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Review

The role of TNF-receptor family members and other TRAF-dependent receptors in bone resorption

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Abstract

The contribution of osteoclasts to the process of bone loss in inflammatory arthritis has recently been demonstrated. Studies in osteoclast biology have led to the identification of factors responsible for the differentiation and activation of osteoclasts, the most important of which is the receptor activator of NF-κB ligand/osteoclast differentiation factor (RANKL/ODF), a tumor necrosis factor (TNF)-like protein. The RANKL/ODF receptor, receptor activator of NF-κB (RANK), is a TNF-receptor family member present on both osteoclast precursors and mature osteoclasts. Like other TNF-family receptors and the IL-1 receptor, RANK mediates its signal transduction via TNF receptor-associated factor (TRAF) proteins, suggesting that the signaling pathways activated by RANK and other inflammatory cytokines involved in osteoclast differentiation and activation are interconnected.

Keywords: osteoclasts, RANK, RANKL, TNF-α, TRAF

Introduction

The pathogenesis of focal bone loss in inflammatory processes such as rheumatoid arthritis (RA) is a subject of recent interest. Osteoclasts are known to contribute to focal bone erosion in RA [1–3] and in animal models of arthritis [4–6]. The role of osteoclasts in normal physiologic bone remodeling is well established. During this process, focal areas of bone are resorbed by osteoclasts and repopulated by osteoblasts, which synthesize new bone matrix [7]. The focal net loss of bone at sites of inflammation in conditions such as RA suggests that there is an imbalance in favor of bone resorption. Considerable effort has been made in elucidating the factors responsible for this increased bone resorption and in defining the mechanisms involved in the differentiation and activation of osteoclasts at sites of inflammation. This review focuses on a newly described and essential factor for osteoclast differentiation-ODF, a member of the TNF ligand family of cytokines [8]. ODF was independently identified as RANKL [9]. We will refer to this factor as RANKL/ODF. Its cognate receptor is RANK. The role of this receptor–ligand pair in bone resorption is reviewed, and the signal

DD = death domain; FADD = Fas-associated death domain protein; IKK = I-kB kinase; IL = interleukin; IRAK = IL-1 receptor-associated kinase; JNK = c-jun amino-terminal kinase; LPS = lipopolysaccharide; M-CSF = macrophage-colony stimulating factor; NF-κB = nuclear factor-kappa B; OCIF = osteoclastogenesis inhibitory factor; ODAR = osteoclast differentiation and activation receptor; ODF = osteoclast differentiation factor; OPG = osteoprotegerin; OPGL = osteoprotegerin ligand; PDK = PI3 kinase-dependent kinase; PGE_2 = prostaglandin E_2; PI3 = phosphatidylinositol 3; PIP3 = phosphatidylinositol-(3,4,5)-triphosphate; PTH = parathyroid hormone; RA = rheumatoid arthritis; RANK = receptor activator of NF-κB; RANKL = receptor activator of NF-κB ligand; RIP = receptor-interacting protein; RZF = RING(C_3HC_4)-zinc finger; TIM = TRAF interaction motif; TIR = Toll/IL-1/IL-18 receptor; TLR = Toll-like receptor; TNF = tumor necrosis factor; TNFR = TNF receptor; TRADD = TNFR-associated death domain protein; TRAF = TNF receptor-associated factor; TRANCE = TNF-related activation-induced cytokine.

transduction pathways involved in signaling through the RANK receptor are discussed in relation to common and interconnected pathways activated by other receptors.

The role of RANKL/ODF in osteoclast differentiation and activation

RANKL/ODF was originally cloned as an essential factor for osteoclastogenesis by two independent research groups [8,10], who named it, respectively, 'osteoclast differentiation factor' (ODF) and 'osteoprotegerin ligand' (OPGL). Using models of osteoclast differentiation *in vitro*, it has been shown that many of the factors that enhance osteoclast formation or activity, including $1,25(OH)_2D_3$, parathyroid hormone (PTH), interleukin (IL)-11, and prostaglandin E_2 (PGE_2), mediate these effects at least in part by inducing the expression of RANKL/ODF by osteoblasts and/or bone lining cells [11–14]. Interestingly, RANKL/ODF had also previously been independently identified as TNF-related activation-induced cytokine (TRANCE) [15], a T cell product upregulated after T cell-receptor stimulation. TRANCE enhances the proliferation of naïve T cells through interactions of T cells with dendritic cells [16].

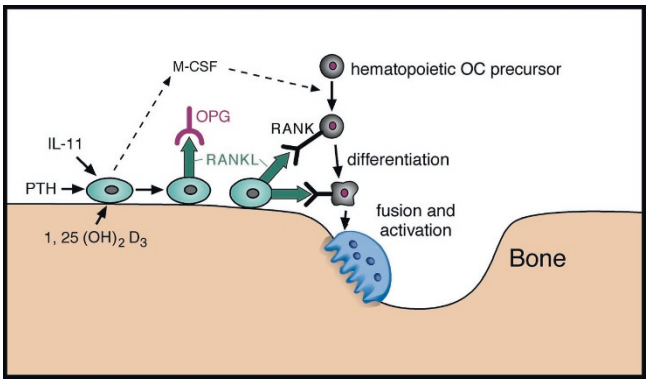
Activated T lymphocytes also express membrane-bound RANKL/ODF and can secrete a soluble form of RANKL/ODF [17]. RANKL/ODF messenger RNA is expressed at high levels in cells in trabecular bone and bone marrow, including bone lining cells and osteoblasts, as well as in lymph node, thymus, and Peyer's patches [8–10]. RANKL/ODF$^{-/-}$ mice exhibit a dramatic phenotype supporting the essential role of this factor in osteoclast differentiation. These mice have defective tooth eruption and severe osteopetrosis associated with the absence of osteoclasts [18].They also have no peripheral lymph nodes, have defects in B cell and T cell maturation, and have thymic hypoplasia, supporting the argument that this factor plays a role in immune-cell differentiation.

The signaling receptor for RANKL/ODF, a member of the TNF receptor (TNFR) family, was originally described as a receptor on T cells and dendritic cells, and was named RANK [also known as osteoclast differentiation and activation receptor (ODAR) and TRANCE receptor], because binding of RANKL/ODF to this receptor leads to activation of the transcription factor NF-κB [9,14,19]. In addition to being expressed on T cells and dendritic cells, RANK is also expressed on osteoclasts and osteoclast precursor cells, and on certain B cells [9,19,20]. RANK$^{-/-}$ mice have a phenotype similar to that of RANKL/ODF$^{-/-}$ mice, including the presence of osteopetrosis, absence of peripheral lymph nodes, and a deficiency of B cells [21,22].

Osteoprotegerin inhibits the actions of RANKL/ODF

A decoy receptor for RANKL/ODF has been identified and named osteoprotegerin (OPG) and osteoclastogenesis inhibitory factor (OCIF) [23,24]. OPG is a secreted member of the TNFR family that lacks a transmembrane domain and is structurally distinct from RANK. It is active as either a soluble monomer or a disulfide-linked homodimer [25]. OPG binds RANKL/ODF with high affinity, thereby preventing RANKL/ODF from interacting with its cognate receptor (RANK) (Fig. 1). Overexpression of OPG in transgenic mice blocks the activity of endogenous RANKL/ODF, resulting in the development of osteopetrosis, although there does not appear to be a defect in lymphoid tissue development [23]. OPG$^{-/-}$ mice demonstrate severe osteoporosis, which is a result of the unopposed activity of endogenous RANKL/ODF, leading to excessive osteoclast differentiation and activity [26,27]. Under physiologic conditions and in disorders associated with disturbed bone remodeling, osteoclast-mediated bone resorption can be modulated by altering the balance between OPG and RANKL/ODF. This has obvious implications for the development of therapeutic strategies for controlling physiologic and pathologic bone loss [11].

Figure 1

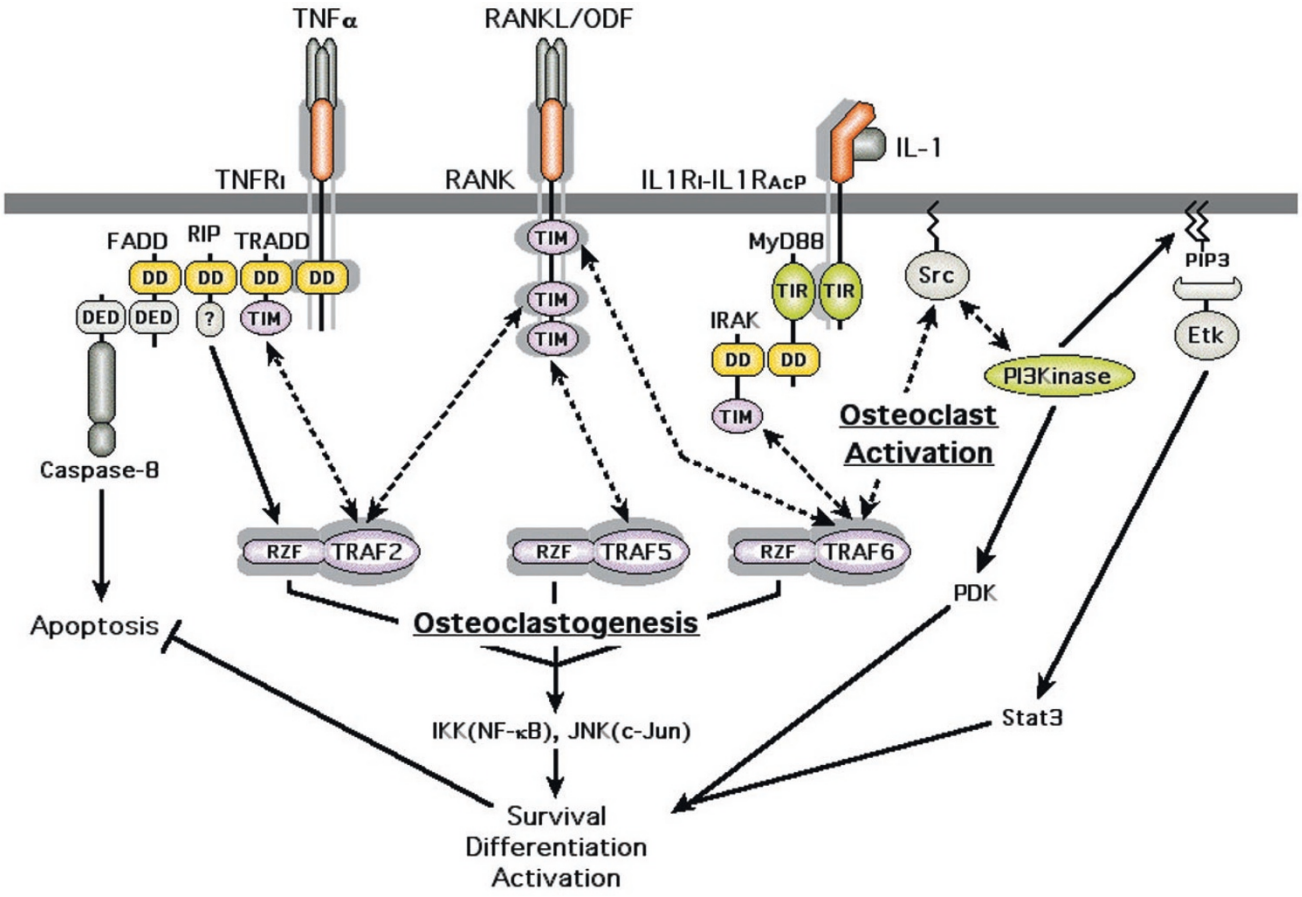


Simplified schematic view of the interactions between membrane-bound RANKL on osteoblast/bone stromal cells and its receptor RANK on osteoclast precursor cells. This interaction leads to the differentiation of osteoclast precursor cells and the activation of osteoclasts to resorb bone. OPG can inhibit this interaction by binding to membrane-bound RANKL and blocking the RANKL–RANK interaction. D_3, vitamin D_3; M-CSF, macrophage-colony stimulating factor; OC, osteoclast.

Two classes of tumor necrosis factor receptors regulate life and death

Binding of RANKL/ODF to RANK activates signal transduction pathways that ultimately lead to osteoclast differentiation and increased osteoclastic activity. It is useful to review other members of the TNFR family and their associated signal transduction pathways to examine the common and interconnected pathways by which these receptors regulate diverse cellular activities. Members of the TNFR family all share an extracellular ligand-binding

Figure 2



Interrelationships among receptors that signal via TRAFs. Double-headed, dashed arrows indicate known direct protein–protein interactions; solid arrows represent enzymatic and functional pathways. Emphasis is placed on distinctions between osteoclastogenesis and osteoclast activation. Abbreviations and terms are defined in the text.

domain containing at least two repeats of a signature module consisting of a disulfide-rich anti-parallel beta-strand structure of approximately 40 amino acids. The structural analysis of the ligand interaction reveals that a trimeric ligand interacts intimately with two of these modules from each of three receptors, resulting in a 3:3 complex [28] that leads to intracellular signal transduction.

With regard to intracellular signaling, the TNFR family can be subdivided into two groups, those that directly induce apoptosis (eg $TNFR_I$/p55 TNF-α receptor and Fas), and those that typically do not (eg RANK, $TNFR_{II}$/p75 TNF-α receptor, and CD40) (Fig. 2). The apoptotic response is dependent upon the presence of death domains (DDs) within the cytoplasmic region of the receptor. These domains mediate protein–protein interactions resulting in dimeric and tetrameric complexes among proteins containing nonidentical DDs [29]. The nonapoptotic TNFRs do not contain DDs but, in contrast to the apoptotic forms, do contain a short-sequence motif [30] necessary for the recruitment of a family of related TRAFs essential for many downstream intracellular signaling events [31,32]. These sequence motifs are referred to here as TRAF interaction motifs (TIMs). Activation of TRAFs appears to be dependent upon membrane localization [33] and trimerization of amino-terminal RING-zinc finger (RZF) domains mediated by the carboxyl-terminal ligand-interaction region [34,35]. Although apoptotic TNFRs do not contain any TIMs, they can recruit TRAFs indirectly via DD-containing adapter molecules.

In the case of the apoptotic receptors, TRAFs appear to be important for cell death, probably by supporting the action of Fas-associated death domain protein (FADD), which is critical for caspase-8 activation. As shown in Fig. 2, the apoptotic TNFRs (eg $TNFR_I$) have the potential to induce both death and survival signals. The specific outcome (ie survival versus death) appears to depend upon mechanisms that shift the balance of cytoplasmic signaling components. For example, the activation of caspase-8 not only activates apoptosis, but also inhibits

the NF-κB survival pathway by degrading receptor-interacting protein (RIP). RIP is a DD kinase essential for TNF-α-dependent activation of NF-κB [36], possibly through the TRAF amino-terminal RZF domain, in a kinase-independent manner [37].

Lipopolysaccharides and IL-1 receptors also signal via TRAF

TRAF recruitment and activation are also mediated by the Toll/IL-1/IL-18 receptor family (TIR), which is distinct from the TNFR family and includes the homodimeric Toll-like receptors (TLRs) that bind microbiological products (eg the lipopolysaccharide receptor TLR4); the heterodimeric IL-1 receptor (consisting of two related molecules, IL-1R_I and IL-1R_{AcP}); and receptors for IL-18 (Fig. 2). The TIRs all contain a specific domain structure (TIR domain) that is critical for the recruitment of MyD88, an adapter molecule that possesses both a TIR and a DD [38,39]. The MyD88 DD can recruit one or more IL-1R-associated kinases (IRAKs) that bind TRAF6. The kinase function of IRAK appears not to be important for TRAF recruitment but may serve to facilitate receptor recycling [40]. Therefore, IL-1, LPS, and TNF-α, which have long been known to have overlapping functions, may mediate these shared activities via the recruitment of TRAFs.

There are at least six distinct TRAF forms, not all of which induce intracellular signaling. TRAF2, 5, and 6 have been shown to be involved in TNFR and TIR downstream activation of NF-κB and the c-jun amino-terminal kinase (JNK), whereas TRAF1, 3, and 4 have not. TRAF4 is restricted to the nucleus, and TRAF1, which can heterotrimerize with TRAF2, is deficient in an amino-terminal region necessary for NF-κB activation [31]. TRAF3, however, which can be activated by engineered membrane localization [33], binds to the CD40 TNF receptor [34], and is required for T cell-dependent immune responses [41]. Although the receptor-binding specificities for TRAF2, 3, and 5 appear to be similar, TRAF6 possesses a distinct binding specificity. Therefore, although TRAF2, 3, and 5 bind either directly or indirectly to most TNFRs, TRAF6 is unique in its ability to bind directly only to IRAK, RANK, and CD40 [30].

RANK binds and activates a full complement of TRAFs and activates Src

The cytoplasmic domain of RANK is unique in that it has three independent TIMs, for binding TRAF2, 5, and 6 [42]. The membrane-proximal TRAF6 binding site appears to be highly specific. The other two sites each bind TRAF2 and 5. However, the carboxyl-terminal site is most specific for TRAF5 and the more amino-proximal site is most specific for TRAF2 [43]. The amino-terminal RZF domain of TRAF2 and 6 has been shown to be capable of activating downstream signals to I-κB kinase (IKK), JNK, and p38 kinase [35]. Osteoclast differentiation is blocked in mice deficient in the p50 and p52 forms of NF-κB, demonstrating the critical role of this factor [44,45]. However, RANK activation of both the JNK and p38 kinase pathways has also been demonstrated to be important for osteoclast differentiation and function [46,47]. TRAF2 and 5 appear to have similar activities. Interestingly, TRAF2$^{-/-}$ mice do not exhibit osteopetrosis [48], an observation indicating that this TRAF is either not important for osteoclastogenesis or, more likely, is complemented by other TRAFs.

The carboxy-terminal receptor-binding/trimerization domain of TRAF6 is distinct from that of other TRAFs in that it contains a short, proline-rich loop capable of binding to the SH3 domain of the Src tyrosine kinase [42]. This loop provides a means for the activation of Src, which, though constitutively membrane-associated via amino-terminal myristylation, is inhibited by intramolecular SH2 and SH3 interactions [49]. The activation of Src by TRAF6-mediated SH3 competition provides a mechanism for the reported activation of phosphatidylinositol 3-kinase (PI3 kinase) by both RANK [42] and IL-1R [39]. This activation may not require involvement of the Src kinase function and may depend only upon interaction between the SH3 domain of Src and the proline-rich sequence of the PI3 kinase p85 regulatory subunit [50]. This could explain how *src*$^{-/-}$ mice, which exhibit a severe osteopetrotic phenotype [51], can be rescued by a kinase-defective Src [52]. The functional association between TRAF6 and Src is also supported by the observation that both *src*$^{-/-}$ and TRAF6$^{-/-}$ mice exhibit a similar phenotype of osteopetrosis in which there are abundant osteoclasts, but a defect in osteoclastic bone resorption [52,53]. This is in contrast to both the RANK$^{-/-}$ and RANKL/ODF$^{-/-}$ mice, which lack osteoclasts [18,21].

Src is also capable of direct activation of the Stat3 transcription factor via tyrosine phosphorylation as well as indirect activation via Tec tyrosine kinases like Etk [54]. The tyrosine kinase Etk is activated by binding to phosphatidylinositol-(3,4,5)-triphosphate (PIP3), a product of activated PI3 kinase, through an amino-terminal plextrin homology domain [49]. The Src–Etk pathway could provide a mechanism for the reported tyrosine phosphorylation-dependent activation of the Stat3 transcription factor by IL-1 via the TRAF6-dependent IL-1R [39]. Also, PI3 kinase phospholipid products can activate many other kinases via PI3 kinase-dependent kinase (PDK) and protein kinases A, B, and C. PI3 kinase may therefore activate many pathways that are probably essential to osteoclast development and activation, including apoptosis inhibition, cell proliferation, endocytosis, and vesicular trafficking [55].

Cross-talk between TNF and Toll/IL-1 receptors probably modulates osteoclast action

The distinction between the activation of osteoclasts to resorb bone and osteoclast differentiation (osteoclasto-

genesis) is underscored by the observed differences between, on the one hand, *src*$^{-/-}$ and TRAF6$^{-/-}$ mice (which exhibit abundant but poorly functioning osteoclasts) and, on the other hand, RANK$^{-/-}$ and RANKL/ODF$^{-/-}$ mice (which lack osteoclasts). RANKL/ODF can support both osteoclast differentiation and activation. In contrast, TNF-α, primarily through $TNFR_I$, supports osteoclast differentiation, while both IL-1 and LPS can activate preformed osteoclasts to resorb bone [56–60]. Interestingly, $TNFR_I$ directly recruits only TRAF2 via the TNFR-associated death domain protein (TRADD) adapter [61], whereas both the LPS TLR4 [38] and IL-1R recruit only TRAF6 via IRAK [31] (Fig. 2). Therefore, because only TRAF6 activates Src, TRAF6 (and receptor ligands that can effect its signaling) may be a key component for the activation of osteoclasts to resorb bone. In this model, RANK induction of osteoclast differentiation does not require TRAF6 when other TRAFs are present. It is not yet clear whether TNF-α/$TNFR_I$ can replace all of the osteoclastogenic activities of RANK. Similarly, it is not yet known whether TRAF5 plays a distinct role in osteoclast differentiation.

A recent publication supports the involvement of $TNFR_I$ but not $TNFR_{II}$ in enhancing both basal and soluble TNF-α-induced osteoclastogenesis in marrow cultures [56], suggesting that TNF-α can synergize with RANKL. Consequently, if differences exist between RANK- and $TNFR_I$-induced osteoclastogenesis, they may be due either to another element in the pathway (such as RIP) or to a dosage effect resulting from the activation of more than one TRAF. Regardless, the ability to fine-tune osteoclast differentiation and activation by $TNFR_I$ and the TRAF6-specific receptors for either IL-1 or LPS, which can each act to deliver a portion of the complete signal that is provided via activation of the RANK signal transduction pathway, may be an important mechanism for regulating osteoclast-mediated bone resorption.

Potential contributions of RANKL/ODF to bone erosions in rheumatoid arthritis

Given the critical role of RANKL/ODF in the regulation of osteoclastogenesis in physiologic bone remodeling, the potential role of interactions between RANKL/ODF and RANK in the generation of bone erosions in RA has been explored. We and others have shown that RANKL/ODF is expressed in cultured synovial fibroblasts from patients with RA [62,63]. This factor is also expressed in CD4+ and CD8+ T lymphocyte subsets in RA synovium [17,64] and in activated CD4+ lymphocytes derived from RA synovium [62]. A potential direct role for RA synovial fibroblasts and/or T lymphocytes in inducing osteoclast differentiation is suggested by recent studies in which coculture of either synovial fibroblasts or T lymphocytes with osteoclast precursors in the presence of cofactors resulted in the generation of multinucleated cells with the phenotypic features of osteoclasts [17,63,64].

Important evidence suggesting that RANKL/ODF plays a role in the pathogenesis of osteoclastic resorption in inflammatory arthritis comes from studies in the rat adjuvant-arthritis model [17]. Arthritic rats treated with OPG at disease onset had minimal loss of cortical and trabecular bone, whereas bone loss was severe in untreated control animals. A dramatic decrease in osteoclast numbers was also observed in the OPG-treated animals [17]. OPG treatment did not appear to decrease joint inflammation, suggesting that the prevention of focal bone destruction was related to a specific effect on osteoclast-mediated bone resorption. These findings are important with respect to treatment strategies that target RANKL–RANK signaling pathways to prevent focal bone destruction in RA. The use of OPG, which binds RANKL and prevents activation of the RANK signaling pathway, represents one therapeutic approach. Additional strategies could target signal transduction pathways shared by TNF-α, IL-1, and RANKL, with the goal of inhibiting more broadly both inflammatory cascades and osteoclast-mediated bone resorption.

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