Review

The molecular mechanism of osteoclastogenesis in rheumatoid arthritis

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Abstract

Bone-resorbing osteoclasts are formed from hemopoietic cells of the monocyte–macrophage lineage under the control of bone-forming osteoblasts. We have cloned an osteoblast-derived factor essential for osteoclastogenesis, the receptor activator of NF-κB ligand (RANKL). Synovial fibroblasts and activated T lymphocytes from patients with rheumatoid arthritis also express RANKL, which appears to trigger bone destruction in rheumatoid arthritis as well. Recent studies have shown that T lymphocytes produce cytokines other than RANKL such as IL-17, granulocyte–macrophage colony-stimulating factor and IFN-γ, which have powerful regulatory effects on osteoclastogenesis. The possible roles of RANKL and other cytokines produced by T lymphocytes in bone destruction are described.

Keywords: granulocyte–macrophage colony-stimulating factor, IFN-γ, IL-17, IL-18, RANKL

Introduction

Bone-resorbing osteoclasts originate from hemopoietic cells probably of the colony-forming-unit megakaryocyte (CFU-M)-derived monocyte–macrophage family. Osteoclasts are large multinucleated giant cells that express tartrate-resistant acid phosphatase (TRAP) activity and calcitonin receptors, and they have the ability to form resorption pits on bone and dentine slices. The characteristics of osteoclasts thus differ from those of macrophage polykaryons.

We have developed a mouse coculture system of hemopoietic cells and primary osteoblasts to investigate osteoclast formation *in vitro* [1–3]. In this coculture system, several systemic and local factors induced formation of TRAP-positive multinucleated cells, which satisfied most of the osteoclast criteria [4]. Subsequent experiments established that the target cells of osteotropic factors for inducing osteoclast formation in the coculture were osteoblasts/stromal cells but not osteoclast precursors. In the coculture system, cell-to-cell contact between osteoblastic cells and osteoclast progenitors was essential for inducing osteoclastogenesis.

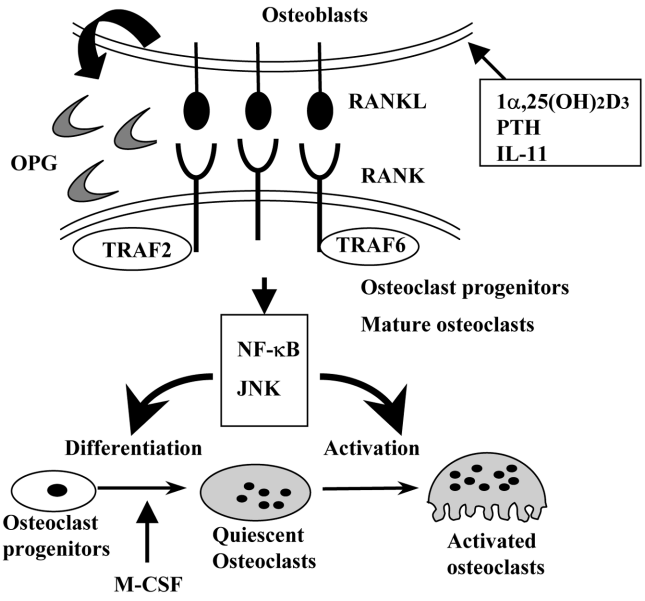
From these experimental findings, we proposed in 1992 that osteoblastic cells induce osteoclast differentiation factor as a membrane-associated factor in response to various osteotropic factors [4]. Six years later, we succeeded in the molecular cloning of osteoclast differentiation factor from a cDNA library of mouse stromal ST2 cells treated with bone-resorbing factors [5]. Osteoclast differentiation factor is a member of the tumor necrosis factor (TNF) ligand family, and was found to be identical to RANKL, TNF-related activation-induced cytokine and

CFU-M = colony-forming-unit megakaryocyte; COX-2 = cyclooxygenase; ELISA = enzyme-linked immunosorbent assay; GM-CSF = granulocyte–macrophage colony-stimulating factor; HuPBL-NOD/SCID = human peripheral blood lymphocyte-nonobese diabetic/severe combined immunodeficiency; IFN = interferon; IL = interleukin; MAP = mitogen-activated protein; M-CSF = macrophage colony-stimulating factor; NF = nuclear factor; OA = osteoarthritis; OPG = osteoprotegerin; PBMC = peripheral blood mononuclear cell; PCR = polymerase chain reaction; PGE_2 = prostaglandin E_2; RA = rheumatoid arthritis; RANK = receptor for RANKL; RANKL = receptor activator of NF-κB ligand; sIL-6R = soluble IL-6 receptors; TNF = tumor necrosis factor; TNFR1 = TNF receptor type 1 (p55); TNFR2 = TNF receptor type 2 (p75); TRAF = TNF receptor-associated factor; TRAP = tartrate-resistant acid phosphatase.

osteoprotegerin (OPG) ligand, which were independently identified by three other research groups [5–9]. The *ad hoc* Committee of the American Society of Bone and Mineral Research has recommended using RANKL as the standardized name [10].

RANKL induced osteoclast differentiation from mouse hemopoietic cells and human peripheral blood mononuclear cells (PBMCs) in the presence of macrophage colony-stimulating factor (M-CSF) [5,8,11,12]. RANK, a receptor for RANKL, is the sole signaling receptor for RANKL in inducing development and activation of osteoclasts [9] (Fig. 1). OPG, which is produced by a variety of cells including osteoblasts, is a soluble decoy receptor for RANKL. OPG inhibits osteoclastogenesis to compete against RANK [9]. The present review article describes the possible roles of members of the TNF receptor and ligand superfamily in osteoclastic bone resorption, especially in rheumatoid arthritis (RA).

Figure 1



A schematic representation of osteoclast differentiation supported by osteoblasts/stromal cells. RANKL, which is induced by bone resorbing factors such as 1-α,25$(OH)_2D_3$, parathyroid hormone (PTH) and IL-11 on the plasma membrane of osteoblasts/stromal cells, binds its receptor RANK present in osteoclast progenitors and mature osteoclasts. Osteoprotegerin (OPG), a decoy receptor for RANKL, strongly and competitively inhibits the RANKL–RANK interaction. The RANK signaling is transduced via TNF receptor-associated factor 2 (TRAF2) and TNF receptor-associated factor 6 (TRAF6), leading to the activation of NF-κB and Jun kinase (JNK), which in turn stimulates differentiation and activation of osteoclasts. M-CSF, macrophage colony-stimulating factor.

Possible roles of TNF receptor and ligand superfamily members in osteoclastic bone resorption in RA

RA is a chronic inflammatory disease characterized by the destruction of articular cartilage and bone in a chronic phase. Although histologic analyses of periarticular trabecular bone have demonstrated that osteoclastic bone resorption is greatly stimulated in RA patients, the mechanism of the joint destruction in RA patients remains to be elucidated.

The levels of monocyte/macrophage-derived cytokines such as IL-1 and IL-6, together with soluble IL-6 receptors (sIL-6R), are significantly elevated in the synovial fluids of patients with RA compared with those patients with osteoarthritis (OA) [13]. These cytokines may play important roles not only in immune responses and in development of inflammation, but also in joint destruction.

The role of T cells in the pathogenesis of RA at a chronic stage, however, has not yet been determined, because T-cell-derived cytokines such as IL-2 and IFN-γ are undetectable in the synovial tissues and fluids [14]. We recently reported that T cells indirectly regulate osteoclastogenesis via IL-17 and IL-18. IL-18 inhibits osteoclast formation by inducing granulocyte–macrophage colony-stimulating factor (GM-CSF) in T cells [15,16]. In contrast, IL-17 first acts on osteoblastic cells, then stimulates cyclooxgenase (COX)-2-dependent prostaglandin E_2 (PGE_2) synthesis, and subsequently induces RANKL gene expression, which in turn induces differentiation of osteoclast progenitors into mature osteoclasts [17].

It has been reported that RANKL is expressed in activated T cells as well as in osteoblastic cells [6,7,18]. These activated T cells are in fact capable of triggering osteoclastogenesis directly through RANKL [18–20]. Kong *et al*. [8] found that systemic activation of T cells *in vivo* leads to a RANKL-mediated increase in osteoclastogenesis and bone loss. In a T-cell-dependent model of rat adjuvant arthritis characterized by severe joint destruction, OPG treatment prevented bone destruction but not inflammation [18]. In addition, we demonstrated that the level of the soluble form of RANKL is elevated, while the level of OPG is decreased in synovial fluids from RA patients [20]. It is thus possible to postulate that T cells directly and indirectly stimulate osteoclastogenesis. Takayanagi *et al*. [21] recently reported that T-cell production of IFN-γ strongly suppresses osteoclastogenesis by disrupting the RANKL–RANK signaling pathway. They showed that there is a crosstalk between the TNF and IFN families of cytokines in bone resorption.

A potential role of IL-17 in joint destruction of RA patients

We previously reported that IL-6 alone did not induce osteoclast formation, but sIL-6R together with IL-6 markedly stimulated osteoclast formation in a mouse

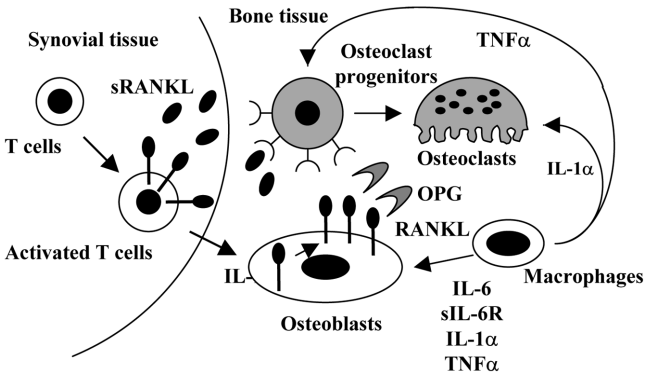
coculture system [22,23]. We also examined whether sIL-6R and IL-6 are involved in joint destruction in RA patients [13]. The number of osteoclast-like multinucleated cells found in the synovial tissues derived from the knee joint was greater in RA patients than in OA patients. These multinucleated cells from RA synovial tissues expressed the osteoclast-specific phenotypes such as TRAP, carbonic anhydrase II, vacuolar type proton-ATPase and vitronectin receptors at similar levels to those from a human giant cell tumor of bone. The concentrations of both IL-6 and sIL-6R were significantly higher in the synovial fluids of patients with RA than in those of patients with OA. The concentrations of IL-6 and sIL-6R were correlated well with the roentgenologic grades of joint destruction [13]. These results suggest that IL-6 in RA synovial fluids is responsible, at least in part, for joint destruction in the presence of sIL-6R through osteoclastogenesis (Fig. 2).

IL-17 is a newly discovered cytokine that is secreted by activated memory $CD4^+$ T cells and modulates an early stage of immune responses [24]. Rouvier *et al*. [25] cloned cytotoxic T-lymphocyte-associated antigen 8 (rat IL-17) from a T-cell subtraction library. Mouse IL-17 was subsequently cloned from a thymus-derived, activated T-cell cDNA library [26]. Fossiez *et al*. [27] reported that IL-17 stimulated epithelial cells, endothelial cells and fibroblastic stromal cells to secrete several cytokines, including IL-6, IL-8, granuloctye colony-stimulating factor and PGE_2. In addition, IL-17 greatly promoted the proliferation of $CD34^+$ hemopoietic progenitors in cocultures with synovial fibroblastic cells collected from RA patients [27].

We examined potential roles of IL-17 in osteoclastogenesis using a mouse coculture system. IL-17 greatly stimulated osteoclast formation via a cell-to-cell interaction between osteoclast progenitors and osteoblasts [17]. IL-17 increased PGE_2 synthesis in cultures of osteoblasts. In addition, IL-17 induced the expression of RANKL mRNA in osteoblasts. Like OPG, NS398 (a selective inhibitor of COX-2) completely inhibited IL-17-induced osteoclast differentiation in the cocultures.

IL-17 levels were significantly higher in the synovial fluids of RA patients compared with those of OA patients. Anti-IL-17 antibody significantly inhibited osteoclast formation induced by conditioned media of the cultures of RA synovial tissues in cocultures. Immunostaining of the synovial tissues from RA patients demonstrated that IL-17-positive cells were detected in a subset of $CD4^+$, $CD45RO^+$ T cells in the specimens [17]. These findings suggest that IL-17 first acts on osteoblasts, which stimulates COX-2-dependent PGE_2 synthesis, and it then induces RANKL gene expression, which in turn induces differentiation of osteoclast progenitors into mature osteoclasts. It is probable that IL-17 is a crucial cytokine for osteoclastic bone resorption in RA patients (Fig. 2).

Figure 2



A possible mechanism of osteoclast formation by activated T cells in rheumatoid arthritis. Activated T cells present in the synovial tissues also produce membrane-associated RANKL, some of which are cleaved enzymatically from the plasma membrane, resulting in soluble RANKL (sRANKL). Activated T cells also produce IL-17, which induces RANKL via prostaglandin E_2 synthesis in osteoblasts. IL-6 together with soluble IL-6 receptors (sIL-6R), IL-1-α and TNF-α derived from macrophages induce RANKL in osteoblasts. In addition, TNF-α directly acts on osteoclast progenitors, which then differentiate into osteoclasts by a mechanism independent of the RANKL–RANK interaction. IL-1 also induces osteoclast activation directly. OPG, osteoprotegerin.

Chabaud and co-workers [28,29] examined the contribution of soluble factors in the interaction between T cells and synoviocytes in RA patients. IL-17 induced production of IL-6 and leukemia inhibitory factor in synovial fibroblasts [28]. IL-17 increased bone resorption and decreased bone formation in human RA bone explants [29]. Chabaud *et al*. also reported that IL-17 was spontaneously produced in organ cultures of synovial tissues derived from RA patients.

Addition of anti-inflammatory cytokines IL-4 and IL-13 completely inhibited the production of IL-17 in synovial tissues [30]. Lubberts *et al*. [31] recently reported the IL-4 gene therapy for collagen-induced arthritis in mice, using a gene transfer with an IL-4-expressing adenoviral vector. Local treatment with IL-4 greatly prevented joint damage and bone erosion, although severe inflammation remained unchanged. The protective effect of IL-4 was associated with the decreased formation of osteoclasts and the downregulation of IL-17 mRNA and RANKL protein expression [31].

Jovanovic *et al*. [32] reported that IL-17 induced production of matrix metalloproteinase 9 in human monocyte/macrophages through PGE_2 synthesis. This stimulation was involved in both phosphorylation of p38 mitogen-activated protein (MAP) kinase and in NF-κB activation [32]. They also found that IL-17 stimulated the production and expression of inflammatory cytokines such as IL-1β, IL-6, and TNF-β by human macrophages [33]. Ziolkowska *et al*. [34] reported that high concentrations of IL-17 were

strongly correlated with those of IL-15 in synovial fluids of RA patients. IL-15 stimulates IL-17 production by human PBMCs in primary cultures [34]. These results together with our recent findings suggest that IL-17 plays an important role in the joint destruction of RA patients.

Osteoclastogenesis by activated T cells in RA

Kong *et al*. [8] reported that RANKL knockout (–/–) mice showed severe osteopetrosis with total occlusion of the bone marrow space within endosteal bone. RANKL (–/–) mice lack osteoclasts but have normal osteoclast progenitors that can differentiate into functionally active osteoclasts when cocultured with wild-type osteoblasts. In addition, RANKL (–/–) mice exhibited defects in early differentiation of T cells and B cells, and they lacked all lymph nodes but had normal splenic structures and Peyer's patches [8]. These results suggest that RANKL is not only a prerequisite for osteoclast development, but that it also plays an important role in early differentiation of T cells and B cells.

Several reports have demonstrated that RANKL is detected in the synovial fibroblasts and activated T lymphocytes derived from RA patients [18,20,35–37]. Horwood *et al*. [19] reported that human PBMC-derived T cells activated by concanavalin A expressed RANKL, and that these cells supported osteoclast formation in cocultures with murine hemopoietic cells. Romas *et al*. [38] found that RANKL mRNA was highly expressed by the synovial cell infiltrate in arthritic joints, as well as by osteoclasts at the sites of bone erosion in collagen-induced arthritis. It was recently reported that the degree of bone erosion in RANKL (–/–) mice was greatly reduced in a serum transfer model of arthritis, when compared with the control mice [39].

To elucidate the direct effect of human T cells in inducing osteoclastogenesis in RA, we conducted coculture experiments of activated human T cells and human adherent PBMCs [20]. When PBMCs were cultured in the presence of M-CSF for 3 days and further cocultured for 7 days with activated $CD3^+$ T cells, vitronectin receptor (CD51)-positive osteoclasts were formed even in the absence of exogenous RANKL. Osteoclast formation induced by activated T cells was completely inhibited by adding OPG.

Using an ELISA system, we measured the level of a soluble form of RANKL in the synovial fluids. Concentrations of soluble RANKL in the synovial fluids were significantly higher in patients with RA than in patients with other arthropathies including OA, gout, and trauma. In contrast, a decreased concentration of OPG was detected in the synovial fluids from RA patients. The ratio of the concentration of soluble RANKL to that of OPG was significantly higher in the synovial fluids of RA patients than in those of patients with OA or gout [20]. These results suggest that excess production of RANKL by activated T lymphocytes may contribute to the pathogenesis of bone destruction in these patients (Fig. 2). Regulation of RANKL and/or OPG expression in RA patients will provide a clue for the strategy of the development of new treatment for inhibiting of bone destruction in this disease.

In a T-cell-dependent model of rat adjuvant arthritis characterized by severe joint inflammation and bone and cartilage destruction, OPG treatment at the onset of the disease prevented bone and cartilage destruction but not inflammation [18]. Teng *et al*. [40] also reported that $CD4^+$ T-cell-mediated immunity is involved in the modulation of periodontal bone destruction in HuPBL-NOD/SCID mice after oral inoculation of *Actinobacillus actinomycetemcomitans*, a well-known Gram-negative anaerobic microorganism that causes human periodontitis. OPG treatment significantly reduced the number of osteoclasts at the sites of local periodontal infection.

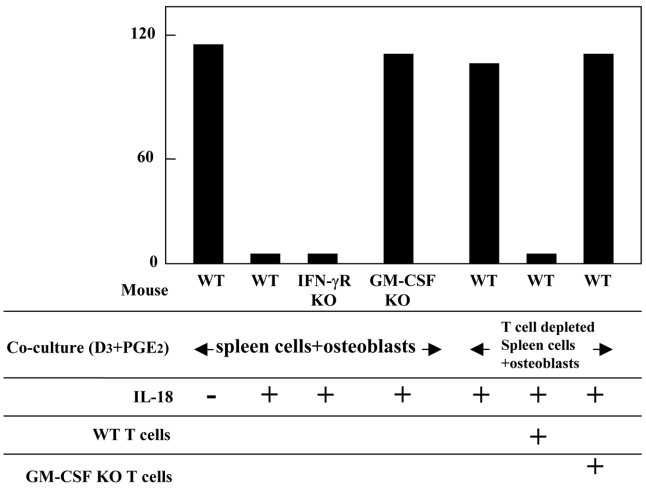
Juji *et al*. [41] recently reported a simple and effective method of active immunization against self RANKL as a potential treatment of bone diseases. Immunization with RANKL vaccines almost completely prevented the bone destruction in RA model mice (SKG mice). These results suggest that a therapeutic vaccine approach targeting RANKL may be useful for inhibiting bone destruction in a variety of pathological bone diseases.

Inhibitory cytokines produced by T cells on osteoclast differentiation

We previously reported that bone-marrow-derived stromal cell lines, MC3T3-G2/PA6 and ST2, had the capacity to support osteoclast formation in cocultures with hemopoietic cells [2,3]. Chambers and co-workers established several bone-marrow-derived stromal cell lines from a transgenic mouse, in which the IFN-inducible major mouse histocompatibility complex H-2Kb promoter drives the temperature-sensitive immortalizing gene of simian virus 40 [42,43]. These cell lines differed in their osteoclast-inductive activity in cocultures with hematopoietic cells.

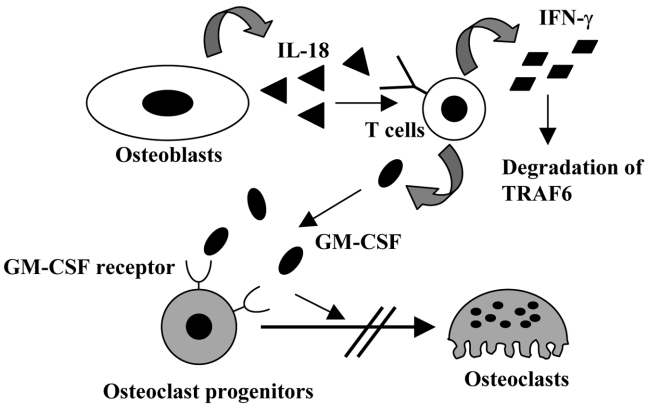
To identify genes in osteoblasts/stromal cells that are involved in the process of osteoclastogenesis, we used differential display of PCR to compare mRNA populations between osteoclast-inductive and osteoclast-noninductive cell lines [15]. Using this approach, we identified IL-18 (IFN-γ-inducing factor) as a product of osteoblastic stromal cells. IL-18 has been reported to induce production of IFN-γ and GM-CSF in T cells, both of which exhibit a potent inhibitory activity of osteoclastogenesis, at least *in vitro* [44]. IL-18 strongly inhibited osteoclast formation induced by bone-resorbing factors in cocultures. IL-18

Figure 3



Effects of IL-18 on osteoclast formation. Mouse spleen cells and osteoblasts from wild-type mice (WT), IFN-γ receptor type II-knockout mice (IFNγR KO) or granulocyte–macrophage colony-stimulating factor-knockout mice (GM-CSF KO) were cocultured with 1-α,25(OH)$_2$D$_3$ and prostaglandin E$_2$ (PGE$_2$) in the presence or absence of IL-18. In some cocultures, T-cell-depleted spleen cells and osteoblasts were cocultured in the presence and absence of WT T cells or GM-CSF KO T cells. In each coculture, numbers of tartrate-resistant acid phosphatase-positive osteoclasts formed were scored.

Figure 4



A proposed mechanism of the inhibitory action of IL-18 on osteoclast differentiation. IL-18 secreted from osteoblasts acts on T lymphocytes, which generate granulocyte–macrophage colony-stimulating factor (GM-CSF) and IFN-γ. Both GM-CSF and IFN-γ are potent inhibitors of osteoclast formation, at least *in vitro*. When GM-CSF binds its receptor, GM-CSFR (present in osteoclast progenitors), osteoclast formation is completely inhibited. In contrast, the target molecule of IFN-γ is TNF receptor-associated factor 6 (TRAF6). The degradation of TRAF6 by IFN-γ leads to the inhibition of osteoclastogenesis. The inhibitory action of IL-18 on osteoclast differentiation occurs via GM-CSF, but not via IFN-γ.

was found to inhibit osteoclast formation in cocultures with osteoblasts and spleen cells from IFN-γ receptor type II-deficient mice, similarly to those with wild-type osteoblasts and spleen cells In contrast, IL-18 was unable to inhibit osteoclast formation in cocultures of osteoblasts and spleen cells from GM-CSF-deficient mice (Fig. 3).

Since T cells comprise a large proportion of the spleen cell population, the role of T cells in osteoclastogenesis was examined. T cells were removed from spleen cell preparations using a monoclonal antibody against Thy 1.2 membrane antigen, which was predominantly expressed on T lymphocytes. The complete absence of T cells abolished the action of IL-18 on osteoclast formation in cocultures of osteoblasts and spleen cells from wild-type mice (Fig. 3). Addition of wild-type T cells but not GM-CSF-deficient T cells to the coculture restored the inhibition by IL-18 of osteoclastogenesis (Fig. 3). These results suggest that IL-18 inhibits osteoclast formation by making T cells promote the release of GM-CSF, which then acts on osteoclast precursors to limit osteoclast differentiation [15,16] (Fig. 4).

Horwood *et al*. [45] found that, like IL-18, IL-12 strongly inhibited osteoclast formation in cocultures, as well as in spleen cell cultures treated with M-CSF and RANKL. An unknown inhibitory molecule was found to be secreted from T cells in response to IL-12 and IL-18. Transwell experiments in which T cells were separated from hemopoietic cells suggested that the inhibitory molecule was a secreted factor, but not a membrane-associated factor. Although a number of cytokines (IL-4, IL-10, IL-13, GM-CSF and IFN-γ) expressed by T cells have the capacity to inhibit osteoclast formation, the present inhibitory factor has not been identified. IL-12 and IL-18 are detected in the RA synovial membrane [46]. It was also reported that IL-18 stimulated expression of OPG mRNA in osteoblasts and bone marrow stromal cells [47]. IL-12 and IL-18 may therefore protect the joint destruction via osteoclast-mediated erosion. IL-18 is effective in inhibiting bone destruction in murine models of breast cancer metastasis in bone [48]. These results suggest that IL-12 and/or IL-18 therapy may be useful for reducing pathological bone loss.

Takayanagi *et al*. [21] demonstrated that activated T cells are capable of inhibiting osteoclastogenesis through IFN-γ production, which interferes with the RANKL–RANK signaling pathway. In that study, osteoclast formation was strongly inhibited in the coculture of activated T cells and bone marrow cells in the presence of RANKL and M-CSF [21]. When activated T cells were cocultured with bone marrow cells derived from IFN-γ receptor knockout mice in the presence of RANKL and M-CSF, the inhibitory effect of activated T cells was completely canceled.

The expression of TNF receptor-associated factor (TRAF)6 was markedly inhibited by IFN-γ in osteoclast progenitors stimulated by RANKL and M-CSF, indicating that TRAF6 is a target molecule of IFN-γ. IFN-γ appears to inhibit osteoclastogenesis by decomposing TRAF6. In fact, TRAF6-deficient mice exhibited severe osteopetrosis [49,50]. It was also reported that IFN-γ receptor knockout mice developed collagen-induced arthritis more readily than wild-type mice [51]. These results suggest that TRAF6 is the critical target molecule in the IFN-γ-mediated suppression of osteoclast formation, and that the balance between RANKL and IFN-γ action may regulate osteoclastogenesis (Fig. 4).

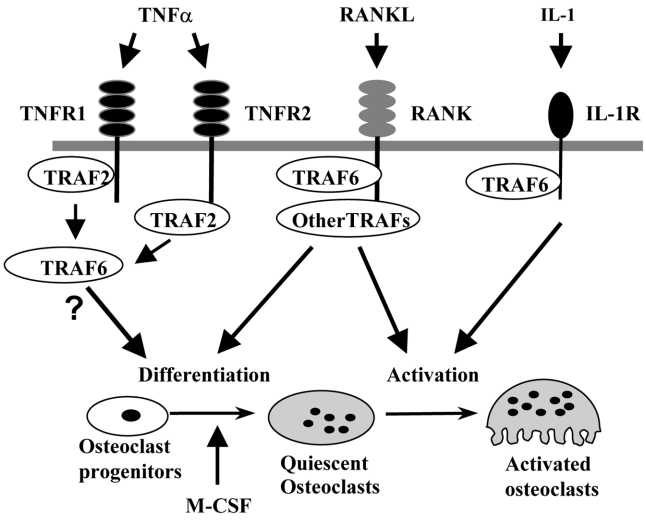
Possible roles of TNF-α in osteoclast differentiation

We have reported that TNF-α induced osteoclast formation via a mechanism independent of the RANKL–RANK signaling pathway [52] (Fig. 5). When mouse bone marrow cells were cultured with M-CSF for 3 days and nonadherent cells removed, adherent cells of uniform size and shape remained on the culture dish. The M-CSF-dependent bone marrow macrophage preparation contained no appreciable number of alkaline phosphatase-positive osteoblastic cells. When M-CSF-dependent bone marrow macrophages were further cultured for 3 days with several bone-resorbing cytokines, mouse TNF-α as well as RANKL induced osteoclast formation in the presence of M-CSF.

IL-1-α failed to induce osteoclast formation in macrophage cultures even in the presence of M-CSF. These osteoclast progenitors expressed not only RANK and c-Fms (M-CSF receptor), but also TNF receptor type 1 (TNFR1, p55) and TNF receptor type 2 (TNFR2, p75). Osteoclast formation induced by RANKL was completely inhibited by adding OPG, but osteoclastogenesis induced by TNF-α was not. Adding antibodies against TNFR1 and TNFR2 blocked osteoclast formation induced by TNF-α but not by RANKL. Bone marrow macrophages prepared from TNFR1 knockout mice differentiated into osteoclasts in response to RANKL, but they failed to differentiate into osteoclasts in response to TNF-α. Similarly, TNFR2 knockout mouse-derived bone marrow macrophages differentiated into osteoclasts in response to RANKL, but osteoclast differentiation induced by TNF-α was markedly decreased in TNFR2 knockout mouse-derived macrophage cultures [52].

These results suggest that TNF-α stimulates osteoclast formation via a mechanism independent of the RANKL pathway (Fig. 5). It was also shown that TNF-α as well as RANKL stimulated differentiation of RAW 264.7 cells into osteoclasts [53,54]. RANK-mediated signals for osteoclastogenesis are transduced via either TRAF6 or TRAF2, whereas TNFR1-mediated and TNFR2-mediated signals are transduced via TRAF2. TRAF-2-mediated signals may play important roles in osteoclast differentiation induced by TNF-α. Using TRAF6-deficient mice, Kaji *et al.* [55] recently found that TRAF6 is also involved in TNF-α-induced osteoclastogenesis (Fig. 5). Further studies are necessary to determine the relationship between TRAF2 and TRAF6 in TNF-α-induced osteoclastogenesis.

Figure 5

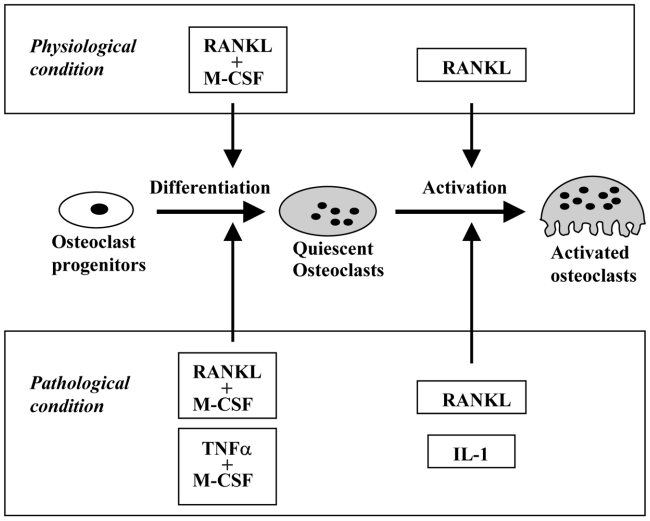


Signal transduction of TNF-α, RANKL and IL-1 in osteoclast differentiation and activation. TNF-α binds TNF receptor type 1 (TNFR1) and TNF receptor type 2 (TNFR2), RANKL binds RANK, and IL-1 binds IL-1 receptor (IL-1R). Both TNFR1 and TNFR2 bind TNF receptor-associated factor 2 (TRAF2), whereas IL-1R binds TNF receptor-associated factor 6 (TRAF6). RANK binds not only TRAF6, but also TRAF2 and other TNF receptor-associated factors (TRAFs). M-CSF, macrophage colony-stimulating factor.

To examine, whether TNF-α induces not only osteoclast differentiation, but also osteoclast activation, macrophages were cultured on dentine slices in the presence of TNF-α, M-CSF, and OPG [52]. Some cultures were also treated with IL-1-α. After culture for 6 days, similar numbers of osteoclasts were formed on dentine slices irrespective of the presence or absence of IL-1-α. However, no resorption pits were detected in macrophage cultures treated with TNF-α and M-CSF. Resorption pits on dentine slices were observed only in the presence of TNF-α and M-CSF together with IL-1-α.

These results suggest that TNF-α stimulates differentiation, but not activation, of osteoclasts. In contrast, IL-1-α does not induce differentiation of osteoclasts in macrophage cultures that do not contain osteoblasts/stromal cells, but it does stimulate pit-forming activity of the osteoclasts formed [52,56] (Fig. 5). Since IL-1R binds TRAF6 but not TRAF2, these results indicate that TRAF6 is a prerequisite for osteoclast activation.

Figure 6



Involvement of TNF ligand family members in physiological and pathological bone resorption. RANKL appears to play a major role in physiological bone resorption. In contrast, both RANKL-dependent and RANKL-independent pathways appear to be involved in pathological bone resorption. At present, the contribution ratio of the RANKL-dependent and RANKL-independent pathways to the pathological bone resorption is not known. M-CSF, macrophage colony-stimulating factor.

Pacifici and co-workers [57,58] recently demonstrated that estrogen deficiency induces bone loss by enhancing TNF-α production by T cells. Ovariectomy failed to induce bone loss in T-cell-deficient athymic nude (nu/nu) mice as well as in TNFR1 knockout mice. They also found that ovariectomy increased the number of TNF-producing T cells in the bone marrow of normal mice without altering the TNF production per T cell [58]. These results suggest that T-cell-produced TNF and its interaction with TNFR1 play a key role in bone loss induced by estrogen deficiency.

Conclusion

Under physiological conditions, osteoclast formation requires cell-to-cell contact between hemopoietic cells (osteoclast progenitors) and osteoblastic cells, in which osteoblastic cells generate RANKL as a membrane-bound factor in response to several bone resorbing factors (Fig. 6). In contrast, like in RA, T cells appear to secrete a soluble form of RANKL in pathological bone resorption that acts directly on osteoclast progenitors without cell-to-cell contact. Furthermore, TNF-α directly stimulates osteoclast differentiation via a mechanism independent of the RANKL–RANK interaction. IL-1-α induces osteoclast activation via its own receptors (Fig. 6).

Takeuchi *et al*. [59] recently established a coculture system with nurse-like cells obtained from synovial tissues of patients with RA. These cells promote survival of B cells and maintain the growth of myeloid cells. In addition, the nurse-like cells supported the generation of TRAP-positive osteoclasts from PBMCs [60]. These results suggest that, like bone-marrow-derived stromal cells, the nurse-like cells from RA synovial tissues also possess a novel ability to support osteoclast differentiation.

In conclusion, control of the production of RANKL, of OPG and of other T-cell-derived cytokines in RA patients will provide a clue for strategies of the development of new treatment for inhibiting bone destruction in this disease.

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