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Bone Damage in Rheumatoid Arthritis – Mechanistic Insights and Approaches to Prevention

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Synopsis

In rheumatoid arthritis, cells within the inflamed synovium and pannus elaborate a variety of cytokines, including TNF α , IL-1, IL-6 and IL-17, that contribute to inflammation, and may directly impact bone. The RANKL/RANK/OPG pathway plays a critical role in regulating osteoclastogenesis in articular bone erosions in RA. Pro-inflammatory cytokines can modulate this pathway, and may also affect the ability of the osteoblast to repair bone at sites of articular erosion. In this review, we discuss the current understanding of pathogenic mechanisms of bone erosion in RA and examine current therapeutic approaches to prevent this damage.

Keywords

rheumatoid arthritis; therapy; osteoclasts; osteoblasts; Wnt; cytokines

Introduction

Rheumatoid arthritis (RA) is a systemic disease process that is characterized by inflammation of the synovial tissues lining the joints. Proliferation of synovial lining cells and infiltration of inflammatory cells, including monocytes and activated leukocytes, into the joint tissues results in formation of “pannus” tissue that covers the surfaces of articular cartilage and bone (1) and produces proinflammatory factors that lead to destruction of both cartilage and bone matrix.

A common and characteristic feature of RA is focal articular bone loss, or erosion, that becomes evident early on in the disease process (2). In RA, inflamed joints are also often associated with periarticular bone loss, which precedes focal bone erosion. Erosions occur at sites where

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pannus invades cortical and subchondral bone and the adjacent marrow spaces. Ultimately, trabecular bone is lost as well. Systemic bone loss also commonly affects both the appendicular and axial skeleton in RA and, over time, results in increased fracture risk (3,4). When RA is not detected and treated early, focal bone erosions progress rapidly and result in joint deformity and functional disability (2,5). Thus, early treatment to prevent focal bone erosions is a critical objective in caring for patients with RA. In this review, we summarize the mechanisms, cell types and pro-inflammatory factors implicated in the pathogenesis of focal articular bone loss and review recent advances in targeted biologic therapies to prevent progression of structural damage in RA.

Role of osteoclasts in focal articular bone loss in RA

Osteoclasts are specialized multinucleated cells that arise from cells of monocyte-macrophage lineage. After attaching to bone matrix proteins, osteoclasts secrete proteinases and create a local acidic environment that mediates bone destruction. During physiologic bone remodeling, bone loss mediated by osteoclasts is balanced by bone formation mediated by osteoblasts. Focal bone loss in RA is mediated by osteoclasts located at the pannus-bone interface and in subchondral locations (6,7). These cells express typical markers of the osteoclast lineage, including cathepsin K and tartrate-resistant acid phosphatase (TRAP), as well as the calcitonin receptor, a marker of terminal osteoclast differentiation (7,8).

The receptor activator of NF- κ B (RANK) ligand (RANKL) pathway regulates osteoclast differentiation and function during normal physiological bone remodeling. RANKL promotes osteoclastogenesis by binding to its cognate receptor RANK on osteoclast precursor cells. Osteoprotegerin (OPG) is a soluble decoy receptor for RANKL that blocks the pro-osteoclastogenic activity of RANKL. In inflammatory arthritis, the RANK/RANKL pathway is activated, resulting in dysregulated bone remodeling. RA synovial tissues exhibit an increased ratio of RANKL:OPG mRNA expression, indicating that pro-osteoclastogenic conditions dominate within the microenvironment of the RA joint (9).

Support for the critical role of osteoclasts in the process of bone erosion in RA is provided by studies using mice deficient in RANKL, an essential factor for osteoclast differentiation. These mice are devoid of osteoclasts and thus have an osteopetrotic bone phenotype (10). Induction of serum transfer arthritis in RANKL-deficient mice resulted in full-blown arthritis, but these osteoclast-deficient mice were protected from focal articular bone loss (11). This finding was confirmed in transgenic mice expressing human TNF (hTNF.tg) that develop a spontaneous, severe and destructive polyarthritis that results in extensive focal articular bone erosion (12). When crossed with osteopetrotic c-fos-deficient mice that have no osteoclasts, the resulting mutant mice developed TNF-dependent arthritis without focal articular bone loss, despite developing inflammation comparable to that of littermate controls expressing c-fos (13). In a T-cell-dependent model of rat adjuvant arthritis characterized by severe joint inflammation accompanied by bone and cartilage destruction, administration of OPG prevented bone and cartilage destruction but not inflammation, indicating the ability of OPG to block RANK signaling and focal bone erosion (14). Further support for a critical role of osteoclasts in mediating focal bone erosion in RA has arisen from murine studies that used bisphosphosphonates to block osteoclast-mediated bone resorption. hTNF.Tg mice treated with pamidronate or zoledronic acid demonstrated inhibition of focal articular bone loss without any significant change in inflammation (15,16). This was also demonstrated with zoledronic acid treatment of mice with collagen-induced arthritis (17), a model of RA that is T cell and antibody-driven.

In addition to RANKL, macrophage-colony stimulating factor (M-CSF) is also required for osteoclastogenesis and serves to amplify the pool of osteoclast cellular precursors (18). Both

RANKL and M-CSF are synthesized mainly by cells of the osteoblast lineage in the setting of physiologic bone remodeling (19). In both human disease and animal models of RA, activated CD4⁺T cells and synovial fibroblasts are alternative sources of RANKL that can drive osteoclastogenesis (8,14).

Osteoprotegerin (OPG), a decoy receptor for RANKL that is synthesized by osteoblast-lineage cells, acts as a key negative regulator of osteoclastogenesis by binding soluble RANKL and preventing its binding to its receptor RANK, thereby inhibiting osteoclast differentiation (10, 20). In RA, the balance between RANKL and OPG expression levels is a fundamental regulator of osteoclast differentiation and function (21,22). Thus, targeting the RANKL/RANK/OPG pathway to inhibit osteoclast differentiation represents an important potential therapeutic strategy to prevent bone erosion in inflammatory arthritis. However, even with aggressive treatment of RA to stop the progression of articular bone destruction, erosive lesions are not typically “repaired”; only a limited amount of new bone forms at sites of erosion. This suggests that osteoblast-mediated bone formation also may be compromised at sites where both inflammation and erosion occur, thereby contributing to the net loss of bone.

Role of osteoblasts in focal articular bone loss in RA

The role of osteoblasts in focal articular bone loss in RA has received less attention than that of osteoclasts. Osteoblasts are important regulators of bone remodeling. These cells both produce and mineralize bone matrix and also modulate osteoclast differentiation and function by producing RANKL and OPG (23). Osteoblasts arise from mesenchymal stem cells and undergo maturation and differentiation towards cells with the capacity to produce and mineralize bone matrix (24). As osteoblasts mature towards functional bone-forming cells, they diminish their expression of RANKL and increase their expression of OPG, thereby creating a microenvironment that favors bone formation over bone loss (25).

Interestingly, although RA patients receiving disease modifying anti-rheumatic drug (DMARD) therapy demonstrate slowing or inhibition of progression of articular bone erosions on plain radiographs in clinical trials and long-term observational studies (26), healing of erosions has been considered to occur only infrequently. However, in recent years, repair of bone erosions with formation of new bone has been documented in RA patients treated with DMARDs (27–30). Based on results of two studies conducted by the OMERACT Subcommittee on Healing of Erosions, Sharp and colleagues confirmed that repair of erosions does occur in RA (31). Additional studies demonstrate a strong association between clinical remission of disease and radiographic evidence of repair of erosions (32,33), suggesting that joint inflammation may play an important role in suppressing the functional capacity of osteoblasts to produce bone and thereby repair erosions.

These observations are supported by a recent study from our laboratory in a murine model of arthritis which demonstrated that mineralized bone formation was compromised at sites of erosion in areas of bone where there was local inflammation (34). It would be expected that bone formation rates would be increased at sites of erosion, where bone has been lost. However, using dynamic bone histomorphometry, no differences were observed in the rate of mineralized bone formation at bone surfaces in inflamed areas, as compared to similar areas without arthritis. In addition, there was a notable paucity of osteoblast-lineage cells expressing markers of cell maturity at bone surfaces adjacent to inflammation, but there were abundant immature osteoblast-lineage cells expressing Runx2, a marker of early osteoblast-lineage cells. Furthermore, cellular expression of alkaline phosphatase, a marker of the mineralization phase of bone formation, was minimal at sites of bone erosion. Taken together, these observations support the hypothesis that inflammation may inhibit the functional capacity of osteoblasts to repair erosions in RA.

Wnt signaling pathway in RA

To elucidate the effect of inflammation on osteoblasts and bone formation, investigators have focused on the Wingless (Wnt) family of proteins. The Wnt signaling pathway plays an important regulatory role in embryonic development, tissue homeostasis and cancer (35). Wnt proteins are secreted glycoproteins that bind and activate the receptor complex of a 7-transmembrane domain-spanning frizzled receptor and the low-density lipoprotein receptor-related protein 5 and 6 (LRP5/6) co-receptors present on mesenchymal cells. Binding of Wnt proteins to the receptor complex induces the differentiation of osteoblast-lineage cells into mature osteoblasts, leading to bone formation (36). The importance of the Wnt signaling pathway in bone homeostasis is demonstrated by the abnormal bone phenotypes that result from receptor mutations. Mutations inducing loss-of-function in the Lrp5 gene, which encodes the LRP5 receptor, decrease bone formation in both humans and mice (37). In contrast, gain-of-function mutations in the Lrp5 gene result in increased bone mass (38).

β -catenin plays a critical role in bone remodeling. Modulation of Wnt signaling alters β -catenin expression and activity, thereby impacting both bone formation and osteoclast-mediated bone resorption. Mice that do not express β -catenin on differentiated osteoblasts develop severe osteopenia associated with increased numbers of activated osteoclasts resulting from an increased RANKL:OPG ratio (39,40). In contrast, mice expressing constitutively active β -catenin-mediated Wnt signaling in differentiated osteoblasts have increased OPG expression and exhibit a decrease in bone resorption and an osteopetrotic phenotype (39,40). Interestingly, the functional dysregulation of osteoblasts seen in mice deficient in β -catenin signaling resembles the osteoblast phenotype found at sites of focal bone loss in inflammatory arthritis, suggesting that inhibition of Wnt signaling may be a mechanism whereby bone formation is compromised in inflammatory states.

Several families of endogenous inhibitors of the Wnt signaling pathway have been identified; these function by limiting the effects of Wnt signaling. The Dickkopf (DKK) and secreted frizzled-related protein (sFRP) family members are the best characterized. Diarra and colleagues (41) showed that DKK1, a member of the DKK family of Wnt antagonists, plays a central role in regulating bone remodeling in RA. Administration of a neutralizing antibody specific to DKK1 protected against focal bone erosion in the hTNF.Tg RA mouse model. In fact, in the setting of DKK1 blockade, new bone formation was observed at sites where erosion would typically occur. However, these effects were due, at least in part, to upregulation of OPG expression, which would result in inhibition of bone resorption; the effect of inhibition of Wnt signaling on osteoblast function at sites of erosion was not directly assessed in this study.

The possible role of DKK1 in promoting bone loss is supported by the finding of elevated serum levels of DKK1 in patients with RA (42) and in postmenopausal women with established osteoporosis (43). Recently, Uderhardt and colleagues (44) showed that blocking TNF α in hTNF.Tg mice did not promote ankylosis of sacroiliac joints. In contrast, blocking DKK1 promoted cartilage formation and ankylosis of sacroiliac joints but had no effect on inflammation, indicating a potential role of DKK1 in the anabolic pattern of axial skeletal remodeling that is observed in ankylosing spondylitis. These results indicate that DKK1 may be one of many factors regulating bone remodeling in the rheumatic diseases. In a recent study from our laboratory, we found that several members of the DKK and sFRP families of Wnt signaling inhibitors were expressed at sites of inflammation-induced bone erosion in arthritic mice (34), indicating their putative roles in regulating bone erosion at arthritic sites.

Proinflammatory cytokines in RA, and their effects on bone

Synovial tissue in inflamed joints, infiltrated with activated macrophages and leukocytes, is a rich source of pro-inflammatory cytokines, including TNF α , IL-1, IL-6 and IL-17, and of

growth factors, such as M-CSF, that impact bone remodeling within the RA bone microenvironment (23). The pro-inflammatory cytokines expressed in the microenvironment of the arthritic joint exert their influence both on osteoclast differentiation and activation and on osteoblasts, thereby contributing to progressive joint destruction in RA. Several key cytokines that both mediate inflammation in RA and likely play a role in bone remodeling will be discussed in the following section.

Tumor necrosis factor-alpha (TNF α)

Within the inflamed bone microenvironment in RA, TNF α is a dominant pro-inflammatory cytokine with pleiotropic effects. Among its diverse pathologic effects, TNF α induces the production of other proinflammatory cytokines (IL-1, IL-6, IL-8) (45), stimulates synovial fibroblast cells to express adhesion molecules that attract leukocytes into affected joints (46), increases the rate of synthesis of metalloproteinases by synovial macrophages and fibroblasts (47), and inhibits the synthesis of proteoglycans in cartilage (48). TNF α is synthesized mainly by macrophages and synovial lining cells, as well as by activated T cells, within the microenvironment of the RA joint (49,50). TNF α also exerts important effects on bone remodeling: it regulates the abundance of osteoclast precursors in the bone marrow directly by upregulating c-Fms expression (51) and activates osteoclasts by enhancing RANK signaling mechanisms (52).

TNF α also indirectly influences osteoclastogenesis by inducing expression of RANKL and M-CSF by bone marrow stromal cells of the osteoblast lineage (53). TNF α is considered to be an “upstream,” dominant pro-inflammatory cytokine, based on the observation that anti-TNF α antibodies significantly reduce IL-1 production in cultures of synovial cells from patients with RA (54). TNF α also impacts osteoblasts by inhibiting osteoblast differentiation (55) and maturation (56,57), with an associated decrease in alkaline phosphatase and osteocalcin expression. Addition of TNF α to fetal calvarial precursor cells or MC3T3-E1 pre-osteoblastic cell cultures led to a dose-dependent suppression of mRNA for the critical osteoblast transcription factor Runx2 (58). TNF α also induced apoptosis of MC3T3-E1 cells when added to cell cultures (59).

The role of TNF α in inflammatory arthritis and bone erosion has been studied in detail, using the hTNF.tg mouse model. Both hTNF.tg mice and wild type mice treated with exogenous TNF α have increased numbers of CD11b⁺ osteoclast precursor cells (60,61), an effect of TNF α that is independent of RANKL. Interestingly, differentiation of CD11b⁺ osteoclast precursors into activated osteoclasts requires the presence of functional RANKL/RANK signaling (60). Blocking either TNF α (using anti-TNF α antibodies) or RANKL (using OPG.Fc) signaling in the hTNF.tg murine model results in reduced osteoclastogenesis and reduced bone erosion (62). Interestingly however, blocking both cytokines together (using both anti-TNF α antibodies and OPG.Fc) did not result in a synergistic reduction of osteoclastogenesis (63). In human disease, data from clinical trials confirm that inhibiting TNF α activity in RA effectively protects against bone erosion (64). Although this is due in large part to the anti-inflammatory effects of TNF inhibition, direct reduction of osteoclast-mediated bone loss and augmentation of osteoblast-mediated bone formation are potential mechanisms by which TNF inhibition reduces structural damage in RA.

Interleukin-1 (IL-1)

The pro-inflammatory cytokine IL-1 belongs to a family of cytokines that includes IL-1 α , IL-1 β , the soluble antagonist IL-1Ra, and IL-18. Activated macrophages and synovial fibroblasts present within the inflamed joint are the main sources of IL-1 in RA (49). IL-1 signals through its cognate receptor, IL-1R1. IL-1Ra competes with IL-1 for binding to the IL-1R1 receptor. More than 95% occupancy of the IL-1R1 receptor by IL-1Ra is required to

effectively block IL-1 signaling; however, this may be difficult to achieve in patients with RA (65).

Data from various animal models suggest an important role for IL-1 in the pathogenesis of inflammatory arthritis. Overexpression of IL-1 α or IL-1 β or deficiency of the soluble IL-1 receptor antagonist (IL-1Ra) in murine RA models resulted in the development of arthritis with associated bone and cartilage destruction (66–68). Blocking IL-1 in the hTNF.tg mouse model of RA using recombinant IL-1Ra was not as effective in reducing numbers of osteoclasts or bone erosions as was blocking TNF α , although IL-1Ra effectively reduced inflammation (63). Blocking both IL-1 and TNF α resulted in almost complete suppression of osteoclast differentiation, inflammation, and bone destruction, indicating that the biological effects of these two pro-inflammatory cytokines are additive *in vivo* (63). *In vitro* studies have shown that addition of IL-1Ra inhibited TNF α -mediated induction of RANKL expression in bone marrow stromal cells (53). Furthermore, the addition of TNF α to these stromal cells induced the expression of IL-1 whereas the addition of both IL-1 and TNF α resulted in upregulation of IL-1R1 expression on these cells, indicating a positive feedback loop. Similarly, treating osteoclast precursor cells with TNF α induced the expression of IL-1 and IL-1R1 in these cells and, in turn, IL-1 augmented RANKL-mediated osteoclast differentiation. Also, treating mice deficient in IL-1R1 expression with TNF α resulted in almost 50% reduction in osteoclastogenesis, indicating the existence of IL-1-independent signaling pathways (53). Taken together, these studies provide evidence that IL-1 may be downstream of TNF α in RA.

Addition of IL-1 decreased the apoptotic rate of osteoclast-like cells *in vitro* (69). In addition, IL-1 has been shown to impact osteoblasts/osteoblast lineage cells *in vitro*. Addition of IL-1 α to rat osteoblast cultures decreased formation of mineralized nodules (70). Fetal rat calvarial cell cultures treated with IL-1 β resulted in potent inhibition of mineralized nodule formation (71). These *in-vitro* effects may also be relevant in inflammatory arthritis, though this has not been shown directly.

Interleukin-6 (IL-6)

Interleukin-6 (IL-6) belongs to the family of cytokines that signal via a gp130-dependent mechanism which also includes IL-11, leukemia inhibitory factor (LIF) and oncostatin M (OSM). These cytokines share common receptor subunits and signaling pathways. IL-6 is a pleiotropic proinflammatory cytokine produced by a variety of cell types in the inflamed RA bone microenvironment including macrophages, fibroblast-like synoviocytes, and chondrocytes (72). Synovial fluid levels of IL-6 are elevated in patients with RA and circulating levels of IL-6 correlate with progressive joint damage in RA (73,74), indicating an important role for IL-6 in the pathogenesis of RA. Furthermore, in RA patients, levels of IL-6 and its soluble receptor (sIL-6R) have been correlated with the degree of bone loss evident on plain radiographs (75).

IL-6 modulates osteoclast differentiation by modulating its interaction with the sIL-6R complex that is present on osteoblast lineage cells, resulting in upregulation of cyclooxygenase (COX)-2-dependent PGE₂ synthesis. This, in turn, upregulates RANKL expression while downregulating OPG expression, leading to enhanced osteoclastogenesis (76). In a recent study, *in vitro* blocking of IL-6R reduced osteoclast formation in mouse monocyte cells stimulated with either RANKL or RANKL plus TNF (77). Addition of IL-6 also stimulated osteoclast-like multinucleated cell formation in long term human bone marrow cultures by inducing synthesis of IL-1 β (78). Furthermore, administration of blocking antibodies directed against IL-6R in hTNF.tg mice significantly reduced osteoclast formation and bone erosion, while not reducing joint inflammation, indicating that IL-6 exerts a specific and direct inhibitory effect on osteoclastogenesis both *in vitro* and *in vivo*. That IL-6 is an important

effector of inflammation-induced joint destruction in arthritis is supported by the observation that mice deficient in IL-6 were protected against bone destruction in an antigen-induced arthritis model (79,80). Blocking the IL-6 receptor in a murine collagen-induced arthritis (CIA) model delayed the onset of inflammation and reduced joint destruction. In addition, administration of IL-6R-neutralizing antibody at the time of CIA induction completely abolished the inflammatory response (81), indicating that IL-6 plays an important role in the initiation of arthritis. Accordingly, blockade of the interaction between IL-6 and its cognate receptor has been developed as a treatment for RA.

Interleukin-17 (IL-17)

The IL-17 cytokine family, which has at least six members, plays an important role in the pathogenesis of RA (82). IL-17 is synthesized by Th17 cells, a novel population of T helper cells that is distinct from the previously described Th1 and Th2 cell populations and that has a unique pattern of cytokine expression (83). High levels of IL-17 have been detected in synovial fluid specimens obtained from RA patients (84). In RA synovium, IL-17 is expressed in areas that are rich in T-cells (85). The addition of IL-17 to osteoblast-lineage cells induces the expression of RANKL and downregulates OPG expression (84). Furthermore, in cultured RA synoviocytes, the addition of exogenous IL-17 synergized with IL-1 and TNF α to induce the expression of IL-1, IL-6 and TNF α *in vitro* (86). IL-17 promoted bone erosion in a murine CIA model by upregulating the expression of RANKL and RANK, thereby enhancing osteoclastogenesis (87). In mice, blocking IL-17 after the onset of CIA reduced joint inflammation and bone erosion (88), whereas blocking IL-17 during reactivation of antigen-induced arthritis reduced both joint inflammation and bone erosion by suppressing RANKL, IL-1 and TNF α production (89). Interestingly, the development of spontaneous arthritis was completely suppressed in the progeny of IL-1Ra-deficient mice crossed with IL-17 deficient mice, indicating that both IL-17 and IL-1 are necessary for this spontaneous development of arthritis (90). IL-17 has also been shown to impact osteoblast lineage cells. Addition of IL-17 enhanced TNF α -stimulated IL-6 synthesis in osteoblast-like cells via activation of the p38 mitogen-activated protein (91) and also induced the expression of RANKL mRNA in mouse osteoblasts (84). Because blocking IL-17 attenuates both inflammation and bone erosion in murine models of inflammatory arthritis, IL-17 inhibition has emerged as an approach to treat RA. Table 1 lists the reported effects of pro-inflammatory cytokines on osteoclasts and osteoblasts.

EFFECTS OF THERAPEUTIC INTERVENTIONS ON BONE REMODELING IN RA

Over the past decade, the introduction of targeted biologic therapy has resulted in significantly improved clinical and structural outcomes for patients with RA. These therapeutic agents have specific mechanisms of action, including inhibiting the action of individual cytokines, blocking cell-cell interactions, and depleting certain cell types. Observations of the effect of each targeted therapy on bone loss in patients with RA has provided further information about the role of each of these pathways in the pathophysiology of bone destruction in this disease.

RANKL blockade

The therapeutic potential of blocking of the biologic actions of RANKL was initially demonstrated in postmenopausal women (92). A single injection of OPG.Fc resulted in a rapid and sustained reduction in urinary NTX, an indicator of bone resorption. In a subsequent phase I study conducted in patients with multiple myeloma (MM) or breast cancer-related bone metastases, administration of a recombinant OPG.Fc construct also resulted in a rapid, sustained, dose-dependent reduction in urinary NTX (93). These results suggested the

therapeutic potential of blocking RANKL to prevent further bone loss in patients with osteoporosis and bone malignancies. However, because OPG.Fc is a large, complex protein that requires frequent dosing in patients, further development of OPG.Fc as a therapeutic agent was curtailed in favor of the development of antibodies that directly target RANKL. Denosumab (formerly known as AMG 162), is a fully humanized IgG2 monoclonal antibody that inhibits the biologic activity of RANKL and has been studied in clinical trials in patients with RA and focal bone loss as well as in patients with osteoporosis.

In a study of 412 postmenopausal women with low bone mineral density, denosumab administration increased bone mineral density and decreased bone resorption (94). This finding was confirmed in the recently concluded Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months (FREEDOM) study, which enrolled 7868 women between the ages of 60 and 90. Blockade of RANKL with denosumab reduced the risk of spinal and hip fractures by 68% and 40%, respectively, in postmenopausal women with osteoporosis as compared to placebo-treated subjects (95). In a phase II clinical trial, administration of denosumab to patients with RA inhibited the development of structural damage evident on MRI, produced a sustained decrease in markers of bone turnover, and resulted in increased bone mineral density (96). Radiographic progression of structural damage over a period of 12 months was also significantly decreased among RA patients who received denosumab, as compared to those who received placebo. These studies suggest the promise of osteoclast inhibition as a therapeutic modality to protect against both focal and systemic bone loss in RA, osteoporosis, and other diseases in which bone is destroyed.

Targeting pro-inflammatory cytokines

Anti-TNF biologic agents—TNF α is a dominant pro-inflammatory cytokine in the pathophysiology of RA and is the target of five biologic agents now used to treat RA: adalimumab, certolizumab pegol, etanercept, golimumab, and infliximab. The efficacy of these TNF α antagonists has been demonstrated primarily in two different populations of patients with RA: individuals with active early RA who had not yet been treated with methotrexate and those with RA of longer disease duration who were inadequately responsive to methotrexate. The administration of adalimumab (97), etanercept (98,99), golimumab (100), and infliximab (101) to patients with active early RA significantly reduced clinical signs of disease activity and effected clinical remission in some patients. Among these patients, adalimumab (97), etanercept (102,103), and infliximab (64) each also slowed progression of structural damage, more so when administered in combination with methotrexate than when used as monotherapy (97,103). Among patients with active RA of longer duration, adalimumab (104), etanercept (105), and infliximab (106,107), each administered in combination with methotrexate, have provided long-term sustained improvement in clinical signs and symptoms of RA and slowed progression of structural damage. Both of the newer TNF antagonists, certolizumab pegol (108–110) and golimumab (100,111), when administered either alone or in combination with methotrexate, have also significantly reduced clinical evidence of disease activity and effected clinical remission in some patients.

The goal of RA treatment is to achieve complete remission of disease activity and halt progression of structural damage. Despite “tight control” of clinical disease activity, treating to the target of a “low” disease activity state, progressive joint destruction becomes evident on radiographs of patients not receiving TNF antagonist therapy (112,113). The addition of a TNF antagonist to the therapeutic regimen decreases the rate at which structural damage progresses among patients with active RA (97,103,113). The combination of methotrexate and TNF antagonist therapy slows this radiographic progression more than treatment with methotrexate alone, at any level of clinical response (97,103,114). In a study comparing methotrexate and etanercept monotherapy with the combination of etanercept plus methotrexate in patients with

active RA, erosions did not progress over two years in 86% of patients treated with combination therapy, as compared to 75% of patients receiving etanercept alone and 66% of those receiving methotrexate alone. The combination of etanercept plus methotrexate resulted in a negative mean change in the total Sharp score over two years, suggesting that structural damage improves when a TNF antagonist is used in combination with methotrexate therapy (103). In another study that compared the combination of infliximab plus methotrexate to methotrexate monotherapy, structural damage progressed less among RA patients treated with infliximab plus methotrexate than among those treated with methotrexate alone, regardless of disease activity. Among those patients treated with methotrexate alone, progression of structural damage was associated with elevated levels of acute phase reactants or persistent disease activity (64). In fact, RA patients may demonstrate slowing of radiographic progression with etanercept or infliximab therapy, despite experiencing a suboptimal clinical response (106, 115). Thus, the effect of TNF antagonist therapy on reducing the progression of structural damage may be dissociated from the effect of these agents on decreasing signs and symptoms of RA. This is likely due to the independent effects of TNF α on osteoclast differentiation and function.

Inhibition of IL-1—Interleukin-1 (IL-1) plays an important role in the progression of structural damage in RA by contributing to destruction of cartilage, bone, and periarticular tissues. Recombinant human IL-1Ra (anakinra) reduces the rate of radiographic progression of RA, as compared to treatment with placebo. (116). However, anakinra provides only modest benefit in improving signs and symptoms of active RA as monotherapy (117) or in combination with methotrexate (118). Treatment with anakinra in combination with etanercept was no more effective in controlling signs and symptoms of RA than treatment with etanercept alone. Because the incidence of serious infections, injection site reactions, and neutropenia was higher among patients treated with combination therapy, the use of anakinra together with other biologic therapies is not recommended (119).

Anti-IL-6 receptor biologic agent—Tocilizumab is a humanized anti-IL-6 receptor monoclonal antibody that binds to both the membrane-bound and soluble forms of the IL-6 receptor and blocks binding of IL-6 to its receptor, thereby preventing signaling. The combination of tocilizumab and methotrexate (120,121) or of tocilizumab and DMARD therapy (122) was superior to methotrexate or DMARD monotherapy in controlling signs and symptoms of active RA among patients inadequately responsive to either methotrexate or DMARDs, respectively. The combination of tocilizumab and methotrexate also was more effective than methotrexate monotherapy in controlling signs and symptoms of RA among patients who previously had been exposed to a TNF antagonist (123). Both among patients with active RA who were naïve to methotrexate or had discontinued methotrexate for reasons other than lack of efficacy or toxicity (124) and among patients with active RA who were inadequately responsive to methotrexate monotherapy (120), tocilizumab monotherapy was superior to methotrexate monotherapy in improving signs and symptoms of disease. Based upon the results of these clinical trials, tocilizumab has been approved for the treatment of patients with active RA.

In a study of Japanese RA patients inadequately responsive to DMARDs, including low-dose weekly methotrexate, tocilizumab monotherapy improved signs and symptoms of disease and reduced progression of bone erosion and joint cartilage space narrowing on plain radiographs significantly more than did continuation of DMARD therapy alone (125). Similarly, in a multinational clinical trial that enrolled patients with active RA who were inadequately responsive to methotrexate monotherapy, tocilizumab in combination with methotrexate was superior to methotrexate alone both in improving signs and symptoms of RA and in slowing the progression of radiographic evidence of structural damage (126). These findings support a significant role for IL-6 in the pathogenesis of joint destruction in RA.

Anti-IL-17 biologic agents—Initial results of phase II clinical trials of two monoclonal antibodies that neutralize IL-17 suggest that treatment with anti-IL-17 antibodies reduces the signs and symptoms of RA without notable adverse effects. In a randomized, placebo-controlled study of 77 RA patients treated with the monoclonal anti-IL-17 antibody LY2439821, clinical improvement was evident within a week of beginning drug therapy and persisted for 8 weeks (127). In another randomized, placebo-controlled study of 52 RA patients treated with the monoclonal anti-IL-17 antibody AIN457 for 6 weeks, signs and symptoms of RA also improved (128). However, both of these studies were of inadequate duration to assess progression of structural damage. Larger and longer multicenter, placebo-controlled clinical trials of these antibodies are needed to confirm these promising initial results.

Targeting B and T cells

B-cell-depleting monoclonal antibody—Rituximab is a chimeric monoclonal anti-CD20 monoclonal antibody that depletes B cells. Rituximab improved the signs and symptoms of RA significantly better than placebo, 24 weeks after two intravenous infusions of 1000 mg administered 14 days apart in combination with background methotrexate therapy, both in patients who had responded inadequately to treatment with a TNF antagonist (129) and in those who had responded inadequately to methotrexate therapy (130). Among patients inadequately responsive to TNF antagonist therapy, treatment with rituximab and methotrexate was superior to methotrexate alone in slowing the progression of both bone erosion and joint cartilage space narrowing on plain radiographs (131). This finding supports a significant role for B cells in the pathogenesis of bone and joint destruction in RA. However, the occurrence of the rare, usually fatal, central nervous system demyelinating disease progressive multifocal leukoencephalopathy in some patients with RA who were treated with rituximab may limit its widespread use, especially in patients with early disease who have not yet failed treatment with other medications (132).

Inhibition of T-cell co-stimulation—Abatacept is a fusion protein of recombinant, dimerized cytotoxic T-lymphocyte antigen 4 (CTLA-4), a natural inhibitor of T-cell activation, and the hinge, CH2, and CH3 domains of IgG1. By blocking the interaction of CD28 on a T-cell with B7 on an antigen presenting cell, abatacept interferes with T-cell co-stimulation. In patients with active RA inadequately responsive to a TNF antagonist, abatacept in combination with background DMARD therapy was more effective than background DMARD therapy alone in reducing the signs and symptoms of RA and improving physical function (133). The combination of abatacept and methotrexate also was more effective than methotrexate monotherapy in controlling signs and symptoms of RA and in improving physical function among patients with RA active despite methotrexate therapy (134). In combination with background methotrexate therapy, abatacept slowed the progression of both bone erosion and joint cartilage space narrowing significantly on plain radiographs (134,135). These findings support a role for activated T cells in the pathogenesis of joint destruction in RA.

CONCLUSION

We have reviewed the current understanding of pathogenic mechanisms of focal articular bone damage in RA and discussed current therapeutic approaches to prevent this damage. In RA, cells within the inflamed synovium and pannus elaborate a variety of cytokines and factors that impact both osteoclast-mediated bone erosion and osteoblast-mediated bone repair. The RANKL/RANK/OPG pathway plays a critical role in regulating osteoclastogenesis in RA. Pro-inflammatory cytokines, including TNF- α , IL-1, IL-6 and IL-17, are expressed by various cell types in the inflamed synovium and exert effects on osteoclastogenesis as well as on osteoclast function and survival. Taken together, the results of clinical trials of targeted biologic therapies for RA indicate that multiple cytokines and cell types contribute to the pathogenesis

of inflammation, and suggest possible direct effects on destruction of bone. Further study of these and additional novel targeted therapies, both in animal models and in human subjects, will clarify the molecular mechanisms of this important pathological process.

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References

1. Gravallesse, EM.; Monach, PA. Rheumatoid synovitis and pannus. In: Hochberg, MSA.; Smolen, J.; Weinblatt, M.; Weisman, M., editors. Rheumatology. 4. London, UK: Elsevier Ltd; 2008. p. 841-65.
2. Scott DL. Radiological progression in established rheumatoid arthritis. J Rheumatol Suppl 2004 Mar; 69:55-65. [PubMed: 15053455]
3. Sambrook PN. The skeleton in rheumatoid arthritis: common mechanisms for bone erosion and osteoporosis? J Rheumatol 2000 Nov;27(11):2541-2. [PubMed: 11093430]
4. Joffe I, Epstein S. Osteoporosis associated with rheumatoid arthritis: pathogenesis and management. Semin Arthritis Rheum 1991 Feb;20(4):256-72. [PubMed: 2042057]
5. Wollheim FA. Established and new biochemical tools for diagnosis and monitoring of rheumatoid arthritis. Curr Opin Rheumatol 1996 May;8(3):221-5. [PubMed: 8796981]
6. Bromley M, Woolley DE. Chondroclasts and osteoclasts at subchondral sites of erosion in the rheumatoid joint. Arthritis Rheum 1984 Sep;27(9):968-75. [PubMed: 6236824]
7. Gravallesse EM, Harada Y, Wang, et al. Identification of cell types responsible for bone resorption in rheumatoid arthritis and juvenile rheumatoid arthritis. Am J Pathol 1998 Apr;152(4):943-51. [PubMed: 9546355]
8. Gravallesse EM, Manning C, Tsay A, et al. Synovial tissue in rheumatoid arthritis is a source of osteoclast differentiation factor. Arthritis Rheum 2000 Feb;43(2):250-8. [PubMed: 10693863]
9. Haynes DR, Crotti TN, Loric M, et al. Osteoprotegerin and receptor activator of nuclear factor kappaB ligand (RANKL) regulate osteoclast formation by cells in the human rheumatoid arthritic joint. Rheumatology (Oxford) 2001 Jun;40(6):623-30. [PubMed: 11426018]
10. Kong YY, Yoshida H, Sarosi I, et al. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 1999 Jan 28;397(6717):315-23. [PubMed: 9950424]
11. Pettit AR, Ji H, von Stechow D, et al. TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. Am J Pathol 2001 Nov;159(5):1689-99. [PubMed: 11696430]
12. Keffer J, Probert L, Cazlaris H, et al. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. EMBO J 1991 Dec;10(13):4025-31. [PubMed: 1721867]
13. Redlich K, Hayer S, Ricci R, et al. Osteoclasts are essential for TNF-alpha-mediated joint destruction. J Clin Invest 2002 Nov;110(10):1419-27. [PubMed: 12438440]
14. Kong YY, Feige U, Sarosi I, et al. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 1999 Nov 18;402(6759):304-9. [PubMed: 10580503]
15. Schett G, Redlich K, Hayer S, et al. Osteoprotegerin protects against generalized bone loss in tumor necrosis factor-transgenic mice. Arthritis Rheum 2003 Jul;48(7):2042-51. [PubMed: 12847699]
16. Herrak P, Gortz B, Hayer S, et al. Zoledronic acid protects against local and systemic bone loss in tumor necrosis factor-mediated arthritis. Arthritis Rheum 2004 Jul;50(7):2327-37. [PubMed: 15248234]
17. Sims NA, Green JR, Glatt M, et al. Targeting osteoclasts with zoledronic acid prevents bone destruction in collagen-induced arthritis. Arthritis Rheum 2004 Jul;50(7):2338-46. [PubMed: 15248235]

18. Tsurukai T, Udagawa N, Matsuzaki K, et al. Roles of macrophage-colony stimulating factor and osteoclast differentiation factor in osteoclastogenesis. *J Bone Miner Metab* 2000;18(4):177–84. [PubMed: 10874596]
19. Tanaka S, Takahashi N, Udagawa N, et al. Macrophage colony-stimulating factor is indispensable for both proliferation and differentiation of osteoclast progenitors. *J Clin Invest* 1993 Jan;91(1):257–63. [PubMed: 8423223]
20. Simonet WS, Lacey DL, Dunstan CR, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 1997 Apr 18;89(2):309–19. [PubMed: 9108485]
21. Skoumal M, Kolarz G, Haberhauer G, et al. Osteoprotegerin and the receptor activator of NF-kappa B ligand in the serum and synovial fluid. A comparison of patients with longstanding rheumatoid arthritis and osteoarthritis. *Rheumatol Int* 2005 Nov;26(1):63–9. [PubMed: 15889303]
22. Geusens PP, Landewe RB, Garnero P, et al. The ratio of circulating osteoprotegerin to RANKL in early rheumatoid arthritis predicts later joint destruction. *Arthritis Rheum* 2006 Jun;54(6):1772–7. [PubMed: 16736519]
23. Lorenzo J, Horowitz M, Choi Y. Osteoimmunology: interactions of the bone and immune system. *Endocr Rev* 2008 Jun;29(4):403–40. [PubMed: 18451259]
24. Aubin JE. Bone stem cells. *J Cell Biochem Suppl* 1998;30–31:73–82. [PubMed: 19594448]
25. Atkins GJ, Kostakis P, Pan B, et al. RANKL expression is related to the differentiation state of human osteoblasts. *J Bone Miner Res* 2003 Jun;18(6):1088–98. [PubMed: 12817763]
26. Pincus T, Ferraccioli G, Sokka T, et al. Evidence from clinical trials and long-term observational studies that disease-modifying anti-rheumatic drugs slow radiographic progression in rheumatoid arthritis: updating a 1983 review. *Rheumatology (Oxford)* 2002 Dec;41(12):1346–56. [PubMed: 12468813]
27. Menninger H, Meixner C, Sondgen W. Progression and repair in radiographs of hands and forefeet in early rheumatoid arthritis. *J Rheumatol* 1995 Jun;22(6):1048–54. [PubMed: 7674229]
28. Rau R, Wassenberg S, Herborn G, et al. Identification of radiologic healing phenomena in patients with rheumatoid arthritis. *J Rheumatol* 2001 Dec;28(12):2608–15. [PubMed: 11764205]
29. Sokka T, Hannonen P. Healing of erosions in rheumatoid arthritis. *Ann Rheum Dis* 2000 Aug;59(8):647–9. [PubMed: 10913064]
30. Ideguchi H, Ohno S, Hattori H, et al. Bone erosions in rheumatoid arthritis can be repaired through reduction in disease activity with conventional disease-modifying antirheumatic drugs. *Arthritis Res Ther* 2006;8(3):R76. [PubMed: 16646983]
31. Sharp JT, Van Der Heijde D, Boers M, et al. Repair of erosions in rheumatoid arthritis does occur. Results from 2 studies by the OMERACT Subcommittee on Healing of Erosions. *J Rheumatol* 2003 May;30(5):1102–7. [PubMed: 12734916]
32. Voskuyl AE, Dijkmans BA. Remission and radiographic progression in rheumatoid arthritis. *Clin Exp Rheumatol* 2006 Nov–Dec;24(6 Suppl 43):S-37–40.
33. Rau R. Is remission in rheumatoid arthritis associated with radiographic healing? *Clin Exp Rheumatol* 2006 Nov–Dec;24(6 Suppl 43):S-41–4. [PubMed: 16859596]
34. Walsh NC, Reinwald S, Manning CA, et al. Osteoblast function is compromised at sites of focal bone erosion in inflammatory arthritis. *J Bone Miner Res* 2009 Sep;24(9):1572–85. [PubMed: 19338457]
35. Clevers H. Wnt/beta-catenin signaling in development and disease. *Cell* 2006 Nov 3;127(3):469–80. [PubMed: 17081971]
36. Schett G, Zwerina J, David JP. The role of Wnt proteins in arthritis. *Nat Clin Pract Rheumatol* 2008 Sep;4(9):473–80. [PubMed: 18756273]
37. Gong Y, Slee RB, Fukai N, et al. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. *Cell* 2001 Nov 16;107(4):513–23. [PubMed: 11719191]
38. Johnson ML. The high bone mass family--the role of Wnt/Lrp5 signaling in the regulation of bone mass. *J Musculoskelet Neuronal Interact* 2004 Jun;4(2):135–8. [PubMed: 15615112]
39. Glass DA 2nd, Bialek P, Ahn JD, et al. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. *Dev Cell* 2005 May;8(5):751–64. [PubMed: 15866165]
40. Holmen SL, Zylstra CR, Mukherjee A, et al. Essential role of beta-catenin in postnatal bone acquisition. *J Biol Chem* 2005 Jun 3;280(22):21162–8. [PubMed: 15802266]

41. Diarra D, Stolina M, Polzer K, et al. Dickkopf-1 is a master regulator of joint remodeling. *Nat Med* 2007 Feb;13(2):156–63. [PubMed: 17237793]
42. Daoussis D, Liossis SN, Solomou EE, et al. Evidence that Dkk-1 is dysfunctional in ankylosing spondylitis. *Arthritis Rheum* 2010 Jan;62(1):150–8. [PubMed: 20039407]
43. Anastasilakis AD, Polyzos SA, Avramidis A, et al. The effect of teriparatide on serum Dickkopf-1 levels in postmenopausal women with established osteoporosis. *Clin Endocrinol (Oxf)*. 2009 Oct 15;
44. Uderhardt S, Diarra D, Katzenbeisser J, et al. Blockade of Dickkopf-1 induces fusion of sacroiliac joints. *Ann Rheum Dis*. 2009 Mar 26;
45. Butler DM, Maini RN, Feldmann M, et al. Modulation of proinflammatory cytokine release in rheumatoid synovial membrane cell cultures. Comparison of monoclonal anti TNF-alpha antibody with the interleukin-1 receptor antagonist. *Eur Cytokine Netw* 1995 Jul–Dec;6(4):225–30. [PubMed: 8789287]
46. Chin JE, Winterrowd GE, Krzesicki RF, et al. Role of cytokines in inflammatory synovitis. The coordinate regulation of intercellular adhesion molecule 1 and HLA class I and class II antigens in rheumatoid synovial fibroblasts. *Arthritis Rheum* 1990 Dec;33(12):1776–86. [PubMed: 1701992]
47. Burrage PS, Mix KS, Brinckerhoff CE. Matrix metalloproteinases: role in arthritis. *Front Biosci* 2006;11:529–43. [PubMed: 16146751]
48. Hardingham TE, Bayliss MT, Rayan V, et al. Effects of growth factors and cytokines on proteoglycan turnover in articular cartilage. *Br J Rheumatol* 1992;31(Suppl 1):1–6. [PubMed: 1532522]
49. MacNaul KL, Hutchinson NI, Parsons JN, et al. Analysis of IL-1 and TNF-alpha gene expression in human rheumatoid synoviocytes and normal monocytes by in situ hybridization. *J Immunol* 1990 Dec 15;145(12):4154–66. [PubMed: 2258613]
50. Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. *N Engl J Med* 2001 Mar 22;344(12):907–16. [PubMed: 11259725]
51. Yao Z, Li P, Zhang Q, et al. Tumor necrosis factor-alpha increases circulating osteoclast precursor numbers by promoting their proliferation and differentiation in the bone marrow through up-regulation of c-Fms expression. *J Biol Chem* 2006 Apr 28;281(17):11846–55. [PubMed: 16461346]
52. Lam J, Takeshita S, Barker JE, et al. TNF-alpha induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. *J Clin Invest* 2000 Dec;106(12):1481–8. [PubMed: 11120755]
53. Wei S, Kitaura H, Zhou P, et al. IL-1 mediates TNF-induced osteoclastogenesis. *J Clin Invest* 2005 Feb;115(2):282–90. [PubMed: 15668736]
54. Brennan FM, Chantry D, Jackson A, et al. Inhibitory effect of TNF alpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. *Lancet* 1989 Jul 29;2(8657):244–7. [PubMed: 2569055]
55. Gilbert L, He X, Farmer P, et al. Inhibition of osteoblast differentiation by tumor necrosis factor-alpha. *Endocrinology* 2000 Nov;141(11):3956–64. [PubMed: 11089525]
56. Kuroki T, Shingu M, Koshihara Y, et al. Effects of cytokines on alkaline phosphatase and osteocalcin production, calcification and calcium release by human osteoblastic cells. *Br J Rheumatol* 1994 Mar; 33(3):224–30. [PubMed: 8156283]
57. Panagakos FS, Hinojosa LP, Kumar S. Formation and mineralization of extracellular matrix secreted by an immortal human osteoblastic cell line: modulation by tumor necrosis factor-alpha. *Inflammation* 1994 Jun;18(3):267–84. [PubMed: 8088924]
58. Gilbert L, He X, Farmer P, et al. Expression of the osteoblast differentiation factor RUNX2 (Cbfa1/ AML3/Pebp2alpha A) is inhibited by tumor necrosis factor-alpha. *J Biol Chem* 2002 Jan 25;277(4): 2695–701. [PubMed: 11723115]
59. Jilka RL, Weinstein RS, Bellido T, Parfitt AM, Manolagas SC. Osteoblast programmed cell death (apoptosis): modulation by growth factors and cytokines. *J Bone Miner Res* 1998 May;13(5):793–802. [PubMed: 9610743]
60. Li P, Schwarz EM, O'Keefe RJ, et al. 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 Feb;19(2):207–13. [PubMed: 14969390]

61. Li P, Schwarz EM, O'Keefe RJ, et al. Systemic tumor necrosis factor alpha mediates an increase in peripheral CD11b^{high} osteoclast precursors in tumor necrosis factor alpha-transgenic mice. *Arthritis Rheum* 2004 Jan;50(1):265–76. [PubMed: 14730625]
62. Redlich K, Hayer S, Maier A, et al. Tumor necrosis factor alpha-mediated joint destruction is inhibited by targeting osteoclasts with osteoprotegerin. *Arthritis Rheum* 2002 Mar;46(3):785–92. [PubMed: 11920416]
63. Zwerina J, Hayer S, Tohidast-Akrad M, 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 Jan;50(1):277–90. [PubMed: 14730626]
64. Smolen JS, Van Der Heijde DM, St Clair EW, et al. Predictors of joint damage in patients with early rheumatoid arthritis treated with high-dose methotrexate with or without concomitant infliximab: results from the ASPIRE trial. *Arthritis Rheum* 2006 Mar;54(3):702–10. [PubMed: 16508926]
65. Abramson SB, Amin A. Blocking the effects of IL-1 in rheumatoid arthritis protects bone and cartilage. *Rheumatology (Oxford)* 2002 Sep;41(9):972–80. [PubMed: 12209029]
66. Niki Y, Yamada H, Kikuchi T, et al. Membrane-associated IL-1 contributes to chronic synovitis and cartilage destruction in human IL-1 alpha transgenic mice. *J Immunol* 2004 Jan 1;172(1):577–84. [PubMed: 14688369]
67. Ghivizzani SC, Kang R, Georgescu HI, et al. Constitutive intra-articular expression of human IL-1 beta following gene transfer to rabbit synovium produces all major pathologies of human rheumatoid arthritis. *J Immunol* 1997 Oct 1;159(7):3604–12. [PubMed: 9317160]
68. Horai R, Saijo S, Tanioka H, et al. Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *J Exp Med* 2000 Jan 17;191(2):313–20. [PubMed: 10637275]
69. Jimi E, Shuto T, Koga T. Macrophage colony-stimulating factor and interleukin-1 alpha maintain the survival of osteoclast-like cells. *Endocrinology* 1995 Feb;136(2):808–11. [PubMed: 7835314]
70. Tanabe N, Ito-Kato E, Suzuki N, et al. IL-1alpha affects mineralized nodule formation by rat osteoblasts. *Life Sci* 2004 Sep 24;75(19):2317–27. [PubMed: 15350829]
71. Stashenko P, Dewhirst FE, Rooney ML, et al. Interleukin-1 beta is a potent inhibitor of bone formation in vitro. *J Bone Miner Res* 1987 Dec;2(6):559–65. [PubMed: 3502684]
72. Okamoto H, Yamamura M, Morita Y, et al. The synovial expression and serum levels of interleukin-6, interleukin-11, leukemia inhibitory factor, and oncostatin M in rheumatoid arthritis. *Arthritis Rheum* 1997 Jun;40(6):1096–105. [PubMed: 9182920]
73. Hirano T, Matsuda T, Turner M, et al. Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis. *Eur J Immunol* 1988 Nov;18(11):1797–801. [PubMed: 2462501]
74. Dasgupta B, Corkill M, Kirkham B, et al. Serial estimation of interleukin 6 as a measure of systemic disease in rheumatoid arthritis. *J Rheumatol* 1992 Jan;19(1):22–5. [PubMed: 1556695]
75. Kotake S, Sato K, Kim KJ, 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 Jan;11(1):88–95. [PubMed: 8770701]
76. Liu XH, Kirschenbaum A, Yao S, et al. Cross-talk between the interleukin-6 and prostaglandin E(2) signaling systems results in enhancement of osteoclastogenesis through effects on the osteoprotegerin/receptor activator of nuclear factor- κ B (RANK) ligand/RANK system. *Endocrinology* 2005 Apr;146(4):1991–8. [PubMed: 15618359]
77. Axmann R, Bohm C, Kronke G, et al. Inhibition of interleukin-6 receptor directly blocks osteoclast formation in vitro and in vivo. *Arthritis Rheum* 2009 Sep;60(9):2747–56. [PubMed: 19714627]
78. Kurihara N, Bertolini D, Suda T, et al. IL-6 stimulates osteoclast-like multinucleated cell formation in long term human marrow cultures by inducing IL-1 release. *J Immunol* 1990 Jun 1;144(11):4226–30. [PubMed: 2341718]
79. Boe A, Baiocchi M, Carbonatto M, et al. Interleukin 6 knock-out mice are resistant to antigen-induced experimental arthritis. *Cytokine* 1999 Dec;11(12):1057–64. [PubMed: 10623431]
80. Ohshima S, Saeki Y, Mima T, et al. Interleukin 6 plays a key role in the development of antigen-induced arthritis. *Proc Natl Acad Sci U S A* 1998 Jul 7;95(14):8222–6. [PubMed: 9653168]

81. Takagi N, Mihara M, Moriya Y, et al. Blockage of interleukin-6 receptor ameliorates joint disease in murine collagen-induced arthritis. *Arthritis Rheum* 1998 Dec;41(12):2117–21. [PubMed: 9870868]
82. Lubberts E. IL-17/Th17 targeting: on the road to prevent chronic destructive arthritis? *Cytokine* 2008 Feb;41(2):84–91. [PubMed: 18039580]
83. Harrington LE, Hatton RD, Mangan PR, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005 Nov;6(11):1123–32. [PubMed: 16200070]
84. Kotake S, Udagawa N, Takahashi N, et al. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. *J Clin Invest* 1999 May;103(9):1345–52. [PubMed: 10225978]
85. Chabaud M, Durand JM, Buchs N, et al. Human interleukin-17: A T cell-derived proinflammatory cytokine produced by the rheumatoid synovium. *Arthritis Rheum* 1999 May;42(5):963–70. [PubMed: 10323452]
86. Kehlen A, Pachnio A, Thiele K, et al. Gene expression induced by interleukin-17 in fibroblast-like synoviocytes of patients with rheumatoid arthritis: upregulation of hyaluronan-binding protein TSG-6. *Arthritis Res Ther* 2003;5(4):R186–92. [PubMed: 12823853]
87. Lubberts E, van den Bersselaar L, Oppers-Walgreen B, et al. IL-17 promotes bone erosion in murine collagen-induced arthritis through loss of the receptor activator of NF-kappa B ligand/osteoprotegerin balance. *J Immunol* 2003 Mar 1;170(5):2655–62. [PubMed: 12594294]
88. Lubberts E, Koenders MI, Oppers-Walgreen B, et al. Treatment with a neutralizing anti-murine interleukin-17 antibody after the onset of collagen-induced arthritis reduces joint inflammation, cartilage destruction, and bone erosion. *Arthritis Rheum* 2004 Feb;50(2):650–9. [PubMed: 14872510]
89. Koenders MI, Lubberts E, Oppers-Walgreen B, et al. Blocking of interleukin-17 during reactivation of experimental arthritis prevents joint inflammation and bone erosion by decreasing RANKL and interleukin-1. *Am J Pathol* 2005 Jul;167(1):141–9. [PubMed: 15972960]
90. Nakae S, Saijo S, Horai R, et al. IL-17 production from activated T cells is required for the spontaneous development of destructive arthritis in mice deficient in IL-1 receptor antagonist. *Proc Natl Acad Sci U S A* 2003 May 13;100(10):5986–90. [PubMed: 12721360]
91. Tokuda H, Kanno Y, Ishisaki A, et al. Interleukin (IL)-17 enhances tumor necrosis factor-alpha-stimulated IL-6 synthesis via p38 mitogen-activated protein kinase in osteoblasts. *J Cell Biochem* 2004 Apr 1;91(5):1053–61. [PubMed: 15034939]
92. Bekker PJ, Holloway D, Nakanishi A, et al. The effect of a single dose of osteoprotegerin in postmenopausal women. *J Bone Miner Res* 2001 Feb;16(2):348–60. [PubMed: 11204435]
93. Body JJ, Greipp P, Coleman RE, et al. A phase I study of AMGN-0007, a recombinant osteoprotegerin construct, in patients with multiple myeloma or breast carcinoma related bone metastases. *Cancer* 2003 Feb 1;97(3 Suppl):887–92. [PubMed: 12548591]
94. McClung MR, Lewiecki EM, Cohen SB, et al. Denosumab in postmenopausal women with low bone mineral density. *N Engl J Med* 2006 Feb 23;354(8):821–31. [PubMed: 16495394]
95. Cummings SR, San Martin J, McClung MR, et al. Denosumab for prevention of fractures in postmenopausal women with osteoporosis. *N Engl J Med* 2009 Aug 20;361(8):756–65. [PubMed: 19671655]
96. Cohen SB, Dore RK, Lane NE, et al. Denosumab treatment effects on structural damage, bone mineral density, and bone turnover in rheumatoid arthritis: a twelve-month, multicenter, randomized, double-blind, placebo-controlled, phase II clinical trial. *Arthritis Rheum* 2008 May;58(5):1299–309. [PubMed: 18438830]
97. Breedveld FC, Weisman MH, Kavanaugh AF, et al. The PREMIER study: A multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. *Arthritis Rheum* 2006 Jan;54(1):26–37. [PubMed: 16385520]
98. Bathon JM, Martin RW, Fleischmann RM, et al. A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. *N Engl J Med* 2000 Nov 30;343(22):1586–93. [PubMed: 11096165]

99. Klareskog L, van der Heijde D, de Jager JP, et al. Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. *Lancet* 2004 Feb 28;363(9410):675–81. [PubMed: 15001324]
100. Emery P, Fleischmann RM, Moreland LW, et al. Golimumab, a human anti-tumor necrosis factor alpha monoclonal antibody, injected subcutaneously every four weeks in methotrexate-naïve patients with active rheumatoid arthritis: twenty-four-week results of a phase III, multicenter, randomized, double-blind, placebo-controlled study of golimumab before methotrexate as first-line therapy for early-onset rheumatoid arthritis. *Arthritis Rheum* 2009 Aug;60(8):2272–83. [PubMed: 19644849]
101. St Clair EW, van der Heijde DM, Smolen JS, et al. Combination of infliximab and methotrexate therapy for early rheumatoid arthritis: a randomized, controlled trial. *Arthritis Rheum* 2004 Nov; 50(11):3432–43. [PubMed: 15529377]
102. Genovese MC, Bathon JM, Fleischmann RM, et al. Longterm safety, efficacy, and radiographic outcome with etanercept treatment in patients with early rheumatoid arthritis. *J Rheumatol* 2005 Jul;32(7):1232–42. [PubMed: 15996057]
103. van der Heijde D, Klareskog L, Rodriguez-Valverde V, et al. Comparison of etanercept and methotrexate, alone and combined, in the treatment of rheumatoid arthritis: two-year clinical and radiographic results from the TEMPO study, a double-blind, randomized trial. *Arthritis Rheum* 2006 Apr;54(4):1063–74. [PubMed: 16572441]
104. Weinblatt ME, Keystone EC, Furst DE, et al. Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. *Arthritis Rheum* 2003 Jan;48(1):35–45. [PubMed: 12528101]
105. Weinblatt ME, Kremer JM, Bankhurst AD, et al. A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N Engl J Med* 1999 Jan 28;340(4):253–9. [PubMed: 9920948]
106. Smolen JS, Han C, Bala M, et al. Evidence of radiographic benefit of treatment with infliximab plus methotrexate in rheumatoid arthritis patients who had no clinical improvement: a detailed subanalysis of data from the anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy study. *Arthritis Rheum* 2005 Apr;52(4):1020–30. [PubMed: 15818697]
107. Maini RN, Breedveld FC, Kalden JR, et al. Sustained improvement over two years in physical function, structural damage, and signs and symptoms among patients with rheumatoid arthritis treated with infliximab and methotrexate. *Arthritis Rheum* 2004 Apr;50(4):1051–65. [PubMed: 15077287]
108. Fleischmann R, Vencovsky J, van Vollenhoven RF, et al. Efficacy and safety of certolizumab pegol monotherapy every 4 weeks in patients with rheumatoid arthritis failing previous disease-modifying antirheumatic therapy: the FAST4WARD study. *Ann Rheum Dis* 2009 Jun;68(6):805–11. [PubMed: 19015206]
109. Keystone E, Heijde D, Mason D Jr, et al. Certolizumab pegol plus methotrexate is significantly more effective than placebo plus methotrexate in active rheumatoid arthritis: findings of a fifty-two-week, phase III, multicenter, randomized, double-blind, placebo-controlled, parallel-group study. *Arthritis Rheum* 2008 Nov;58(11):3319–29. [PubMed: 18975346]
110. Smolen J, Landewe RB, Mease P, et al. Efficacy and safety of certolizumab pegol plus methotrexate in active rheumatoid arthritis: the RAPID 2 study. A randomised controlled trial. *Ann Rheum Dis* 2009 Jun;68(6):797–804. [PubMed: 19015207]
111. Kay J, Matteson EL, Dasgupta B, et al. Golimumab in patients with active rheumatoid arthritis despite treatment with methotrexate: a randomized, double-blind, placebo-controlled, dose-ranging study. *Arthritis Rheum* 2008 Apr;58(4):964–75. [PubMed: 18383539]
112. Grigor C, Capell H, Stirling A, et al. Effect of a treatment strategy of tight control for rheumatoid arthritis (the TICORA study): a single-blind randomised controlled trial. *Lancet* 2004 Jul 17–23;364(9430):263–9. [PubMed: 15262104]
113. Goekoop-Ruiterman YP, de Vries-Bouwstra JK, Allaart CF, et al. Clinical and radiographic outcomes of four different treatment strategies in patients with early rheumatoid arthritis (the BeSt study): A randomized, controlled trial. *Arthritis Rheum* 2008 Feb;58(2 Suppl):S126–35. [PubMed: 18240203]

114. Lipsky PE, van der Heijde DM, St Clair EW, et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. *N Engl J Med* 2000 Nov 30;343(22):1594–602. [PubMed: 11096166]
115. Landewe R, van der Heijde D, Klareskog L, et al. Disconnect between inflammation and joint destruction after treatment with etanercept plus methotrexate: results from the trial of etanercept and methotrexate with radiographic and patient outcomes. *Arthritis Rheum* 2006 Oct;54(10):3119–25. [PubMed: 17009230]
116. Jiang Y, Genant HK, Watt I, et al. A multicenter, double-blind, dose-ranging, randomized, placebo-controlled study of recombinant human interleukin-1 receptor antagonist in patients with rheumatoid arthritis: radiologic progression and correlation of Genant and Larsen scores. *Arthritis Rheum* 2000 May;43(5):1001–9. [PubMed: 10817552]
117. Bresnihan B, Alvaro-Gracia JM, Cobby M, et al. Treatment of rheumatoid arthritis with recombinant human interleukin-1 receptor antagonist. *Arthritis Rheum* 1998 Dec;41(12):2196–204. [PubMed: 9870876]
118. Cohen S, Hurd E, Cush J, et al. Treatment of rheumatoid arthritis with anakinra, a recombinant human interleukin-1 receptor antagonist, in combination with methotrexate: results of a twenty-four-week, multicenter, randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* 2002 Mar;46(3):614–24. [PubMed: 11920396]
119. Genovese MC, Cohen S, Moreland L, et al. Combination therapy with etanercept and anakinra in the treatment of patients with rheumatoid arthritis who have been treated unsuccessfully with methotrexate. *Arthritis Rheum* 2004 May;50(5):1412–9. [PubMed: 15146410]
120. Maini RN, Taylor PC, Szechinski J, et al. Double-blind randomized controlled clinical trial of the interleukin-6 receptor antagonist, tocilizumab, in European patients with rheumatoid arthritis who had an incomplete response to methotrexate. *Arthritis Rheum* 2006 Sep;54(9):2817–29. [PubMed: 16947782]
121. Smolen JS, Beaulieu A, Rubbert-Roth A, et al. Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial. *Lancet* 2008 Mar 22;371(9617):987–97. [PubMed: 18358926]
122. Genovese MC, McKay JD, Nasonov EL, et al. Interleukin-6 receptor inhibition with tocilizumab reduces disease activity in rheumatoid arthritis with inadequate response to disease-modifying antirheumatic drugs: the tocilizumab in combination with traditional disease-modifying antirheumatic drug therapy study. *Arthritis Rheum* 2008 Oct;58(10):2968–80. [PubMed: 18821691]
123. Emery P, Keystone E, Tony HP, et al. IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: results from a 24-week multicentre randomised placebo-controlled trial. *Ann Rheum Dis* 2008 Nov; 67(11):1516–23. [PubMed: 18625622]
124. Jones G, Sebba A, Gu J, et al. Comparison of tocilizumab monotherapy versus methotrexate monotherapy in patients with moderate to severe rheumatoid arthritis: the AMBITION study. *Ann Rheum Dis* 2010 Jan;69(1):88–96. [PubMed: 19297346]
125. Nishimoto N, Hashimoto J, Miyasaka N, et al. Study of active controlled monotherapy used for rheumatoid arthritis, an IL-6 inhibitor (SAMURAI): evidence of clinical and radiographic benefit from an x ray reader-blinded randomised controlled trial of tocilizumab. *Ann Rheum Dis* 2007 Sep; 66(9):1162–7. [PubMed: 17485422]
126. Fleischmann R, Burgos-Vargas R, Ambs P, Alecock E, Kremer J. LITHE: Tocilizumab inhibits radiographic progression and improves physical function in rheumatoid arthritis (RA) patients (Pts) at 2 yrs with increasing clinical efficacy over time [abstract]. *Arthritis Rheum* 2009;60(Suppl 10): 637.
127. Sloan-Lancaster JGM, Roberson SA, van den Bosch F. Safety, tolerability and evidence of efficacy of intravenous LY2439821 in patients with rheumatoid arthritis receiving background oral DMARDs. *Ann Rheum Dis* 2009;68(Suppl3):125.
128. Durez PCV, Wittmer B, Cohen M, Tornero J, Kivitz A, Coddling C, DiGiovanni R, Gomez-Reino J. AIN457, an anti-IL-17 antibody, shows good safety and induces clinical responses in patients with active rheumatoid arthritis (RA) despite methotrexate therapy in a randomized, double-blind proof-of-concept trial. *Ann Rheum Dis* 2009;68(Suppl3):125.

129. Cohen SB, Emery P, Greenwald MW, et al. Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. *Arthritis Rheum* 2006 Sep;54(9):2793–806. [PubMed: 16947627]
130. Emery P, Fleischmann R, Filipowicz-Sosnowska A, et al. The efficacy and safety of rituximab in patients with active rheumatoid arthritis despite methotrexate treatment: results of a phase IIB randomized, double-blind, placebo-controlled, dose-ranging trial. *Arthritis Rheum* 2006 May;54(5):1390–400. [PubMed: 16649186]
131. Keystone E, Emery P, Peterfy CG, et al. Rituximab inhibits structural joint damage in patients with rheumatoid arthritis with an inadequate response to tumour necrosis factor inhibitor therapies. *Ann Rheum Dis* 2009 Feb;68(2):216–21. [PubMed: 18388156]
132. Fleischmann RM. Progressive multifocal leukoencephalopathy following rituximab treatment in a patient with rheumatoid arthritis. *Arthritis Rheum* 2009 Nov;60(11):3225–8. [PubMed: 19877057]
133. Genovese MC, Becker JC, Schiff M, et al. Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. *N Engl J Med* 2005 Sep 15;353(11):1114–23. [PubMed: 16162882]
134. Kremer JM, Genant HK, Moreland LW, et al. Effects of abatacept in patients with methotrexate-resistant active rheumatoid arthritis: a randomized trial. *Ann Intern Med* 2006 Jun 20;144(12):865–76. [PubMed: 16785475]
135. Kremer JM, Genant HK, Moreland LW, et al. Results of a two-year followup study of patients with rheumatoid arthritis who received a combination of abatacept and methotrexate. *Arthritis Rheum* 2008 Apr;58(4):953–63. [PubMed: 18383390]

Table 1

Reported effects of pro-inflammatory cytokines on cells within bone

Cytokine	Effects on osteoclasts (OC)	Effects on osteoblasts (OB)
RANKL	Required for OC activation and differentiation (10)	
TNF	Stimulates OC precursor differentiation; synergistic with RANKL in OC differentiation (52) Increases CD11b high osteoclast precursors (61) Increases osteoclast precursor numbers through upregulation of c-Fms expression (51)	Inhibits OB differentiation (55) Inhibits Runx2, OB differentiation factor (58) Induces expression of RANKL, M-CSF in OB-lineage cells (53) Induces OB apoptosis (59)
IL-1 (α and β)	Increases osteoclastogenesis, along with TNF (53) Decreases apoptotic rate of OCs (69)	IL-1 α : Inhibits differentiation, matrix formation <i>in-vitro</i> (70) IL-1 β : Inhibits bone collagen synthesis <i>in-vitro</i> (71)
IL-6	Blocking of IL-6R reduces OC formation (77) Stimulates OC-like multinucleated cell formation in long term human marrow culture (78)	Indirectly increases osteoclastogenesis by stimulating PGE ₂ synthesis, increasing RANKL/OPG in OBs (76)
IL-17	Increases osteoclastogenesis; induces RANKL and RANK (84, 87)	Indirectly upregulates IL-6 via TNF α (91) Increases RANKL/OPG in OB-lineage cells <i>in-vitro</i> (84)