Journal of Cellular Biochemistry 97:226–232 (2006)

PROSPECTS

Anti-RANKL Therapy for Inflammatory Bone Disorders: Mechanisms and Potential Clinical Applications

Allen P. Anandarajah[1]* and Edward M. Schwarz[2]

[1]Department of Allergy, Immunology and Rheumatology, University of Rochester Medical Center, Rochester, New York 14642
[2]Department of Orthopedics, University of Rochester

Abstract Focal bone loss around inflamed joints in patients with autoimmune disease, such as rheumatoid arthritis, remains a serious clinical problem. The recent elucidation of the RANK/RANK-ligand/OPG pathway and its role as the final effector of osteoclastogenesis and bone resorption has brought a tremendous understanding of the pathophysiology of inflammatory bone loss, and has heightened expectation of a novel intervention. Here, we review the etiology of inflammatory bone loss, the RANK/RANK-ligand/OPG pathway, and the clinical development of anti-RANK-ligand therapy. J. Cell. Biochem. 97: 226–232, 2006. © 2005 Wiley-Liss, Inc.

Key words: RANKL; inflammatory bone disease; erosions

The chronic inflammatory arthritides are often associated with localized and generalized bone loss. Localized bone loss, manifests as periarticular osteopenia and subchondral bone erosions, and constitutes an important feature in diagnosing and directing treatment in rheumatoid arthritis (RA), psoriatic arthritis (PsA), and juvenile idiopathic arthritis. The advent of magnetic resonance imaging (MRI) has revealed that erosions occur early in these diseases. Furthermore, erosions tend to correlate with ongoing disease activity and joint destruction. Early intervention to alter the natural progression of joint destruction has been shown to substantially improve functional status [O'Dell, 2002]. These observations led the American College of Rheumatology [Arthritis and Rheum, 2002] to emphasize the importance of preventing early destruction of the periarticular bone and cartilage, in their most recent guidelines on the treatment of rheumatoid arthritis.

The revelation that erosions reflect ongoing disease activity of inflammatory arthritis, and are, therefore, associated with an unfavorable prognosis, increased the efforts to identify the underlying mechanisms behind this pathologic process. The success of the anti-tumor necrosis factor-α (anti-TNF-α) in retarding, and in some cases possibly reversing early focal bone loss, further increased the interest in developing therapies specifically aimed at inhibiting the progression of erosions in the various inflammatory arthritides. Recent advances in identifying and understanding the role of the osteoprotegerin /RANK/RANKL system in bone remodeling has helped create new paradigms aimed at limiting localized bone loss in the inflammatory arthritides.

PATHOGENESIS OF BONE EROSION

Bone remodeling is a continuous process that occurs in adult skeleton that permits the repair of micro damage while still regulating the mechanical strength and structure of bone. In this tightly coupled process, bone resorption is followed by new bone formation. Bone cells responsible for this process include specialized bone-resorbing cells (osteoclasts), and specialized bone-forming cells (osteoblasts). Physiologic bone remodeling is initiated by cells lining

*Correspondence to: Allen P. Anandarajah, Department of Allergy, Immunology and Rheumatology, University of Rochester Medical Center, 601 Elmwood Avenue, BOC 695, Rochester, NY 14642.
E-mail: Allen_Anandarajah@urmc.rochester.edu

Received 29 August 2005; Accepted 6 September 2005
DOI 10.1002/jcb.20674

the bone surface, which are of osteoblastic lineage. When activated, the osteoblastic cells release several cytokines and chemokines that in turn recruit and induce osteoclasts. Following the resorptive phase, orchestrated by the osteoclasts, the bone surface is repopulated by osteoblasts that deposit bone matrix, which eventually undergoes mineralization to form the new bone surface. In physiologic remodeling, the amount of bone removed is exactly matched by the amount of bone that is laid down. An imbalance between these two processes, which favors increased osteoclastic activity, results in focal articular bone loss and generalized osteoporosis.

Role of Osteoclasts in Inflammatory Bone Disease

Osteoclasts are multinucleated cells derived from the mononuclear cell precursors of the monocyte/macrophage lineage [Massey and Flanagan, 1999]. The pivotal role that osteoclasts play in the initiation and progression of bone erosions is well highlighted in the scientific literature. Animal studies reported that mice lacking osteoclasts are resistant to arthritis-induced bone erosion [Pettit et al., 2001; Redich et al., 2002]. Early evidence in humans came in the form of histopathological studies by Bromley et al. demonstrating the presence of mature osteoclasts at the bone pannus junction and subchondral bone marrow of rheumatoid patients [Bromley and Woolley, 1984a,b]. Since then, many other studies have provided ample evidence to implicate the crucial role of osteoclasts in the pathogenesis of erosions in patients with RA [Leisen et al., 1988; Chang et al., 1992; Fujikawa et al., 1996; Romas et al., 2000; Suzuki et al., 2001]. The important role of osteoclasts in bone resorption has been found to be true of other inflammatory arthritis as well. A recent study reported on the role of osteoclasts in the pathogenesis of bone erosions in juvenile rheumatoid arthritis [Gravallese et al., 1998]. Ritchlin et al. [2003] reported that patients with psoriatic arthritis, who have characteristically elevated serum TNF-α levels, have a significant increase in the osteoclast precursor cell pool within the peripheral blood mononuclear cells populations that in turn correlated with the extent of bone destruction. The demonstration that osteoclasts are largely responsible for focal bone erosions has increased efforts to understand the exact role played by a number of cytokines and inflammatory mediators that posses the capacity to induce the recruitment, differentiation, and activation of these osteoclast.

The Role of the OPG/RANK/RANKL System in Bone Resorption

OPG. The discovery of osteoprotegerin (OPG) in 1997 was a major advance in the field of bone biology [Simonet et al., 1997]. OPG is synthesized as a 401 amino-acid peptide, which is cleaved to give a 380 amino acid mature protein. In contrast to other members of the TNF receptor superfamily, OPG lacks transmembrane and cytoplasmic domains and is released in soluble form by stromal cells/osteoblasts. In addition to bone, *OPG* mRNA is expressed by a number of other tissues, including lung, heart, kidney, liver, stomach, intestine, thyroid gland, brain, and spinal cord. The major biologic action of OPG described to date, however, has been only that in bone.

OPG inhibits the differentiation and activity of osteoclasts [Simonet et al., 1997]. It does this by acting as a decoy receptor for the receptor activator of NF-κB ligand (RANKL) thereby downregulating the RANKL signaling through receptor activator of NF-κB (RANK). By neutralizing the biological effects of RANKL, it inhibits osteoclast differentiation, suppresses mature osteoclast activation, and induces their apoptosis. Mice with targeted ablation of OPG develop severe osteoporosis due to markedly increased osteoclast formation and subsequent bone resorption [Bucay et al., 1998; Mizuno et al., 1998]. Furthermore, administration of OPG to Lewis rats with experimentally induced arthritis completely protected the animals from bone loss while untreated animals had extensive local bone and cartilage destruction [Kong et al., 1999]. OPG, however, had no effect on the severity of inflammation.

RANK. RANK and its receptor RANKL had been characterized prior to the discovery of OPG [Anderson et al., 1997]. RANK is a transmembrane protein of 616 amino acids that belongs to the tumor necrosis factor receptor (TNFR) superfamily. It is expressed primarily on the cells of the monocytes/macrophage lineage including osteoclastic precursors, B and T cells, dendritic cells, and fibroblasts [Anderson et al., 1997; Hsu et al., 1999]. RANK is also present on the surface of mature osteoclasts.

RANK, is known to activate a cascade of intracellular signaling events leading to

osteoclast activation. RANK knockout mice have a complete block in osteoclast development resulting in osteopetrosis [Li et al., 2000]. Furthermore, osteoclast development could be restored by reintroduction of RANK into bone marrow progenitor cells. Interestingly, these RANK knockout mice also lacked peripheral lymph nodes and had defective B and T cell maturation. This was in keeping with the initial description of RANK and RANKL that recognized the importance of these cytokines in the regulation of the immune system [Anderson et al., 1997].

RANKL. The discovery of OPG was soon followed by the identification of its ligand, initially termed osteoprotegerin ligand (OPG-L), which turned out to be identical with two previously described members of the TNF superfamily, RANKL and TNF-related activation induced cytokine (TRANCE) [Lacey et al., 1998; Yasuda et al., 1998]. RANKL is a 317 amino acid peptide that also belongs to the TNF-super family. It exists in two forms, a soluble and a membrane bound form [Lacey et al., 1998]. RANKL mRNA is largely expressed by cells of the osteoblastic lineage and lymphoid tissue, most notably by the T lymphocytes. Agents that promote osteoclast development in vitro, such as vitamin D3, IL-1, PGE-2, and parathyroid hormone, increase expression of RANKL by osteoblasts.

The predominant roles of RANKL in bone physiology are the stimulation of osteoclastic activation and differentiation as well as the inhibition of osteoclast apoptosis [Fuller et al., 1998; Lacey et al., 1998; Malyankar et al., 2000]. RANKL in association with macrophage colony stimulating factor (M-CSF) is necessary and sufficient for the complete differentiation of osteoclastic precursors into mature osteoclasts [Lacey et al., 1998; Yasuda et al., 1998]. Consistent with these findings, RANKL knockout mice have a complete absence of osteoclasts and go on to develop severe osteopetrosis [Kong et al., 2000].

RANKL AND OTHER CYTOKINES IN INFLAMMATORY ARTHRITIS

RANKL, therefore, plays a pivotal role in controlling osteoclastogenesis. Inflamed synovial tissue produces a variety of other cytokines and hormones that can also influence osteoclastogenic activity. These factors include interleukin-1α (IL-1α), IL-1β, TNF-α, IL-6, M-CSF, IL-17, and parathyroid hormone related hormone related peptide (PTHrP) [Goldring and Gravallese, 2000; Gravallese et al., 2000]. Of these TNF-α and IL-1 play particularly important roles in the pathogenesis of bone erosions. Both of them upregulate RANKL expression in bone lining cells and marrow stromal cells. In addition, RANKL and TNF-α act in synergy to enhance osteoclast differentiation. Meanwhile IL-1 acts primarily to directly activate osteoclasts and delay osteoclast apoptosis [Suda et al., 1999; Teitelbaum, 2000; Romas et al., 2002]. Furthermore IL-1 and TNF-α impair bone formation by inducing osteoblast apoptosis [Tsuboi et al., 1999]. The role of these cytokines in inflammation and bone erosions provides further evidence to a link between immune system activation and bone resorption.

Once the function of OPG/RANK/RANKL in bone remodeling was perceived, it was hypothesized that RANKL may be of major pathophysiological importance in the bone and joint destruction observed in inflammatory arthritides such as RA. Studies by Goldring and Gravallese [2000] provided initial insights into the role of RANKL in the pathogenesis of focal bone erosions, in patients with rheumatoid arthritis. Other studies have provided compelling evidence that activated T cells from the RA synovium and synovial fibroblasts produce RANKL [Kong et al., 1999; Gravallese et al., 2000; Takayanagi et al., 2000]. Indeed it is now assumed that activated T-cells, which play a central role in the pathogenesis of RA, may contribute to the osteoclast-mediated bone resorption via RANKL expression [Horwood et al., 1999] [see Fig. 1]. Although the role of RANKL in other inflammatory arthritis is not yet known, Ritchlin et al., using immunohistiochemistry, have revealed the striking spatial regulation of RANK, RANKL, and OPG expression in the PsA joints [Ritchlin et al., 2003].

THERAPEUTIC PERSPECTIVES USING RANKL INHIBITION IN INFLAMMATORY ARTHRITIS

As stated earlier, an important aim of treating inflammatory arthritis is to limit the progression of bone erosion. This may be done by suppressing bone resorption and/or by increasing bone formation. Anti-cytokine therapy, in the form of anti-TNF-α and anti-IL-1 blockade, has provided evidence for its ability to retard bone loss, in randomized placebo con-

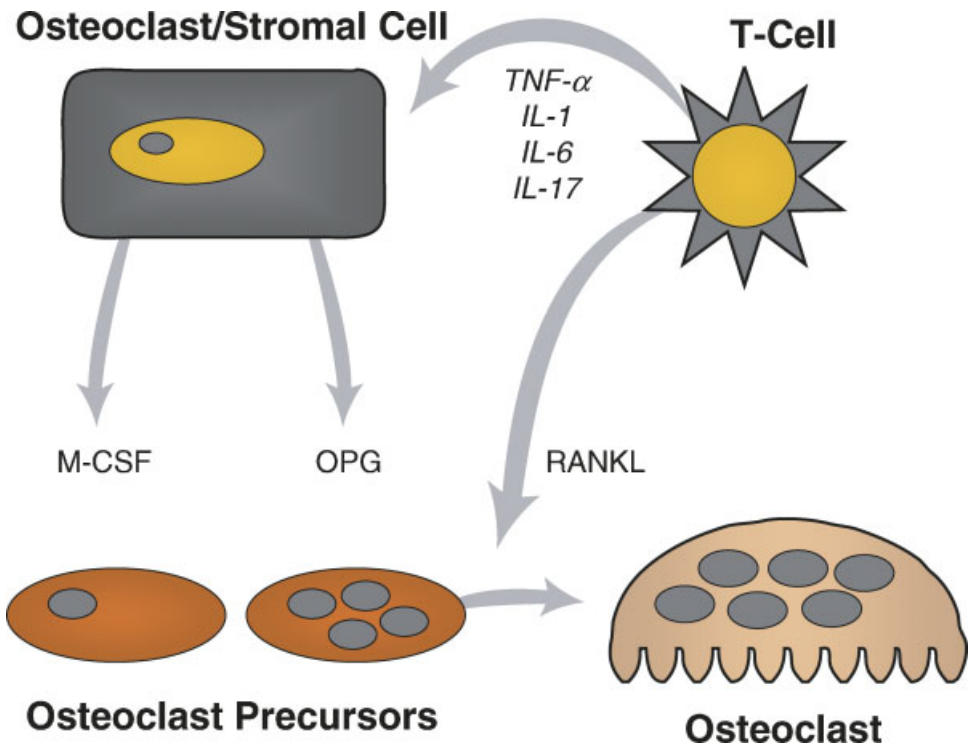

Fig. 1. The OPG/RANK/RANKL system and osteoclastogenesis. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

trolled clinical trials. [Maini et al., 1999; Weinblatt et al., 1999; Mease et al., 2004; Antoni et al., 2005]. The exact mechanism of how anti-TNF-α and anti-IL-1 therapies do so, however, is not yet well understood. Furthermore, there are still a number of patients with inflammatory arthritis who do not respond to these agents. This has led to research into finding alternative therapeutic strategies to control bone loss in the inflammatory arthritides.

The concept that cytokines and hormonal factors implicated in bone resorption, may act via a common final pathway involving RANKL, has led to new therapeutic approaches in several diseases characterized by excessive bone resorption. The modulation of RANKL to inhibit bone loss may be achieved by the suppression of endogenous RANKL expression, blockade of RANKL, interruption of RANKL binding, suppression of post-receptor signaling of RANK, or by enhancing overexpression of OPG.

Most strategies developed to date have aimed to block RANKL. The development of small molecules that mimic OPG action by targeting the RANKL/RANK signaling pathway provided proof-of-principle experiments that these agents may indeed prevent osteoclast-mediated bone loss [Cheng et al., 2004; Onyia et al., 2004]. OPG was thus an obvious first choice, used to combat inflammation-induced bone resorption. The anti-erosive effect of OPG had been demonstrated in rats especially when started early in disease [Campagnuolo et al., 2002]. A trial of OPG in postmenopausal women reported that a single injection of OPG-Fc resulted in sustained suppression of bone resorption, as measured by levels of urinary excretion of deoxypyridinoline levels [Bekker et al., 2001]. There, however, were some potential concerns for using the OPG molecule. One was the risk for generation of anti-OPG antibodies, especially with chronic use, leading to cross-reactivity with endogenous OPG, thereby neutralizing the activity of OPG. Another concern was, the possibility of OPG binding to TNF-related apoptosis-inducing ligand (TRAIL), a survival factor for tumor cells, resulting in an interference with the natural defense mechanism against tumorigenesis. Although two human studies with Fc-OPG constructs have reported good safety profile with the use of these agents [Bekker et al., 2001; Body et al., 2003], both were short-term studies that did not assess specific clinical end points.

The development of an antibody to RANKL has created a lot of excitement in the bone field.

The RANKL antibody specifically binds to human RANKL and does not appear to produce antibodies. AMG-162 (denosumab) is an investigational, fully human monoclonal antibody that specifically targets RANKL. Results from the Phase 2 trial of AMG-162 on bone mineral density in postmenopausal women were presented at the 2004 annual meetings of the American Society for Bone Mineral Research and the American College of Rheumatology [McClung et al., 2004]. A Phase 3 clinical trial of AMG-162 in postmenopausal women with osteoporosis was initiated in 2004. A Phase 2 study to assess efficacy, safety, and tolerability of AMG-62 in the treatment of RA is also being currently undertaken at multiple centers across the United States.

FUTURE PROSPECTS

The realization that the ratio of RANKL to OPG is also important in other metabolic bone diseases, along with recent evidence implicating the OPG/RANKL/ RANK system in vascular diseases has heightened speculation for the use of RANK blockade in a wide range of bone and vascular diseases. Evidence for its value in treating postmenopausal osteoporosis has already started emerging [Bekker et al., 2004; McClung et al., 2004]. Studies are also being conducted to assess the use of agents that block RANKL, for treatment of myeloma bone disease, osteolytic bone metastases, and humoral hypercalcemia of malignancy. Table I provides a list of diseases in which RANKL blockade may be of potential benefit.

A protective effect for OPG on the vascular system was suggested by studies showing that mice deficient in OPG developed arterial calcifications of the aorta and renal arteries [Min et al., 2000]. In human subjects, however, patients with coronary artery diseases have been reported to have higher OPG serum levels compared to healthy patients [Jono et al., 2002]. Furthermore, increased levels of OPG are associated with a three- to fourfold increased risk for cardiovascular mortality [Browner et al., 2001]. Thus the role of OPG in treating vascular diseases in humans is unclear at present.

Another potential use of RANKL and possibly OPG may be its use as serum markers for various bone diseases. Some studies have supported a role for measuring serum OPG levels as markers of disease activity in postmenopausal osteoporosis [Yano et al., 1999], patients with skeletal metastases due to prostatic cancer [Brown et al., 2001] and possibly myeloma patients [Seidel et al., 2001]. These studies, however, were small and are yet to be validated. A large prospective study did report lower serum RANKL levels as an independent predictor for non-traumatic fractures [Schett et al., 2004]. OPG and RANKL may, however, not be ideal markers as they are produced by many tissues in the body. Furthermore age, renal function, and vascular disease may influence their levels.

TABLE I. RANKL Mediated Diseases

Rheumatoid arthritis
Postmenopausal osteoporosis
Glucocorticoid induced osteoporosis
Periprosthetic osteolysis
Hyperparathyroidism
Paget's disease
Myeloma bone disease
Osteolytic bone metastases
Hypercalcemia of malignancy
Periodontal infections
Familial Paget's disease
Juvenile Paget's disease
Expansile skeletal hyperphosphatasia

CONCLUSION

Focal bone resorption in inflammatory arthritis is associated with increased morbidity. Bone loss is secondary to increased osteoclastic bone resorption brought on by the activation of the inflammatory immune response. The discovery that RANKL/RANK signaling is required for osteoclast differentiation and function in both physiologic and pathologic bone remodeling has transformed our understanding of metabolic bone diseases and suggested the potential for RANKL/RANKL signaling as a viable therapeutic target in the treatment of bone erosion in inflammatory arthritis. RANKL inhibition has provided a rational strategy to inhibit bone loss in the inflammatory arthritis and osteoporosis and potentially may be of value in treating other metabolic bone diseases.

REFERENCES

Anderson MS, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D, Gailbert L. 1997. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic cell function. Nature 390:175–179.

Antoni CE, Kavanaugh A, Kirkham B, Tutuncu Z, Burmester GR, Schneider U, Furst DE, Molitor J, Keystone E, Gladman D, Manger B, Wassenberg S,

Weier R, Wallace DJ, Weisman MH, Kalden JR, Smolen J. 2005. Sustained benefits of infliximab therapy for dermatologic and articular manifestations of psoriatic arthritis: Results from the infliximab multinational psoriatic arthritis controlled trial (IMPACT). Arthritis & Rheumatism 52(4):1227–1236.

Arthritis and Rheum. 2002. Guidelines for the management of rheumatoid arthritis 2002 update. Arthritis Rheum 46:328–346.

Bekker PJ, Holloway D, Nakanishi A, Arrighi HM, Else PT, Dunstan CR. 2001. Osteoprotegerin (OPG) has potent and sustained antiresorptive activity in postmenopausal women. J Bone Miner Res 16(2):348–360.

Bekker PJ, Holloway D, Rasmussen AS, Murphy R, Martin SW, Leese PT, Holmes GB, Dunstan CR, DePaoli AM. 2004. A single-dose placebo controlled study of AMG-162, a fully human monoclonal antibody to RANKL, in postmenopausal women. J Bone Miner Res 19(2):1059–1066.

Body JJ, Greipp P, Coleman RE, Facon T, Geurs f, Fermand J-P, Harousseau J-L, Lipton A, Mariette X, Williams CD, Nakanishi A, Holloway D, Martin SW, Dunstan CR, Bekker PJ. 2003. A phase I study of AMG-0007, a recombinant osteoprotegerin construct, in patients with multiple myeloma or breast carcinoma related bone metastases. Cancer 97(3 suppl):887–892.

Bromley M, Woolley DE. 1984. Chondroclasts and osteoclasts at subchondral sites of erosions in the rheumatoid joint. Arthritis Rheum 27:968–975.

Bromley M, Woolley DE. 1984. Histopathology of the rheumatoid lesion; identification of the cell types at sites of cartilage erosion. Arthritis Rheum 27:857–863.

Brown JM, Vessella RL, Kostenuik PJ, Dunstan CR, Lange PH, Corey E. 2001. Serum osteoprotegerin levels are increased in patients with advanced prostate cancer. Clin Cancer Res 7:2977–2983.

Browner WS, Lui LY, Cummings SR. 2001. Association of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures and mortality in elderly women. J Clin Endocrinol Metab 86:631–637.

Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley j, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WS. 1998. Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 12:1260–1268.

Campagnuolo G, Bolon B, Feige U. 2002. Kinetics of bone protection by a recombinant osteoprotegerin therapy in Lewis rats with adjuvant arthritis. Arthritis Rheum 46: 1926–1936.

Chang JS, Quinn JM, Demaziere A, Bulstrode CJ, Francis MJ, Duthie RB, Athanasou NA. 1992. Bone resorption by cells isolated from rheumatoid synovium. Ann Rheum Dis 51:1223–1229.

Cheng X, Kinosaki M, Takami M, Choi Y, Zhang H, Murali R. 2004. Disabling of RANK receptor complex by novel osteoprotegerin-like peptidomimetics restores bone loss in vivo in postmenopausal women. J Biol Chem 279: 8269–8277.

Fujikawa Y, Shingu M, Torisu T, Itonaga I, Masumi S. 1996. Bone resorption by tartrate-resistant acid phosphatase-positive multinuclear cells isolated from rheumatoid synovium. Br J Rheumatol 35:213–217.

Fuller K, Wong B, Fox S, Choi Y, Chambers TJ. 1998. TRANCE is necessary for osteoblast-mediated activation of bone resorption in osteoclasts. J Exp Med 188:997–1001.

Goldring SR, Gravallese EM. 2000. Mechanisms of bone loss in inflammatory arthritis; diagnosis and therapeutic implications. Arthritis Res 2:33–37.

Gravallese EM, Harada Y, Wang JT, Gorn AH, Thornhill TS, Goldring SR. 1998. Identification of cells types responsible for bone resorption in rheumatoid arthritis and juvenile rheumatoid arthritis. Am J Pathol 152:943–951.

Gravallese EM, Manning C, Tsay A, Naito A, Pan C, Amento E, Goldring SR. 2000. Synovial tissue in rheumatoid arthritis is a source of osteoclast differentiation factor. Arthritis Rheum 43:250–258.

Horwood NJ, Kartsogiannis V, Quinn JM, Romas E, Martin E, Martin TJ, Gillespie MT. 1999. Activated T lymphocytes support osteoclast formation *in vitro*. Biochem Biophy Res Commun 265:144–150.

Hsu H, Lacey DL, Dunstan CR, Solovyev I, Colombero A, Timms E, Tan HL, Elliott G, Kelley MJ, Sarosi I, Wang L, Xia XZ, Elliot R, Chiu L, Black T, Scully S, Capparelli C, Morony S, Shimamoto G, Bass MB, Boyle WJ. 1999. Tumor necrosis factor receptor family member RANK mediates osteoblast differentiation and activation induced by osteoprotegerin ligand. Proc Natl Acad Sci USA 96:3540–3545.

Jono S, Ikari Y, Shioi A, Mori K, Miki T, Hara K, Nishizawa Y. 2002. Serum osteoprotegerin levels are associated with the presence and severity of coronary artery disease. Circulation 106:1192–1994.

Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, Capparelli C, Li J, Elliott R, McCabe S, Wong T, Campagnuolo G, Moran E, Bogoch ER, Van G, Nguyen LT, Ohashi PS, Lacey DL, Fish E, Boyle WJ, Penninger JM. 1999. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 402:304–309.

Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A, Khog W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ, Penninger JM. 2000. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph node organogenesis. Nature 397:315–323.

Lacey DL, Timms E, Tan HL, Kelly MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhouh V, Senaldi G, Guo J, Delaney J, Boyle WJ. 1998. Osteoprotegerin (OPG) ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93:165–176.

Leisen JC, Duncan H, Riddle JM, Pitchford WC. 1988. The erosive front: A topographic study of the junction between the pannus and the subchondral plate in the macerated rheumatoid metacarpal head. J Rheumatol 15:17–22.

Li J, Sarosi I, Yan XQ, Morony S, Capparelli C, Tan HL, McCabe S, Elliott R, Sully S, Van G, Kaufman S, Juan SC, Sun Y, Tarpley J, Martin L, Christensen K, McCabe J, Kostenuik S, Hsu H, Fletcher F, Dunstan CR, Lacey DL, Boyle WJ. 2000. RANK is the intrinsic hematopoietic cell surface receptor that controls osteoclastogenesis and regulation of bone mass and calcium metabolism. Proc Natl Acad Sci USA 97:1566–1571.

Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, Smolen J, Emery P, Hamman G, Feldman M, Lipsky P. 1999. Infliximab (chimeric anti-tumor necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis concomitant methotrexate: A randomized phase III trial. ATTRACT Study Group. Lancet 354:1932–1939.

Malyankar UM, Scatena M, Suchland KL, Yun TJ, Clark EA, Giachelli CM. 2000. Osteoprotegerin is an α v β3-induced, NF-κB-dependent survival factor for endothelial cells. J Biol Chem 275:20959–20962.

Massey HM, Flanagan AM. 1999. Human osteoclasts derived from CD 14-positive monocytes. Br J Haematol 106:167–170.

McClung MR, Lewiecki EM, Bolognese MA, et al. 2004. AMG 162 increases bone mineral density (BMD) within 1 month in postmenopausal women with low BMD. J Bone Miner Res 19(Suppl1):S20; Abstract 1072.

Mease PJ, Kivitz AJ, Burch FX, Siegel EL, Cohen SB, Ory P, Salonen D, Rubenstein J, Sharp JT, Tsuji W. 2004. Etanercept treatment of psoriatic arthritis: Safety, efficacy, and effect on disease progression. Arthritis & Rheumatism 50(7):2264–2272.

Min H, Morony S, Sarosi I, Dunstan CR, Capparelli C, Scully S, Van G, Kaufman S, Kostenuik PJ, Lacey DL, Boyle WJ, Simonet WS. 2000. Osteoprotegerin reverses osteoporosis by inhibiting endosteal osteoclasts and prevents vascular calcifications by blocking a process resembling osteoclastogenesis. J Exp- Med 192:463–474.

Mizuno A, Amizuka N, Irie K, Murakami A, Fujise N, Kanno T, Sato Y, Nakagawa N, Yasuda H, Mochizuki S, Gomibuchi T, Yano K, Shima N, Washida N, Tsuda E, Morinaga T, Higashio K, Ozawa H. 1998. Severe osteoporosis in mice lacking osteoclastogenesis inhibitory factor/osteoprotegerin. Biochem Biophys Res Commun 247:610–615.

O'Dell J. 2002. Treating rheumatoid arthritis early: A window of opportunity. Arthritis Rheum 46:283–285.

Onyia JE, Galvin RJ, Ma YL, Halladay DL, Miles RR, Yang X, Fuson T, Cain RL, Zeng QQ, Chandrasekhar S, Emkey R, Xu Y, Thirunavukkarasu K, Bryant HU, Martin TJ. 2004. Novel and selective small molecule stimulators of osteoprotegerin expression inhibit bone resorption. J Pharmacol Exp Ther 309:369–379.

Pettit AR, Ji H, von Stechow D, Muller R, Goldring SR, Choi Y, Benoist C, Gravallese EM. 2001. TRANCE/RANKL knockout mice are protected from bone erosion in serum transfer model of arthritis. Am J Pathol 159: 1689–1699.

Redich K, Hayer S, Ricci R, David JP, Tohidast-Akrad M, Kollias G, Steiner G, Smolen JS, Wagner EF, Schett G. 2002. Osteoclasts are essential for TNF-alpha mediated joint destruction. J Clin Invest 110:1419–1427.

Ritchlin CT, Haas-Smith SA, Li P, Hicks DG, Schwarz EM. 2003. Mechanisms of TNF-α and RANKL-mediated osteoclastogenesis and bone resorption in psoriatic arthritis. J Clin Invest 222:821–831.

Romas E, Bakharevski O, Hards DK, Kartsogiannis V, Quinn JM, Ryan PF, Martin TJ, Gillespie MT. 2000. Expression of osteoclast differentiation factor at sites of bone erosion in collagen-induced arthritis. Arthritis Rheum 43:821–826.

Romas E, Gillespie MT, Martin TJ. 2002. Involvement of receptor activator of NFκB ligand and tumor necrosis factor-α in bone destruction in rheumatoid arthritis. Bone 30:340–346.

Schett G, Kiechi S, Redlich K, Oberhollenzer F, Weger S, Egger G, Mayr A, Jocher J, Xu Q, Pietschmann P, Teitelbaum S, Smolen J, Willeit J. 2004. Soluble RANKL and risk of non traumatic fracture. JAMA 291:1108–1113.

Seidel C, Hjertner O, Abildgaard N, Heickendorff L, Hiorth M, Westin J, Nielsen JL, Hjorth-Hansen H, Waage A, Sundan A, Borset M. 2001. Nordic Myeloma Study Group. Serum osteoprotegerin levels are reduced in patients with multiple myeloma with lytic bone disease. Blood 98:2269–2271.

Simonet WA, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derbt P, Lee R, Boyle WJ. 1997. Osteoprotegerin: A novel secreted protein involved in the regulation of bone density. Cell 89:309–319.

Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ. 1999. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev 20:345–357.

Suzuki Y, Tsutsumi Y, Nakagawa M, Suzuki H, Matsushita K, Beppu M, Aoki H, Ichikawa Y, Mizushima Y. 2001. Osteoclast-like cells in an in vitro model of bone destruction by rheumatoid synovium. Rheumatology (Oxford) 40:673–682.

Takayanagi H, Lizuka H, Juji T, Nakagawa T, Yamamoto A, Miyazaki T, Koshihara Y, Oda H, Nakamura K, Tanaka S. 2000. Involvement of receptor activator of nuclear factor kappa-B ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. Arthritis Rheum 43:259–269.

Teitelbaum SL. 2000. Bone resorption by osteoclasts. Science 289:1504–1508.

Tsuboi M, Kawakami A, Nakashima T, Matsuoka N, Urayama S, Kawabe Y, Fujiyama K, Kiriyama T, Aoyagi T, Maeda K, Eguchi K. 1999. Tumor necrosis factor-α and interleukin-1β increase the Fas-mediated apoptosis of human osteoblasts. J Lab Clin Med 134:222–231.

Weinblatt ME, Kremer JM, Bankhurst AS, Bulpitt KJ, Fleischmann RM, Fox RI, Jackson CG, Lange M, Burge DJ. 1999. A trial of etanercept, a recombinant tumor necrosis factor receptor: Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. N Engl J Med 340:253–259.

Yano K, Tsuda E, Washida N, Kobayashi F, Goto M, Harada A, Ikeda K, Higashio K, Yamada Y. 1999. Immunological characterization of circulating osteoprotegerin/osteoclastogenesis inhibitory factor. J Bone Miner Res 14:518–527.

Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. 1998. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 95:3597–3602.