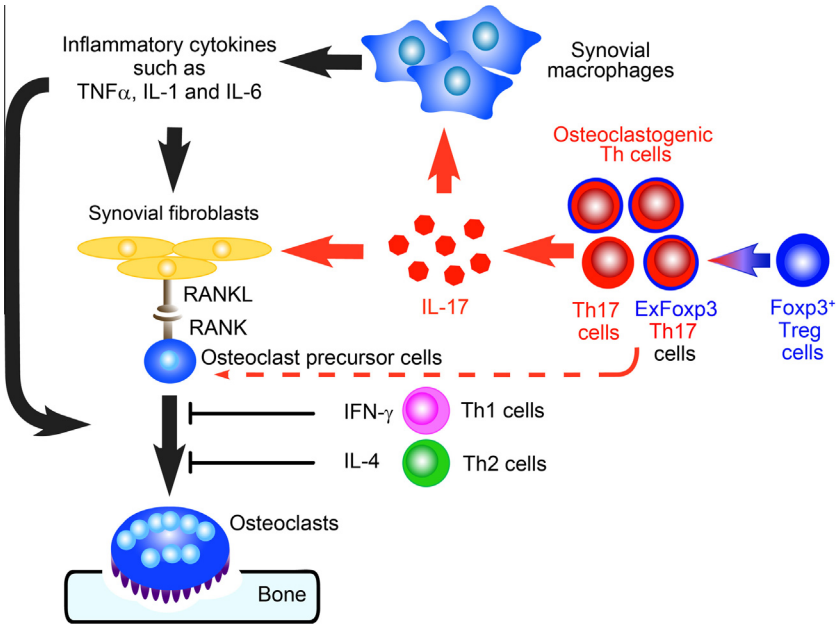

Fig. 1. Signalling pathways downstream M-CSF, RANKL and Ig-like receptors in osteoclast differentiation. (a) Precursor cell stage. M-CSF promotes survival and proliferation of osteoclast precursor cells that express RANK. Immunoglobulin-like receptor might bind specific ligands. (b) Proximal RANK signals. RANKL binds to RANK that recruits TRAF6. RANK in the same time phosphorylates ITAM on DAP12 or FcRγ, which are associated to Ig-like receptors. (c) Initial induction of NFATc1. NF-kB, activated by TRAF6, induces expression of NFATc1 in cooperation with NFATc2. Syk is recruited to the phosphorylated ITAM of DAP12 or FcRγ, resulting in the activation of calcium signaling through Phospholipase Cγ. (d) Auto-amplification of NFATc1. Calcium signaling mediates sustained activation of NFATc1, together with AP-1 cooperation. Activation of AP-1 requires induction of cFos, that is mediated by CaMKIV-activated CREB and c-Fms. NFATc1 binds to a NFAT binding site on its own promoter. (e) In the nucleus, NFATc1, together with AP-1, PU.1, MITF and CREB, induces transcription of osteclast specific genes, such as TRAP, Cathepsin K and Calcitonin receptor.