Mod Rheumatol (2005) 15:232–240
DOI 10.1007/s10165-005-0412-z

REVIEW ARTICLE

David M. Findlay · David R. Haynes

Mechanisms of bone loss in rheumatoid arthritis

Received: May 9, 2005 / Accepted: May 30, 2005

Abstract Rheumatoid arthritis (RA) is an autoimmune inflammatory disease in which destruction of bone in the joints causes major morbidity. Recent research has shed light on the cell and molecular mechanisms that lead to this osteolysis, all due directly or indirectly to the chronic inflammation. The aspects of this research covered in this review include the alteration of cell proliferation and survival that results in growth of the RA synovium. This process depends upon an increase in angiogenesis and local blood flow, which is also a feature of increased bone turnover. In addition, the inflammatory environment increases expression of chemokines, which are involved in the recruitment of monocytic osteoclast precursors. Chronic inflammation also promotes an overall catabolic state, with increased osteoclast differentiation and resorptive activity, driven by disregulation of receptor activator of NF-κB ligand (RANKL) and the synergistic activity of inflammatory cytokines such as tumor necrosis factor-α and interleukin-1. Osteoclast survival is increased in this environment, but osteoblast differentiation and survival are decreased, with a consequent reduction in bone formation and a net loss of bone. Recognition of these processes and the factors involved will enable more effective and targeted treatments for RA.

Key words Bone resorption · Osteoclast · Osteolysis · Rheumatoid arthritis (RA)

D.M. Findlay (✉)
Department of Orthopaedics and Trauma, University of Adelaide, and Hanson Institute, Level 4, Bice Building, Royal Adelaide Hospital, North Terrace, Adelaide 5000, South Australia, Australia
Tel. +61-8-8222-5621; Fax +61-8-8232-3065
e-mail: david.findlay@adelaide.edu.au

D.R. Haynes
Department of Pathology, University of Adelaide, Adelaide, South Australia, Australia

Introduction

In rheumatoid arthritis (RA), inflammation of the synovial tissues can result in the destruction of the joint through the breakdown of bone and cartilage. Although secondary osteoporosis is often seen,[1] the bone erosion seen in RA is largely localized to the inflamed joints.[2,3] The systemic and localized bone loss is distinct from systemic hormonally regulated bone pathologies. Despite RA affecting approximately 2% of the world's population, we still have an incomplete understanding of the processes of this chronic systemic disease and, until recently, studies were largely confined to the inflammation that occurs in the soft tissues. However, recent advances in the understanding of bone metabolism have enabled investigation of the mechanisms involved in the bone and cartilage loss in this disease.

Physiological osteoclast formation

The bone remodeling unit (BMU)

In maturity, the integrity of bones is maintained by ongoing bone removal and replacement, a process termed bone remodeling.[4] Bone remodeling occurs in the "bone remodeling unit (BRU)" or "basic multicellular unit (BMU)," terms coined originally by Frost[5] to describe the structures in which bone removal by osteoclasts and its replacement by osteoblasts occurs in a highly coordinated sequence. The initiation or "activation" of BMUs requires signals that result in the recruitment of osteoclast precursors, and their differentiation and activation, to remove bone matrix.[6] Both targeted and nontargeted (stochastic) remodeling are thought to occur, although the relative extent of either is not known. However, there is evidence that targeted remodeling takes place in response to bone matrix damage and fatigue loading of bone.[7,8] The reason that this remodeling is targeted to a specific site is thought to be due to local loss of osteocyte viability.[9] It has been argued[10] that at the origin of each BMU a local "area code" is turned on that

provides the signals for preosteoclasts, circulating cells of the monocyte/macrophage lineage to exit the circulation at the appropriate site. The nature of such signals is not known, although osteocytic NO[11] and osteopontin[12] have been proposed as possible factors. A cascade of events can be proposed, such that signals elicited by osteocyte death, for example, might be further amplified by bone lining cells or vascular endothelial cells into signals that attract osteoclast precursors. Consistent with this, several cell-attractant molecules have been shown in vitro to attract osteoclast precursors. The chemokine SDF-1 has been reported to increase the recruitment of CXCR4-positive cells, including osteoclast progenitor cells, to regenerating bone.[13,14] Likewise, chemokines that bind the receptor CCR1 (macrophage inflammatory protein [MIP]-1α, regulated on activation of normal T cell expressed [RANTES], monocyte chemoattractant protein [MCP]-1, and MCP-3) have been implicated in the recruitment of osteoclast precursors and in their differentiation (depicted schematically in Fig. 1A).[15–17] In diseases such as RA, some of these processes appear to be overridden, while production of molecular factors that promote bone turnover are disregulated.

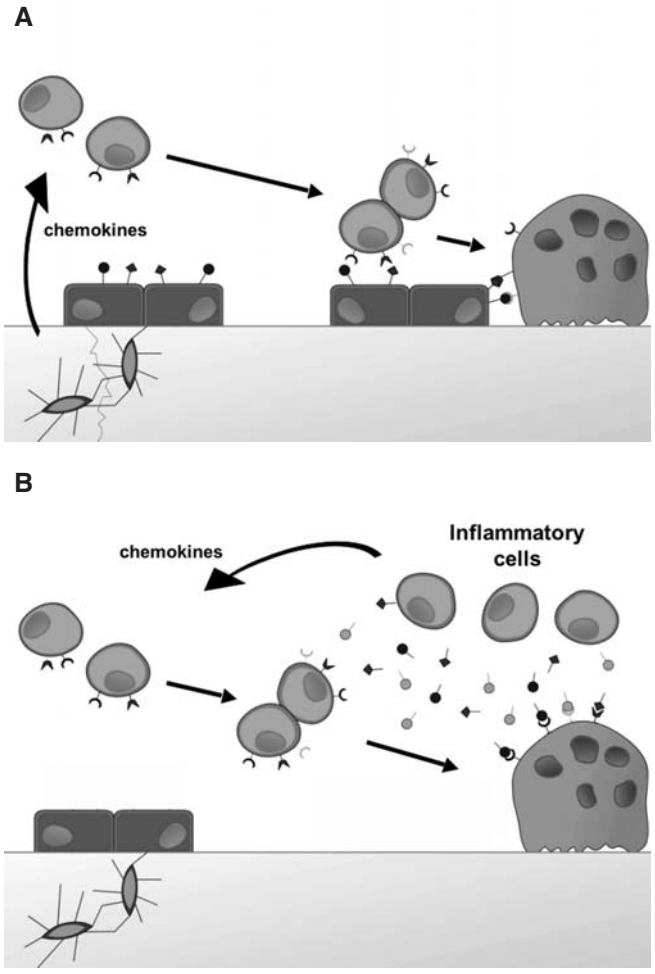

Fig. 1. **A** Proposed initiation of events in physiological bone resorption. Structural damage in the bone matrix results in osteocyte-initiated signals, which stimulate local release of cytokines and chemokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and macrophage inflammatory protein (MIP)-1α. These factors stimulate expression by osteoblasts and/or lining cells of receptor activator of NF-κB ligand (RANKL), while suppressing osteoprotegerin (OPG) production. In addition, chemokines such as regulated on activation of normal T cell expressed (RANTES) and monocyte chemoattractant protein (MCP)-1 are produced that recruit monocyte osteoclast precursors to sites of bone to be repaired, promote their fusion to multinuclear cells, and bring them into proximity to RANKL-expressing osteoblastic cells for maturation to functional osteoclasts. **B** Proposed initiation of events in pathological bone resorption. In inflammatory conditions, such as rheumatoid arthritis, there is prolonged production of inflammatory cytokines and chemokines such as TNF-α, IL-1β, and MIP-1α. The source of these molecules is the inflammatory cells, involving macrophages and lymphocytes as part of an immune response, rather than resident bone cells as part of a bone repair mechanism. In addition, RANKL is produced by the inflammatory cells and OPG is suppressed, resulting in osteoclast formation in the inflammatory tissues in the absence of osteoblasts

Factors required for osteoclast formation

Once recruited to sites of bone remodeling the monocyte osteoclast precursors differentiate in response to local signals. Formation of osteoclasts in vitro was shown to require contact between cells of the osteoblast lineage and osteoclast precursors and the action of pro-resorptive stimuli, such as parathyroid hormone, interleukin (IL)-1, IL-11, prostaglandin E, and $1,25(OH)_2$ vitamin D, is mediated by osteoblastic cells.[18] These factors all increase the production and cell-surface expression of receptor activator of NF-κB ligand (RANKL), by osteoblastic cells. RANKL interacts with its receptor, RANK, located on the surface of osteoclast precursor cells and mature osteoclasts.[19] Experimentally, osteoclast formation occurs in vitro upon exposure of osteoclast precursors to soluble RANKL, a process that also requires the presence of macrophage colony-stimulating factor (M-CSF).[20] A secreted RANKL binding protein, osteoprotegerin (OPG),[21,22] is also produced by osteoblastic cells and inhibits both the formation and the activity of osteoclasts by binding to RANKL and blocking its binding to RANK. There is now a great deal of evidence that the local RANKL/OPG ratio determines the effective activity of RANKL to promote osteoclast formation, which supports the concept that RANKL and OPG occupy a central place in the regulation of bone turnover.[23,24] Interestingly, OPG has also been shown to inhibit monocyte migration towards RANTES and MCP-1.[25] RANKL promotion of osteoclast differentiation requires the presence of M-CSF; however, a large number of other factors have been identified that can modulate the activity of RANKL. These include tumor necrosis factor (TNF)-α[26], molecules such as DAP12 and FcRγ providing ITAM-dependent costimulatory signals,[27] IL-3, IL-10, and osteoclast inhibitory lectin (OCIL).[28] This review focuses on those factors that regulate inflammatory-mediated osteolysis, in particular osteolysis in RA. Evidence is presented that disregulated bone turnover that results in the net loss of bone can be due to RANKL expression by cells not of the osteoblastic lineage. In addition, the inflammatory environ-

ment can upregulate osteoclast formation, activity and survival while inhibiting osteoblastic bone formation. Since excellent reviews are available covering a number of aspects of this topic,[29–32] this review describes the intimate relationship between inflammation and osteolysis in RA, with particular emphasis on some of the more newly recognized factors that may mediate bone resorption in inflammatory environments.

Evidence for involvement of RANKL in RA-induced bone loss

RANKL in animal models of RA

Elegant experiments by Redlich et al.[33] showed that bone erosion, in at least one model of RA, is mediated by osteoclasts. These authors crossed transgenic mice that overexpress human TNF (hTNFtg) and that develop a severe and destructive arthritis, with osteopetrotic, c-fos-deficient mice (c-fos$^{-/-}$) completely lacking osteoclasts. The resulting mutant mice (c-fos$^{-/-}$/hTNFtg) developed a TNF-dependent arthritis, with paw swelling and reduction of grip strength, but were fully protected against bone destruction. Other animal models of RA provide good evidence for a role for RANKL in the osteoclast-mediated joint destruction of this disease. For example, in collagen-induced arthritis (CIA) in mice, cells expressing RANK increased in abundance with the progression of arthritis.[34] In areas of focal bone erosion where RANK-positive cells were abundant, TRAP-positive multinucleated osteoclast-like cells are also present suggesting differentiation of RANK-positive osteoclast precursor cells that are present in the inflamed synovial tissues. In addition, sites of RANK-expressing cells correlated with sites of RANKL expression. There is strong evidence that RANKL is important in the bone destruction in mouse models of RA. Firstly, RANKL gene knock out mice were protected from bone erosions in RA in a serum transfer model of arthritis.[35] It has been found that RANKL expression is increased in activated T cells but not in quiescent cells.[36] Kong et al.[37] reported that both soluble and membrane-spanning forms of RANKL are produced by activated T cells. Secondly, blocking of RANKL with OPG treatment at the onset of induced RA in mice prevented bone and cartilage destruction but had little effect on inflammation.[37] Protection of the cartilage was thought to be secondary to the protection of the subchondral bone. Thus, although none of the mouse models of RA perfectly reflect human RA, these data are nevertheless consistent with a central role for RANKL in the bone erosion side effect of this disease.

RANKL in human tissues in RA

Examination of human RA tissues also provides evidence for a key role for RANKL produced in the inflamed synovial tissues in destructive RA. RANKL appears to be over-expressed in pannus tissue from active RA.[38,39] This finding is consistent with the ready proliferation of macrophage-like cells from these tissues and their rapid differentiation into multinucleated bone resorbing cells.[38,40] In addition, osteoclasts formed from circulating precursors from RA patients produced significantly greater bone resorption than cells from controls, which was ascribed to increased functional activity rather than osteoclast formation.[41] Importantly, cells other than, or in addition to, osteoblasts may be a source of RANKL in inflammatory tissues. Using antibodies directed against RANKL and OPG, several studies have shown "ectopic" production of RANKL by several cell types in a variety of inflamed tissues adjacent to sites of bone resorption.[39,42–45] In particular, RANKL protein was predominant within inflammatory cells in the inflamed tissues adjacent to pathological bone loss in RA.[39] It is important to note that the expression of RANKL in the tissues adjacent to bone loss was significantly greater than in the relevant controls in many of these diseases.[39,42,43,46] Investigation of RANKL expression in human RA pannus tissue showed strong RANKL staining in the inflamed pannus and dual staining showed expression by both CD3 cells and macrophages.[39] These data are consistent with other reports that CD3-positive lymphocytes in synovial tissue of active RA patients are the predominant cell type expressing RANKL protein.[39,46] Moreover, lymphocytes isolated from inflammatory arthritis pannus produce RANKL[47,48] and importantly, there is evidence that activated T cells may regulate osteoclast activity by producing RANKL.[49] RANKL was also associated with monocyte/macrophages, and multinucleated cells expressing CD68 in pannus tissue from patients with rheumatoid arthritis as well as in inflammatory periodontal disease and in peri-prosthetic osteolytic lesions. In addition, a recent report demonstrated RANKL mRNA expression in macrophages and multinucleated cells in diseased tissues in an animal model of periodontitis associated with localized bone loss.[50] Since these cells may also express RANK it is possible that macrophages can independently "auto-stimulate" themselves to become osteoclasts. RANKL is also reported to be expressed by synovial fibroblasts[51] and isolated microvascular endothelial cells.[52] Fibroblasts may contribute to the formation of osteoclasts in inflammation. However, the absence of RANKL protein in endothelial cells residing in a variety of inflamed tissues suggest that endothelial cells may not have a role in directly stimulating osteoclast formation in inflammatory diseases.[43–45] These studies suggest that most of the RANKL present in inflamed tissues associated with osteolytic lesions is produced by activated leucocytes that are recruited to sites of inflammation.

Although it is established that OPG exists in both monomeric and dimeric forms,[53] the significance of this and the roles of the different forms are not yet known. Using antibodies that differentiate between these forms, dimeric OPG was only detected in blood vessels (on factor VIII-positive endothelial cells), whereas monomeric OPG was predominantly detected in the synovium (on CD68 positive type A synoviocytes) in RA and other tissues.[43] The function of OPG produced by endothelial cells or type A synoviocytes is yet to be determined. However, it may be significant that

the expression of OPG was markedly reduced in the blood vessels and synovium of inflamed RA joint tissues in active disease compared to the corresponding tissues in disease remission or the appropriate control tissues. Endothelial OPG may be important in enhancing endothelial cell survival[54,55] and may have a role in regulating angiogenesis in inflammation. OPG also has a potential role in inflammatory disease by providing modulation of the immune system.[30,31] While RANKL has been shown to prolong the survival of dendritic cells and cause proliferation of T cells,[56] OPG can abolish these effects. Thus OPG can decrease the production of cytokines such as IL-6 by RANKL-stimulated dendritic cells and also decrease the generation of cytokines by proliferating T cells.[57]

Factors that stimulate RANKL, or amplify the effectiveness of RANKL/RANK signaling

Together with RANKL, M-CSF also has a central role in the bone destruction of RA. M-CSF is expressed abundantly in the RA joint and is a necessary cofactor for RANKL promotion of osteoclastogenesis. Exogenous addition of M-CSF to animal models of RA exacerbates the disease.[58] Other cytokines, such as TNF-α, that stimulate osteoclast formation in vitro are also present in high levels in RA tissues[59–68] and this cytokine environment is likely to augment the RANKL/RANK pathway in the bone of affected joints. Although TNF-α, like other inflammatory cytokines stimulates both OPG and RANKL expression,[69–72] it has also been reported that TNF-α acts synergistically with RANKL by sensitizing osteoclast precursors to low levels of RANKL[26] and may stimulate osteoclast formation directly in the absence of RANKL.[73] Tumor necrosis factor-α antagonists and antibodies are now an accepted treatment regime for active rheumatoid arthritis. Inhibition of TNF-α activity not only reduces the inflammatory response[74] but may also inhibit osteoclast bone resorption by reducing the activity of RANKL present in inflammatory tissues.

Arachidonate metabolites are important inflammatory mediators produced during inflammation, and these molecules are reported to regulate osteoclast formation and activity. The role of prostaglandin (PG) E2 has been studied extensively and its effects can vary, possibly depending on the stage of osteoclast differentiation.[75] Another inflammatory mediator which has been implicated in destructive arthritis is the T-cell product, IL-17.[76] Iinterleukin-17 is present in abundance in active RA joints and is a potent inducer of RANKL like TNF-α and IL-1. Interleukin-17 is likely to be important in inflammatory mediated osteolysis in those situations characterized by a major contribution by T cells. The actions of IL-18 seem to depend on the environment in which it is expressed. In noninflammatory situations, both in vivo and in vitro, IL-18 inhibits osteoclast formation by inducing naïve T cells to produce inhibitors such as granulocyte (G)M-CSF and interferon (IFN)-γ.[28] However, in inflammatory animal models IL-18 can augment osteoclast formation. Dai et al.[77] have examined the actions of IL-18 in human osteoclast precursors incubated with normal peripheral blood T cells or synovial T cells isolated from RA synovium. In this model, IL-18, IL-1, and TNF-α all increased both soluble RANKL production and membrane bound RANKL expression in PHA prestimulated T cells or RA synovial T cells, but not in resting control T cells.[77] Upregulation of RANKL was accompanied by increased osteoclastogenesis and increased bone resorption in vitro.

A number of chemokines are abundant in inflammatory conditions and are likely to be important in recruitment of monocyte osteoclast precursors into the inflammatory pannus and eventually to sites of bone resorption. They may also potentiate the effects of RANKL in osteoclastogenesis. Monocyte chemotactic protein 1 (MCP-1) is a CC chemokine commonly found at sites of RA bone degradation and other inflammation-induced bone loss.[78] MCP-1 is expressed by mature osteoclasts and its expression is regulated by NF-κB.[79,80] Kim et al.[17] identified MCP-1 by microarray as a RANKL-induced molecule during osteoclast differentiation. Osteoclasts were not formed when production of MCP-1 expression was suppressed by GM-CSF or when RANKL signaling through NFAT-c1 was blocked with cyclosporin A. Furthermore, in the presence of RANKL MCP-1 increased osteoclast formation whereas MCP-1 treatment of monocytes in the absence of RANKL promoted the formation of multinucleated TRAP-positive cells that could not resorb bone.[17] These proposed actions of chemokines are depicted in Fig. 1B.

Role of angiogenic factors in RA

The development of new vasculature is essential to sustain the growth of the rheumatoid pannus and angiogenesis has therefore been proposed as a target in RA management.[81] Inflammatory mediators that have been shown to induce angiogenesis at the site of the inflamed synovium include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), integrin alpha(V)beta(3), TNF-α, IL-1, IL-8, IL-18, hypoxia and angiopoietin, and prostaglandins.[81,82] Significantly, it has also been proposed that RANKL has angiogenic activity.[83] The likely role of angiogenic agents in RA disease progression is suggested by a study, which showed that serum VEGF levels at presentation in patients with early RA correlated with the development of radiographic damage after one year.[84] Based on the likely association between angiogenesis inhibition, and the reduction of signs and symptoms of RA by antiangiogenic drugs, these agents have been considered for the treatment of proliferative synovitis. These include angiostatin, endostatin, IL-1 receptor antagonist, thalidomide, fumagillin, and others.[81]

The vasculature is also important in bone metabolism and bone turnover. The elegant work of Reeve and coworkers persuasively demonstrated a relationship between blood flow and bone turnover[85] and indicated that bone can regulate its own blood flow. They developed a method of measuring blood flow in bone that depends on the complete

extraction of ^{18}F in a single passage through bone. They found that skeletal blood flow (SBF) in untreated Paget's disease of bone was strikingly increased in proportion to the biochemical severity of the disease. Treatment with calcitonin brought a rapid return to normal,[85] which was postulated to explain the rapid pain relief often reported on starting treatment with calcitonin. Likewise, treatment with sodium etidronate reduced the SBF and the responses of patients correlated well with the their reductions in serum alkaline phosphatase.[85] These data indicate that active bone resorption may enhance local blood flow to the region and this could in turn further enhance synovial inflammation.

Regulation of cell survival: relationship to bone erosion in RA

Agents that alter cell survival can change the balance between anabolic and catabolic processes in tissues[86] and can lead to the unregulated proliferation and overgrowth of tissues such as the rheumatoid pannus. The proapoptotic molecules Fas and Fas-ligand have been implicated in RA,[87] since expression of these molecules is elevated in synovial and other cells in active RA disease. The inflammatory environment in RA is likely to influence a cell's susceptibility to Fas/Fas-L and inactivating mutations in these proteins in autoimmune disease have also been associated with proliferative arthritis.[88] It has also been suggested that regulation of apoptosis by TRAIL and TRAIL receptor expression may also be a factor that maintains inflammation in arthritis.[89,90] Abnormal TRAIL receptor expression, principally upregulation of TRAIL decoy receptors, may be an important factor in determining the activity of TRAIL. The fact that TRAIL can inhibit the formation of osteoclasts in vitro[91] suggests that TRAIL receptors on the precursors of osteoclasts may be important in regulating bone loss in RA.

In bone, prolonged survival of osteoclasts may be one mechanism for increased bone resorption, and perhaps a net loss of bone. Several positive and negative regulators of osteoclast life span have been identified, including hormones, cytokines, and growth factors.[92] RANKL,[93] M-CSF,[94] IL-1,[94] and TNF-α[95] all support osteoclast survival in vitro, and, as discussed above, these factors are all elevated in the RA joint. The mechanism by which RANKL, TNF-α, and IL-1[95] increase osteoclast survival is thought to involve the activation of Akt and the inactivation of Bad and caspase 9, thus preventing activation of the apoptosis cascade. M-CSF-mediated osteoclast survival also works through Akt[92] in a different way, resulting in increased expression of the anti-apoptotic genes, Bcl-2, Bcl-xL,[93] and XIAP.[94]

Bone loss in RA would be exacerbated if osteoblast differentiation and survival were reduced in the inflammatory environment. This is not a well-researched area but there are data on the regulation of osteoblast apoptosis in relation to this.[92] The Wnt family of proteins are involved in cell differentiation and tissue morphogenesis. These molecules interact with other types of anabolic molecules, such as BMPs, and have received close attention recently as a group of agonistic and antagonistic molecules in bone formation.[96] Interest was stimulated by the discovery of a mutation in the low-density lipoprotein receptor-related protein 5 (LRP5), which was identified as causal for an autosomal dominant high bone mass trait in a single human family. The same mutation results in increased transcription of OPG in response to loading and may also result in a reduction in osteoclastogenesis. The identification of LRP5/Wnt signaling in bone mechano-sensing has resulted in a new paradigm for understanding bone formation. Since some members of the Wnt (Wnt1 and Wnt5a) and the functionally related frizzled proteins (Fz5) families have been found at elevated levels in RA synovium, it has been proposed that these may play a causal role in synovial hyperplasia and inflammation.[97] A number of Wnt antagonists have also been identified, including the Dickkopf (DKK) family of proteins. While there is no direct experimental evidence as yet for these molecules as suppressors of bone formation in RA, a potential antianabolic role has been proposed for Dkk1 in osteolytic lesions in multiple myeloma,[98] another disease characterized by increased bone resorption and decreased bone formation.

Inflammatory cytokines may also play an anti-anabolic role in RA. There is abundant literature on the likely pathological roles of TNF-α in pathological bone loss and, while it is recognized that inflammatory cytokines have a role in physiological bone tissue repair and turnover,[32] high and/or sustained levels of these may have a net catabolic and anti-anabolic effect on bone tissue.[99] There is also evidence that TNF-α, for example, might reduce bone formation, by inhibiting the differentiation of osteoblasts.[100] A possible mechanism for this is the inhibition by TNF-α of expression of the transcription factor RUNX2/Cbfa1, a critical regulator of osteoblast differentiation.[100] In RA, and in other states of TNF-α excess, TNF-α may therefore decrease the phenotype progression of precursor cells to the osteoblast pathway. Since TNF also promotes osteoclastogenesis (see above), TNF-α suppression of RUNX2/Cbfa1 could decrease bone formation while simultaneously increasing resorption.

Treating bone loss in RA

There are now a large number of different drugs available to treat RA; however, most of these treatments are aimed at reducing inflammation and very few treatments target the debilitating bone loss seen in RA. Now that the key factors regulating osteoclast resorption in RA are known, new therapies specifically targeting bone destruction are being developed. The RANK–RANKL interaction in the formation of osteoclasts is an ideal target for therapy because it is a point at which numerous pathways for osteolysis converge. Accordingly, treatments based on the inhibition of RANKL inhibition by its natural inhibitor, OPG,[37] or soluble RANK,[101] have been successful. Combinations of

therapies that target several factors, such as TNF-α, IL-1β, and RANKL inhibitors have also been used in an animal model of RA[102] and may be more successful than single therapies. Like anti-TNF treatment, methotrexate, sulfasalazine, and IL-4 have recently been shown to inhibit human osteoclastogenesis, and can inhibit RANKL mRNA expression by RA synoviocytes.[103] Selective blockade of potassium channels may also reduce osteoclast formation in T-cell driven osteolysis in inflammatory diseases. These recent animal studies are promising, and preliminary studies based on selective blockade of RANK-RANKL interaction demonstrate the importance of RANKL in bone destruction in RA.[104]

Bisphosphonates, a group of drugs currently used to inhibit osteoclast activity in osteolytic bone tumors and osteoporosis, have also been used to inhibit osteolysis in animal models of osteolysis.[105] In a recent study,[106] zoledronic acid was tested as a potential treatment for the joint destruction in the transgenic mouse RA model produced by over-expression of human tumor necrosis factor (hTNFtg). Untreated, these mice developed severe destructive arthritis as well as osteoporosis. In contrast, bone erosion was retarded by a single dose of zoledronic acid and was almost completely blocked by repeated administration of the drug. Although cartilage damage was partly inhibited, synovial inflammation was not affected by zoledronic acid treatment. These findings are in agreement with several other studies,[107,108] although the success of bisphosphonates in preventing joint damage may depend on the disease model or on the particular bisphosphonate used. For example, in adjuvant-induced arthritis in rats, it was found that while clodronate decreased bone deformation, alendronate did not.[109] However, both bisphosphonates prevented systemic bone loss and strength reduction in this study. In the hTNFtg model, calcitonin had no effect on synovial inflammation, bone erosion, cartilage damage, or systemic bone mass, whereas anti-TNF treatment entirely blocked synovial inflammation, bone erosion, synovial osteoclast formation, and cartilage damage, but had only minor effects on systemic bone mass.[106]

As alluded to above, other potentially rewarding approaches in treating RA may include the use of antiangiogenic agents, as has been trialed in other diseases of bone destruction. For example, in multiple myeloma it has been demonstrated that patients with active disease have an increase in bone marrow angiogenesis that correlates with the progression of disease and an adverse prognosis. Accordingly, therapeutic approaches to target VEGF, as well as a range of other angiogenesis-related factors, are being investigated for this disease.[110] Vascular endothelial growth factor also shows promise as a target in solid tumors. A humanized anti-VEGF monoclonal antibody, bevacizumab, resulted in tumor growth inhibition of human tumor cell lines implanted into nude mice and changes in vascular functions were frequently reported, including decreased vessel diameter, density, and permeability in response to treatment.[111] Safety studies with bevacizumab in cynomolgus monkeys showed that this agent is generally well tolerated, supporting similar studies in RA.

Conclusions

Bone destruction in the joints of RA patients occurs through similar mechanisms as in other inflammatory diseases. The RANKL/RANK pathway of osteoclast differentiation is central to the formation of osteoclasts at these sites of inflammation and is therefore an important target for the pharmaceutical regulation of bone loss in several common inflammatory diseases. Other inflammatory cytokines are likely to augment the RANKL/RANK pathway of osteoclast formation and activity, and some of these factors may also prolong the survival of osteoclasts and decrease the differentiation and survival of osteoblasts. In addition, increased blood flow to the inflamed pannus is likely to promote bone resorption and also represents a potential target in this disease.

Acknowledgements The authors are recipients of research support from the National Health and Medical Research Council of Australia, The University of Adelaide, and the Royal Adelaide Hospital.

References

1. Gough A, Sambrook P, Devlin J, Huissoon A, Njeh C, Robbins S, et al. Osteoclastic activation is the principal mechanism leading to secondary osteoporosis in rheumatoid arthritis. J Rheumatol 1998;25:1282–9.
2. Gravallese EM, Harada Y, Wang J-T, Gorn AH, Thornhill TS, Goldring SR. Identification of cell types responsible for bone resorption in rheumatoid arthritis and juvenile rheumatoid arthritis. Am J Pathol 1998;152:943–51.
3. Goldring SR, Gravallese EM. Pathogenesis of bone lesions in rheumaotid arthritis. Curr Rheum Rep 2002;4:226–31.
4. Burr DB. Targeted and nontargeted remodeling. Bone 2002;30(1):2–4.
5. Frost HM. Tetracycline-based histological analysis of bone remodeling. Calcif Tissue Res 1969;3(3):211–37.
6. Parfitt AM. Targeted and nontargeted bone remodeling: relationship to basic multicellular unit origination and progression. Bone 2002;30(1):5–7.
7. Bentolila V, Boyce TM, Fyhrie DP, Drumb R, Skerry TM, Schaffler MB. Intracortical remodeling in adult rat long bones after fatigue loading. Bone 1998;23(3):275–81.
8. Burr DB. Remodeling and the repair of fatigue damage. Calcif Tissue Int 1993;53 Suppl 1:S75–80; discussion S80–1.
9. Noble B. Bone microdamage and cell apoptosis. Eur Cell Mater 2003;6:46–55; discussion 55.
10. Parfitt AM. Osteoclast precursors as leukocytes: importance of the area code. Bone 1998;23(6):491–4.
11. Burger EH, Klein-Nulend J, Smit TH. Strain-derived canalicular fluid flow regulates osteoclast activity in a remodelling osteon – a proposal. J Biomech 2003;36(10):1453–9.
12. Gross TS, King KA, Rabaia NA, Pathare P, Srinivasan S. Upregulation of osteopontin by osteocytes deprived of mechanical loading or oxygen. J Bone Miner Res 2005;20(2):250–6.
13. Yu X, Huang Y, Collin-Osdoby P, Osdoby P. Stromal cell-derived factor-1 (SDF-1) recruits osteoclast precursors by inducing chemotaxis, matrix metalloproteinase-9 (MMP-9) activity, and collagen transmigration. J Bone Miner Res 2003;18(8):1404–18.
14. Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 2004;10(8):858–64.
15. Yano K, Tsuda E, Washida N, Kobayashi F, Goto M, Harada A, et al. Immunological characterization of circulating osteoprotegerin/osteoclastogenesis inhibitory factor: Increased serum

concentrations in postmenopausal women with osteoporosis. J Bone Miner Res 1999;14(4):518–27.
16. Yu X, Huang Y, Collin-Osdoby P, Osdoby P. CCR1 chemokines promote the chemotactic recruitment, RANKL development, and motility of osteoclasts and are induced by inflammatory cytokines in osteoblasts. J Bone Miner Res 2004;19(12):2065–77.
17. Kim MS, Day CJ, Morrison NA. MCP-1 is induced by RANKL, promotes osteoclast fusion and rescues GM-CSF suppression of osteoclast formation. J Biol Chem 2005; 280(16):16163–9.
18. Rodan GA, Martin TJ. Role of osteoblasts in hormonal control of bone resorption – a hypothesis. Calcif Tissue Int 1981;33(4):349–51.
19. Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Boyle WJ, Riggs LB. The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. J Bone Miner Res 2000;15(1):2–12.
20. Quinn JMW, Neale SD, Fujikawa Y, McGee JOD, Athanasou NA. Human osteoclast formation from blood monocytes, peritoneal macrophages, and bone marrow cells. Calcif Tissue Int 1998;62(6):527–31.
21. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang M-S, Luthy R, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997;89:309–19.
22. Tsuda E, Goto M, Mochizuki S-i, Yano K, Kobayashi F, Moringa T, et al. Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. Biochem Biophys Res Commun 1997;234:137–42.
23. Fazzalari NL, Kuliwaba JS, Atkins GJ, Forwood MR, Findlay DM. The ratio of messenger RNA levels of receptor activator of nuclear factor kappaB ligand to osteoprotegerin correlates with bone remodeling indices in normal human cancellous bone but not in osteoarthritis. J Bone Miner Res 2001;16(6):1015–27.
24. Hofbauer LC, Khosla S, Dunstan CR, Lacey DL, Boyle WJ, Riggs BL. The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. J Bone Miner Res 2000;15(1):2–12.
25. Mosheimer BA, Kaneider NC, Feistritzer C, Djanani AM, Sturn DH, Patsch JR, et al. Syndecan-1 is involved in OPG-induced chemotaxis in human peripheral blood monocytes. J Clin Endocrinol Metab 2005; in press.
26. Lam J, Takeshita S, Barker JE, Kanagawa O, Ross FP, Teitelbaum SL. TNF-α induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. J Clin Invest 2000;106:1481–8.
27. Takayanagi H. Mechanistic insight into osteoclast differentiation in osteoimmunology. J Mol Med 2005; in press.
28. Quinn JM, Gillespie MT. Modulation of osteoclast formation. Biochem Biophys Res Commun 2005;328(3):739–45.
29. Romas E, Gillespie MT, Martin TJ. Involvement of receptor activator of NFkappaB ligand and tumor necrosis factor-alpha in bone destruction in rheumatoid arthritis. Bone 2002;30(2):340–6.
30. Hofbauer LC, Schoppet M. Clinical implications of the osteoprotegerin/RANKL/RANK system for bone and vascular diseases. Jama 2004;292(4):490–5.
31. Saidenberg-Kermanac'h N, Cohen-Solal M, Bessis N, De Vernejoul MC, Boissier MC. Role for osteoprotegerin in rheumatoid inflammation. Joint Bone Spine 2004;71(1):9–13.
32. Goldring SR. Inflammatory mediators as essential elements in bone remodeling. Calcif Tissue Int 2003;73(2):97–100.
33. Redlich K, Hayer S, Ricci R, David JP, Tohidast-Akrad M, Kollias G, et al. Osteoclasts are essential for TNF-alpha-mediated joint destruction. J Clin Invest 2002;110(10):1419–27.
34. Lubberts E, Oppers-Walgreen B, Pettit AR, Van Den Bersselaar L, Joosten LA, Goldring SR, et al. Increase in expression of receptor activator of nuclear factor kappaB at sites of bone erosion correlates with progression of inflammation in evolving collagen-induced arthritis. Arthritis Rheum 2002;46(11):3055–64.
35. Pettit AR, Ji H, von Stechow D, Muller R, Goldring SR, Choi Y, et al. TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. Am J Path 2001;159:1689–99.
36. Horwood NJ, Kartsogiannis V, Quinn JM, Romas E, Martin TJ, Gillespie MT. Activated T lymphocytes support osteoclast formation in vitro. Biochem Biophys Res Commun 1999;265(1):144–50.
37. Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, et al. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 1999;402(6759):304–9.
38. Haynes DR, Crotti TN, Loric M, Bain GI, Atkins GJ, Findlay DM. Osteoprotegerin and receptor activator of nuclear factor kappa B ligand (RANKL) regulate osteoclast formation by cells in the human rheumatoid arthritic joint. J Rheumatol 2001;40(6):623–30.
39. Crotti TN, Smith MD, Weedon H, Ahern MJ, Findlay DM, Kraan M, et al. Receptor activator NF-kappaB ligand (RANKL) expression in synovial tissue from patients with rheumatoid arthritis, spondyloarthropathy, osteoarthritis, and from normal patients: semiquantitative and quantitative analysis. Ann Rheum Dis 2002;61(12):1047–54.
40. Suzuki Y, Tsutsumi Y, Nakagawa M, Suzuki H, Matsushita K, Beppu M, et al. Osteoclast-like cells in an in vitro model of bone destruction by rheumatoid synovium. Rheumatology 2001;40(6):673–82.
41. Hirayama T, Danks L, Sabokbar A, Athanasou NA. Osteoclast formation and activity in the pathogenesis in rheumatoid arthritis. Rheumatol 2002;41:1232–9.
42. Haynes DR, Barg E, Crotti TN, Holding C, Weedon H, Atkins GJ, et al. Osteoprotegerin expression in synovial tissue from patients with rheumatoid arthritis, spondyloarthropathies and osteoarthritis and normal controls. Rheumatology (Oxford) 2003;42(1):123–34.
43. Haynes DR, Barg E, Crotti TN, Weedon H, Atkins GJ, Zannettino A, et al. Osteoprotegerin (OPG) Expression in synovial tissue from patients with rheumatoid arthritis, spondyloarthropathies, osteoarthritis and normal controls. Rheumatol 2003;43:1–12.
44. Crotti T, Smith MD, Hirsch R, Soukoulis S, Weedon H, Capone M, et al. Receptor activator NF kappaB ligand (RANKL) and osteoprotegerin (OPG) protein expression in periodontitis. J Periodontal Res 2003;38(4):380–7.
45. Crotti TN, Smith MD, Findlay DM, Zreiqat H, Ahern MJ, Weedon H, et al. Factors regulating osteoclast formation in human tissues adjacent to peri-implant bone loss: expression of receptor activator NFkappaB, RANK ligand and osteoprotegerin. Biomaterials 2004;25(4):565–73.
46. Crotti TN, Smith MD, Hirsch RS, Soukoulis S, Weedon H, Capone M, et al. Receptor activator NF k B ligand (RANKL) and osteoprotegerin (OPG) protein expression in periodontitis. J Periodont Res; in press.
47. Kong Y-Y, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, et al. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 1999;402(18):304–9.
48. Romas E, Bakharevski O, Hards DK, Kartsogiannis V, Quinn JMW, Ryan PFJ, et al. Expression of osteoclast differentiation factor at sites of bone resorption in collagen-induced arthritis. Arthritis Rheum 2000;43(4):821–6.
49. Udagawa N. The mechanism of osteoclast differentiation from macrophages: possible roles of T lymphocytes in osteoclastogenesis. J Bone Miner Metab 2003;21(6):337–43.
50. Ogasawara T, Yoshimine Y, Kiyoshima T, Kobayashi I, Matsuo K, Akamine A, et al. In situ expression of RANKL, RANK, osteoprotegerin and cytokines in osteoclasts of rat periodontal tissue. J Periodontal Res 2004;39(1):42–9.
51. Quinn JMW, Horwood NJ, Elliott J, Gillespie MT, Martin TJ. Fibroblastic stromal cells express receptor activator of NF-kappa B ligand and support osteoclast differentiation. J Bone Miner Res 2000;15(8):1459–66.
52. Collin-Osdoby P, Rothe L, Anderson F, Nelson M, Maloney W, Osdoby P. Receptor activator of NF-κB and osteoprotegerin expression by human microvascular endothelial cells, regulation by inflammatory cytokines, and role in human osteoclastogenesis. J Biol Chem 2001;276(23):20659–72.
53. Tomoyasu A, Goto M, Fujise N, Mochizuki S, Yasuda H, Morinaga T, et al. Characterization of monomeric and homodimeric forms of osteoclastogenesis inhibitory factor. Biochem Biophys Res Commun 1998;245(2):382–7.
54. Malyankar UM, Scatena M, Suchland KL, Yun TJ, Clark EA, Giachelli CM. Osteoprotegerin is an alpha vbeta 3-induced, NF-

kappa_B-dependent survival factor for endothelial cells. Biol Chem 2000;275(28):20959–62.
55. Kim HH, Shin HS, Kwak HJ, Ahn KY, Kim JH, Lee HJ, et al. RANKL regulates endothelial cell survival through the phosphatidylinositol 3′-kinase/Akt signal transduction pathway. FASEB J 2003;17(14):2163–5.
56. Wong BR, Rho J, Arron J, Robinson E, Orlinick J, Chao M, et al. TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun n-terminal kinase in T cells. J Biol Chem 1997;272(40):25190–4.
57. Josien R, Wong BR, Li HL, Steinman RM, Choi Y. TRANCE, a TNF family member, is differentially expressed on T cell subsets and induces cytokine production in dendritic cells. J Immunol 1999;162(5):2562–8.
58. Campbell IK, Rich MJ, Bischof RJ, Hamilton JA. The colony-stimulating factors and collagen-induced arthritis: exacerbation of disease by M-CSF and G-CSF and requirement for endogenous M-CSF. J Leukocyte Biol 2000;68(1):144–50.
59. Chu CQ, Field M, Feldman M, Maini RN. Localization of tumor necrosis factor a in synovial tissues and at the cartilage-pannus junction in patients with rheumatoid arthritis. Arthritis Rheum 1991;34:1125–32.
60. Geivelis M, Turner DW, Pederson ED, Lamberts B. Measurements of interleukin-6 in gingival crevicular fluid from adults with destructive periodontal disease. J Periodontol 1993;64:980–3.
61. Yamazaki K, Nakajima T, Gemmell E, Polak B, Seymour GJ, Hara K. IL-4 and IL-6-producing cells in human periodontal disease tissue. J Oral Pathol Med 1994;23:347–53.
62. al-Saffar N, Revell A. Interleukin-1 production by activated macrophages surrounding loosened orthopaedic implants: A potential role in osteolysis. Br J Rheumatol 1994;33(4):309–16.
63. Arend WP, Dayer J-M. Inhibition of the production and effects of interleukin-1 and tumor necrosis factor-α in rheumatoid arthritis. Arthritis Rheum 1995;38:151–60.
64. Xu JW, Konttinen YT, Lassus J, Natah S, Ceponis A, Solovieva SA, et al. Tumour necrosis factor-alpha (TNF-alpha) in loosening of total hip replacement (THR). Clin Exp Rheumatol 1996;14(6):643–8.
65. Kotake S, Sato K, Kim KJ, Takahashi N, Udagawa N, Nakamura I, et al. Interleukin-6 and soluble interleukin-6 receptors in the synovial fluids from rheumatoid arthritis patients are responsible for osteoclast-like cell formation. J Bone Miner Res 1996;11(1):88–95.
66. Xu JW, Li T-F, Partsch G, Ceponis A, Santavirta S, Konttinen YT. Interleukin-11 (IL-11) in aseptic loosening of total hip replacement (THR). Scand J Rheumatol 1998;27:363–7.
67. Neale S, Sabokbar A, Howie DW, Murray DW, Athanasou NA. Macrophage colony-stimulating factor and interleukin-6 release by periprosthetic cells stimulates osteoclast formation and bone resorption. J Orthop Res 1999;17(5):686–94.
68. Merkel KD, Erdmann JM, Mchugh KP, Abu-Amer Y, Ross FP, Teitelbaum SL. Tumor necrosis factor-α mediates orthopedic implant osteolysis. Am J Pathol 1999;154(1):203–10.
69. Brandstrom H, Jonsson K, Vidal O, Ljunghall S, Ohlsson C, Ljunggren O. Tumor necrosis factor-α and -β upregulate the levels of osteoprotegerin mRNA in human osteosarcoma MG-63 cells. Biochem Biophys Res Commun 1998;248:454–7.
70. Horwood NJ, Elliot J, Martin TJ, Gillespie MT. Osteotropic agents regulate the expression of osteoclast differentiation factor and osteoprotegerin in osteoblastic stromal cells. Endocrinology 1998;139(11):4743–6.
71. Hofbauer LC, Lacey DL, Dunstan CR, Spelsberg TC, Riggs BL, Khosla S. Interleukin-1β and tumor necrosis factor-α, but not interleukin-6, stimulate osteoprotegerin ligand gene expression in human osteoblastic cells. Bone 1999;25(3):255–9.
72. Nakashima T, Kobayashi Y, Yamasaki S, Kawakami A, Eguchi K, Sasaki H, et al. Protein expression and functional difference of membrane-bound and soluble receptor activator of NF-κ_ ligand: Modulation of the expression by osteotropic factors and cytokines. Biochem Biophys Res Commun 2000;275:768–75.
73. Kobayashi K, Takahashi N, Jimi E, Udagawa N, Takami M, Kotake S, et al. Tumor necrosis factor α stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. J Exp Med 2000;191(2):275–85.
74. Assuma R, Oates T, Cochran D, Amar S, Graves DT. IL-1 and TNF antagonists inhibit the inflammation response and bone loss in experimental periodontitis. J Immununol 1998;160:403–9.
75. Quinn JMW, Sabokbar A, Denne M, de Vernejoul MC, McGee JO, Athanasou NA. Inhibitory and stimulatory effects of prostaglandins on osteoclast differentiation. Calcif Tiss Int 1997;60(1):63–70.
76. Lubberts E, Koenders MI, van den Berg WB. The role of T cell interleukin-17 in conducting destructive arthritis: lessons from animal models. Arthritis Res Ther 2005;7(1):29–37.
77. Dai SM, Nishioka K, Yudoh K. Interleukin (IL) 18 stimulates osteoclast formation through synovial T cells in rheumatoid arthritis: comparison with IL1 beta and tumour necrosis factor alpha. Ann Rheum Dis 2004;63(11):1379–86.
78. Wise GE, Frazier-Bowers S, D'Souza RN. Cellular, molecular, and genetic determinants of tooth eruption. Crit Rev Oral Biol Med 2002;13(4):323–34.
79. Cappellen D, Luong-Nguyen NH, Bongiovanni S, Grenet O, Wanke C, Susa M. Transcriptional program of mouse osteoclast differentiation governed by the macrophage colony-stimulating factor and the ligand for the receptor activator of NFkappa B. J Biol Chem 2002;277(24):21971–82.
80. Ishida N, Hayashi K, Hoshijima M, Ogawa T, Koga S, Miyatake Y, et al. Large scale gene expression analysis of osteoclastogenesis in vitro and elucidation of NFAT2 as a key regulator. J Biol Chem 2002;277(43):41147–56.
81. Koch AE. Angiogenesis as a target in rheumatoid arthritis. Ann Rheum Dis 2003;62 Suppl 2:ii60–7.
82. Lainer DT, Brahn E. New antiangiogenic strategies for the treatment of proliferative synovitis. Expert Opin Investig Drugs 2005;14(1):1–17.
83. Min JK, Kim YM, Kim EC, Gho YS, Kang IJ, Lee SY, et al. Vascular endothelial growth factor up-regulates expression of receptor activator of NF-kappa B (RANK) in endothelial cells. Concomitant increase of angiogenic responses to RANK ligand. J Biol Chem 2003;278(41):39548–57.
84. Ballara S, Taylor PC, Reusch P, Marme D, Feldmann M, Maini RN, et al. Raised serum vascular endothelial growth factor levels are associated with destructive change in inflammatory arthritis. Arthritis Rheum 2001;44(9):2055–64.
85. Wootton R, Reeve J, Spellacy E, Tellez-Yudilevich M. Skeletal blood flow in Paget's disease of bone and its response to calcitonin therapy. Clin Sci Mol Med 1978;54(1):69–74.
86. Bouralexis S, Findlay DM, Evdokiou A. Death to the bad guys: targeting cancer via Apo2L/TRAIL. Apoptosis 2005;10(1):35–51.
87. Smith MD, Walker JG. Apoptosis a relevant therapeutic target in rheumatoid arthritis? Rheumatology (Oxford) 2004;43(4):405–7.
88. Rieux-Laucat F, Le Deist F, Hivroz C, Roberts IA, Debatin KM, Fischer A, et al. Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. Science 1995;268(5215):1347–9.
89. Feng X. Regulatory roles and molecular signaling of TNF family members in osteoclasts. Gene 2005; in press.
90. Tsokos GC, Tsokos M. The TRAIL to arthritis. J Clin Invest 2003;112(9):1315–17.
91. Zauli G, Rimondi E, Nicolin V, Melloni E, Celeghini C, Secchiero P. TNF-related apoptosis-inducing ligand (TRAIL) blocks osteoclastic differentiation induced by RANKL plus M-CSF. Blood 2004;104(7):2044–50.
92. Xing L, Boyce BF. Regulation of apoptosis in osteoclasts and osteoblastic cells. Biochem Biophys Res Commun 2005;328(3):709–20.
93. Lacey DL, Tan HL, Lu J, Kaufman S, Van G, Qiu W, et al. Osteoprotegerin ligand modulates murine osteoclast survival in vitro and in vivo. Am J Pathol 2000;157:435–48.
94. Kanaoka K, Kobayashi Y, Hashimoto F, Nakashima T, Shibata M, Kobayashi K, et al. A common downstream signaling activity of osteoclast survival factors that prevent nitric oxide-promoted osteoclast apoptosis. Endocrinology 2000;141(8):2995–3005.
95. Glantschnig H, Fisher JE, Wesolowski G, Rodan GA, Reszka AA. M-CSF, TNFalpha and RANK ligand promote osteoclast survival by signaling through mTOR/S6 kinase. Cell Death Differ 2003;10(10):1165–77.
96. Westendorf JJ, Kahler RA, Schroeder TM. Wnt signaling in osteoblasts and bone diseases. Gene 2004;341:19–39.

97. Sen M. Wnt signalling in rheumatoid arthritis. Rheumatology (Oxford) 2005; in press.
98. Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B, et al. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. N Engl J Med 2003;349(26):2483–94.
99. Pfeilschifter J, Koditz R, Pfohl M, Schatz H. Changes in proinflammatory cytokine activity after menopause. Endocrine Rev 2002;23(1):90–119.
100. Gilbert L, He X, Farmer P, Rubin J, Drissi H, van Wijnen AJ, et al. Expression of the osteoblast differentiation factor RUNX2 (Cbfa1/AML3/Pebp2alpha A) is inhibited by tumor necrosis factor-alpha. J Biol Chem 2002;277(4):2695–701.
101. Childs LM, Paschalis EP, Xing L, Dougall WC, Anderson D, Boskey AL, et al. In vivo RANK signaling blockade using the receptor activator of NF-kappaB:Fc effectively prevents and ameliorates wear debris-induced osteolysis via osteoclast depletion without inhibiting osteogenesis. J Bone Miner Res 2002;17(2): 192–9.
102. Zwerina J, Hayer S, Tohidast-Akrad M, Bergmeister H, Redlich K, Feige U, et al. Single and combined inhibition of tumor necrosis factor, interleukin-1, and RANKL pathways in tumor necrosis factor-induced arthritis: effects on synovial inflammation, bone erosion, and cartilage destruction. Arthritis Rheum 2004;50 (1):277–90.
103. Lee CK, Lee EY, Chung SM, Mun SH, Yoo B, Moon HB. Effects of disease-modifying antirheumatic drugs and antiinflammatory cytokines on human osteoclastogenesis through interaction with receptor activator of nuclear factor kappaB, osteoprotegerin, and receptor activator of nuclear factor kappaB ligand. Arthritis Rheum 2004;50(12):3831–43.
104. Li P, Schwarz EM, O'Keefe RJ, Ma L, Boyce BF, Xing L. RANK signaling is not required for TNFalpha-mediated increase in CD11(hi) osteoclast precursors but is essential for mature osteoclast formation in TNFalpha-mediated inflammatory arthritis. J Bone Miner Res 2004;19(2):207–13.
105. Iwase M, Kim KJ, Kobayashi Y, Itoh M, Itoh T. A novel bisphosphonate inhibits inflammatory bone resorption in a rat osteolysis model with continuous infusion of polyethylene particles. J Orthop Res 2002;20(3):499–505.
106. Herrak P, Gortz B, Hayer S, Redlich K, Reiter E, Gasser J, et al. Zoledronic acid protects against local and systemic bone loss in tumor necrosis factor-mediated arthritis. Arthritis Rheum 2004;50(7):2327–37.
107. Sims NA, Green JR, Glatt M, Schlict S, Martin TJ, Gillespie MT, et al. Targeting osteoclasts with zoledronic acid prevents bone destruction in collagen-induced arthritis. Arthritis Rheum 2004;50(7):2338–46.
108. Harada H, Nakayama T, Nanaka T, Katsumata T. Effects of bisphosphonates on joint damage and bone loss in rat adjuvant-induced arthritis. Inflamm Res 2004;53(2):45–52.
109. Itoh F, Aoyagi S, Kusama H, Kojima M, Kogo H. Effects of clodronate and alendronate on local and systemic changes in bone metabolism in rats with adjuvant arthritis. Inflammation 2004;28(1):15–21.
110. Podar K, Anderson KC. The pathophysiologic role of VEGF in hematologic malignancies: therapeutic implications. Blood 2005;105(4):1383–95.
111. Gerber HP, Ferrara N. Pharmacology and pharmacodynamics of bevacizumab as monotherapy or in combination with cytotoxic therapy in preclinical studies. Cancer Res 2005;65(3):671–80.