Mechanisms of Erosion in Rheumatoid Arthritis

Irreversible joint destruction characterized by cartilage degradation and bone resorption is the major long-term consequence of inflammatory events initiated early in the course of rheumatoid synovitis. Although the relationship between early symptoms and joint destruction is still not completely understood, a significant amount of osteolysis occurs early in the disease and correlates with disease activity. This osteolysis manifests in several forms, including localized or juxtaarticular osteopenia and generalized bone loss in the appendicular and axial skeleton. Localized and generalized bone resorption is initiated and maintained by complex pathways. Activation events initiated by cell–cell contact between infiltrating T lymphocytes and monocytes, along with resident synovial lining cells, trigger intercellular signaling cascades. Elucidation of the molecules involved in these signaling processes has translated into improved therapeutic modalities.

The purpose of this editorial is to describe the molecular and cellular features of rheumatoid cartilage and bone, provide an overview of the molecular basis of bone erosion, and review the clinical efficacy of the new biologic response modifiers (BRM) approved for the treatment of rheumatoid arthritis (RA).

INFLAMMATORY EVENTS IN RHEUMATOID SYNOVIIUM

The normal synovial membrane is one to 2 cell layers thick and composed of synoviocytes derived from monocytoid (type A) and mesenchymal (type B) lineages. In the rheumatoid joint, this delicate tissue is transformed into a proliferating cell mass that destroys surrounding tissue and bone. This remarkable degree of synovial lining hyperplasia is correlated with the influx of infiltrating CD4+ lymphocytes and their subsequent binding to activated endothelial cells, although details of this interaction and the initiating immunologic stimuli are not well understood. The formation of pannus results from migration of these inflammatory cells into the synovium and neovascularization of synovial tissue. Hyperplasia of synovial cells also plays a critical role in pannus formation. Indeed, hyperplasia of type B synoviocytes is a major reason contributing to joint inflammation and matrix degradation, which results in the release of effector molecules that attract leukocytes into the joint and which enhances the production of matrix metalloproteinases (MMP). Additionally, fibroblasts isolated from rheumatoid joints have been shown to release soluble factors known as cytokines that stimulate fibroblast proliferation, a property not observed in fibroblasts isolated from osteoarthritic joints.

Cytokines are small proteins that are involved in many aspects of inflammation, including the initiation and amplification of leukocyte recruitment into the joint space. A complex cascade of cytokine mediated events alters the phenotype of the type A and type B synoviocytes, resulting in widespread tissue destruction. Two main cytokines at the apex of the cytokine network in the rheumatoid joint are tumor necrosis factor (TNF) and interleukin 1 (IL-1). TNF-α is a proinflammatory cytokine produced by monocytes, T lymphocytes, macrophages, and fibroblasts that stimulates prostaglandin E2 (PGE2) and collagenase release from human synovial cells and potentiates osteoclast differentiation (osteoclastogenesis) and activation. IL-1 is typically synthesized and released simultaneously with TNF, and the 2 cytokines share similar biologic properties. Although their functional roles are not entirely coupled, TNF and IL-1 do display highly synergistic and overlapping biological effects. The complex nature of this synergy is not well understood. Moreover, IL-1 and TNF exert autocrine effects on macrophages, thereby leading to upregulated synthesis of both cytokines. IL-1 and TNF bind to distinct receptors and are regulated independently of one another. However, IL-1 can induce the release of TNF in peripheral blood mononuclear cells in vitro and TNF-like activity in the sera of rabbits. Additional evidence suggests that TNF is the primary inducer of IL-1 in human synovial cells, underscoring the pivotal role of TNF as the initiator of downstream proinflammatory pathways.

There is also evidence that TNF can induce the synthesis of IL-6 by monocytes, T lymphocytes, and fibroblasts. IL-6 shares many biologic properties with IL-1 and TNF; however, there are several important differences. First, IL-6 does not stimulate production of PGE2 and collagenase from human fibroblast and synovial cells. Second, IL-6 enhances rather than inhibits the production of tissue inhibitor of metalloproteinases (TIMP), the natural antagonist of MMP. Third, in contrast to the actions of IL-1 and TNF, intraarticular injection of IL-6 has been shown to enhance proteoglycan synthesis, suggesting a protective role...
for IL-6 in the joint. The contribution of IL-6 to MMP actions is complex. For example, Ito, et al. showed that IL-6 stimulates the production of TIMP, while enhancing IL-1 induced production of metalloproteinase proenzymes. Thus, while IL-6 participates in the regulation of cartilage homeostasis, the net effect of IL-6 on cartilage in the inflamed joint remains to be determined.

Elevated concentrations of proinflammatory cytokines have been identified in the peripheral blood and joints of patients with RA. Plasma levels of IL-1 correlated with disease activity and were significantly higher in patients with RA compared with age matched controls. Similarly, synovial fluid concentrations of IL-6 were significantly greater in RA than in patients with osteoarthritis. Further, RA synovial fluid levels of TNF correlated with the amount of bone resorbed in joint tissues. Animal data have shown that the combined blockade of TNF and IL-1 reduces synovial inflammation and joint destruction and preserves bone density in arthritic rats. A study using porcine cartilage explants showed increased resorption and inhibition of proteoglycan synthesis after treatment with TNF or IL-1. These effects were additive when the explants were treated with the 2 cytokines simultaneously. Studies in murine models of arthritis have demonstrated suppression of proteoglycan synthesis after intraarticular injection of IL-1α, IL-1β, and high doses of TNF, further supporting a pleiotropic role for cytokines in rheumatoid joint destruction.

Both IL-1 and TNF, as well as granulocyte/monocyte-colony stimulating factor (GM-CSF), stimulate fibroblast growth. In addition to its promotion of pannus formation via stimulation of fibroblast growth, GM-CSF also enhances the transcription of TNF. In turn, TNF and IL-1 stimulate GM-CSF secretion by synovial cells; such regulatory interdependence may contribute to the accelerated rate of pannus formation in the advanced stages of RA. In addition, TNF and IL-1 increase the expression of cellular adhesion molecules, such as intercellular adhesion molecule-1, endothelial leukocyte adhesion molecule, and vascular cell adhesion molecule, which enhance leukocyte migration into the synovium.

In summary, animal and human studies indicate that both IL-1 and TNF are dominant cytokines that promote the degradation of articular cartilage in the inflamed joint. These 2 cytokines inhibit the osteogenic properties of bone-remodeling cells, accelerate matrix degradation via the activation of proteolytic enzymes, stimulate osteoclastogenesis, and indirectly promote pannus formation and inflammation via interactions with other cytokines.

**A MOLECULAR BASIS FOR THE BREAKDOWN OF CARTILAGE**

Because RA has devastating effects on cartilage as the disease progresses, it is worth reviewing some of the characteristics of cartilage. The basic function of cartilage in the joint is to provide a tough, elastic shock absorber that limits friction and resists wear on the bones as the joints move. Matrix is composed of chondroitin-rich proteoglycan and collagen. Chondrocytes in lacunae synthesize and enzymatically digest matrix, and these are processes that fall out of equilibrium in diseases that destroy articular cartilage. Activation of catabolic enzymes and decreased production of enzyme inhibitors can lead to accelerated matrix breakdown.

Two major processes contribute to the cartilage destruction seen in arthritis: decreased synthetic activity of chondrocytes and increased enzymatic degradation of the matrix. Evidence suggests that cartilage breakdown is mediated by MMP synthesized by transformed fibroblasts and chondrocytes in the synovium and macrophages at the interface of pannus and cartilage. Additional evidence implicates cathepsins and mast cell proteinases in the degradation of matrix components and osteolysis of bone. IL-1 stimulates metalloproteinase synthesis in synovial cells and chondrocytes. These MMP exhibit selectivity for the different components of cartilage. For example, collagen is the primary substrate of MMP-1, while gelatin is the primary substrate of MMP-2. MMP-3 degrades proteoglycans, laminin, and fibronectin. However, cells that produce MMP also produce TIMP, suggesting that the regulation of cartilage turnover is dependent on the balance of matrix degradation by MMP and inhibition of MMP by TIMP. An additional level of regulation is suggested by studies that show that TIMP production is differentially regulated by various cytokines in a cell-specific manner. For example, in endothelial cells, synoviocytes, and chondrocytes, TNF inhibits while IL-6 stimulates the production of TIMP. Calcitonin also opposes this degradative activity by inhibiting bone resorption.

**BONE LOSS IN RA**

The destruction of diarthrodial joints is a prominent manifestation of RA. A high proportion of RA patients develop lesions detectable by magnetic resonance imaging (MRI) of the wrist early in the disease process (median symptom duration 4 months). Bone edema, manifested as increased signals on fat-suppressed T2-weighted MRI images, is almost always associated with synovitis, suggesting that synovial inflammation may be a predecessor to bone damage in RA patients. Radiologic damage progresses at a fairly constant rate over the first 6 years after onset of RA. Compston, et al. have reported that the reduced bone mass in steroid-naïve RA patients results from a negative remodeling balance associated with a reduced rate of bone formation. Other studies have demonstrated a relationship between increased bone resorption and disease activity in RA patients. Focal bone erosions are associated with increased morbidity and may correlate with the severity of disease.
In one study, a majority of patients experienced severe morbidity, with lower grip strength and functional capacity in over 90% of patients, and an increase in mortality relative to expected rates for age and sex matched individuals\textsuperscript{34}. Functional disability was evident at baseline and continued to worsen with time\textsuperscript{34}. Further, the pain and functional impairment associated with RA have substantial effects on quality of life and medical costs\textsuperscript{35,36}, as well as on morbidity and mortality.

**OSTEOCLASTS AS MEDIATORS OF BONE RESORPTION**

Normal remodeling of bone depends on the coordinated activity of bone-forming and bone-resorbing cells. Osteoclasts, multinucleated cells derived from the monocyte/macrophage line (Figure 2), are primarily responsible for bone resorption. Under normal physiologic conditions, mature osteoclasts are solely responsible for bone resorption in matrix lacunae\textsuperscript{37}. Osteoclastic resorption of bone requires the cells to adhere to the bone surface, an interaction enhanced by increased osteoclast number\textsuperscript{38}. The short lifespan (about 2 weeks) of osteoclasts suggests that factors related to the formation and maturation of these cells represent important mechanisms for regulating their activity\textsuperscript{39}.

Histologic analyses suggest that, in addition to their role as mediators of normal bone turnover, osteoclasts also mediate focal bone resorption in RA\textsuperscript{3}. Osteoclasts were originally identified by acid phosphatase staining in rheumatoid joints showing erosion at the junction between cartilage and pannus\textsuperscript{40}. Osteoclasts have been identified
more specifically by tartrate-resistant acid phosphatase staining and expression of calcitonin receptor mRNA in rheumatoid lesions. Further, osteoclast precursors have been identified in bone resorption lacunae adjacent to invading pannus. Together with data suggesting that other cell types (e.g., macrophages) at the bone–pannus junction have a limited ability to resorb mineralized bone, these findings suggest that osteoclasts may be the cell type primarily responsible for bone loss in patients with RA.

The differentiation of osteoclast precursors into mature, bone-resorbing cells depends on a network of cytokines that includes TNF, IL-1, and IL-6. These cytokines have been localized to rheumatoid synovium at the cartilage–pannus junction. Recently, an additional member of the TNF family of cytokines was discovered by 2 independent teams; the factor was named either osteoprotegerin ligand, osteoclast differentiation factor, or receptor activator of nuclear factor κB ligand (RANKL). RANKL is synthesized by osteoblasts and bone stromal cells, and in conjunction with macrophage-colony stimulating factor (M-CSF), is an essential factor for osteoclast differentiation. In an animal model, RANKL has been shown to induce osteoclastogenesis and bone loss. RANKL binds to its cell-surface receptor (RANK) located on dendritic, T lymphocytes, osteoclast precursors, and osteoclasts. In addition, a soluble “decoy” receptor for RANKL has been identified, termed osteoprotegerin. This “decoy” receptor blocks the binding of RANKL, thereby preventing RANK activation and subsequent osteoclastogenesis. Together with data showing that blockade of RANKL signaling prevents destruction of bone and cartilage in arthritic rats, the observation of RANKL transcription in synovial tissue derived from RA patients suggests that this osteoclast differentiation factor may play a key role in promoting bone loss in the rheumatoid joint.

Additional cytokines such as IL-1β, IL-18, and IL-11 may also contribute to the proinflammatory cascade by binding to osteoblasts and stimulating the release of factors that, in turn, lead to the differentiation of osteoclasts. One of these factors is M-CSF, which has been shown to be uniquely essential in osteoclastic differentiation.

TRADITIONAL TREATMENT OF RA

The treatment of RA has centered on relieving the symptoms of inflammation, improving overall function, and preventing disease progression. However, there is little evidence supporting the concept that therapies that improve pain and mobility significantly lessen joint destruction and disease progression. Enthusiasm for the step-up or pyramid approach to treating RA waned when observational and MRI studies revealed that functional decline and joint erosions emerge early in the disease. This realization led to the use of standard disease-modifying antirheumatic drugs (DMARD) early in the disease. The use of combination DMARD regimens also became popular. Despite this aggressive strategy, only modest inhibition of joint destruction has been obtained in trials with individual DMARD, such as sulfasalazine, hydroxychloroquine, and cyclo-
IL-1 and TNF

Leflunomide has been shown to be similar effects via a decrease in proinflammatory cytokines such as decreases in T cell proliferation may have antiinflammatory activity of TNF by competitively inhibiting the association of TNF with its cell-surface receptors, thereby blocking the biologic activity of IL-171,72. Anakinra is an IL-1 receptor antagonist (IL-1Ra) synthesized by recombinant DNA technology. Anakinra competitively inhibits binding of IL-1 to its cell-surface receptor, thus blocking the biologic activity of IL-171,72. Anakinra closely resembles naturally occurring IL-1Ra with respect to structure and prevents interactions between IL-1 and cells involved in inflammation71,73,74.

Clinical trials have provided convincing evidence for the efficacy of the new biologic agents in retarding progression of RA. Etanercept has been shown to improve the inflammatory symptoms of RA and to attenuate radiographic progression of the disease55,67. Further, patients who received etanercept had more rapid improvement than those receiving MTX55. In a 2-year followup study of 512 patients receiving monotherapy of either etanercept or MTX, 72% of etanercept patients achieved American College of Rheumatology 20% (ACR 20) improvement criteria versus 59% of MTX patients (p = 0.005). Etanercept patients also had better outcomes as measured by Sharp scale total score and erosion score (mean change 1.3 and 0.66 units, respectively; vs 3.2 and 1.86 units; p = 0.001). More etanercept patients had improvement in Health Assessment Questionnaire disability index (55% vs 37%; p < 0.001)75. Maini, et al observed a rapid reduction in disease activity in patients who were treated with infliximab and who had failed to respond adequately to previous treatment with MTX. Although infliximab produced dramatic results, one disadvantage of this agent is that it must be used sporine50-53. Based on several comparative trials, a consensus has emerged suggesting that treatment with methotrexate (MTX) limits joint damage more effectively than treatment with the other traditional DMARD54.

Initiation of treatment with MTX early in the course of RA coupled with rapid escalation to a target dose of 17.5 mg/week significantly reduced joint erosions and progression of the disease55. Longterm treatment with MTX, however, has been associated with significant toxicity and treatment discontinuation over a 5 year period of up to 75% of patients56,57.

Leflunomide inhibits dihydroorotate dehydrogenase, an enzyme involved in de novo pyrimidine synthesis58. The active metabolite of leflunomide, A77 1726, inhibits DNA synthesis in lymphocytes by limiting the pool of available pyrimidines, thus resulting in an antiproliferative effect. Decreases in T cell proliferation may have antiinflammatory effects via a decrease in proinflammatory cytokines such as IL-1 and TNF59. Leflunomide has been shown to be similar in efficacy to sulfasalazine and MTX in reducing swollen/tender joint counts, as well as in decreasing radiographic progression as assessed by erosions and joint space narrowing60,61. Concern has been mounting recently, however, on a possible association between leflunomide and liver toxicity62-65.

THE EMERGENCE OF BIOLOGIC AGENTS IN RA

Advances in the understanding of the pathophysiology of RA have resulted in the development of 3 new agents with proven efficacy in halting radiographic progression of the disease: etanercept, infliximab, and anakinra. These agents have all been approved for the treatment of RA. Etanercept and infliximab are TNF-neutralizing agents (Figure 3). Etanercept is a human fusion protein produced by recombinant DNA technology and consists of 2 copies of the soluble form of the p75 TNF receptor (p75 TNFR) fused to the Fc portion of human IgG166. Etanercept blocks the biologic activity of TNF by competitively inhibiting the association of TNF with its cell-surface receptors, thereby mimicking the action of endogenous soluble TNFR that neutralize free TNF67. Infliximab is a chimeric monoclonal antibody that binds with high affinity and selectivity to TNF68. Like etanercept, infliximab prevents the association of TNF with its cell-surface receptor, thereby blocking the signaling activity of TNF. Unlike etanercept, infliximab may cause complement-mediated lysis of TNF-expressing cells69 and does not neutralize the activity of the related proinflammatory cytokine lymphotoxin-α, which also exerts biologic actions via TNFR70. The clinical significance of this finding is under investigation.

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Table 1. Factors regulating osteoclast differentiation. Adapted with permission from Gravallese EM, Goldring SR. Arthritis Rheum 2000; 43:2143-51.

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<tr>
<th>Factor (TRANCE, OPG)</th>
<th>Function</th>
<th>Effect on Osteoclast Differentiation</th>
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<tr>
<td>RANKL (TRANCE, OPG)</td>
<td>Cell-surface ligand that induces osteoclast differentiation and activity</td>
<td>Stimulates</td>
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<tr>
<td>RANK (ODAR)</td>
<td>Receptor for RANKL</td>
<td>Stimulates</td>
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<tr>
<td>OPG (OCIF)</td>
<td>Soluble receptor that binds RANKL and prevents RANK activation</td>
<td>Inhibits</td>
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RANKL: receptor activator of nuclear factor-kB ligand/osteoclast differentiation factor; TRANCE: tumor necrosis factor-related activation-induced cytokine; OPG: osteoprotegerin ligand; RANK: receptor activator of nuclear factor-kB; ODAR: osteoclast differentiation and activation receptor; OPG: osteoprotegerin; OCIF: osteoclastogenesis inhibitory factor.
concomitantly with MTX. Further, many patients become tolerant to the medication, requiring either dose escalation or more frequent administration. Clinical trials with anakinra have also shown that this agent reduces the rate of joint destruction and improves measures of disease activity. Moreover, patients who received longterm (1 year) treatment with anakinra continued to show improvement through the study endpoint.

Although overwhelming evidence suggests that the cytokine family represents a rational target for treating RA, the choice of which individual cytokine/receptor system to target for optimal therapeutic effects remains a central issue. Studies to date suggest that TNF-neutralizing agents produce better symptomatic and functional relief than IL-1-neutralizing agents. In both human studies and animal models of arthritis, the effect of anakinra on measures of inflammation was relatively modest. Intermittent dosing with subcutaneous administration coupled with a short half-life (4 to 6 h) may have contributed to the modest effects, insofar as maximal effects in animal studies necessitated continuous infusion of anakinra. In contrast, etanercept has produced more impressive effects on pain, inflammation, and radiographic progression in RA patients. However, it is not possible to compare the efficacy of anakinra with that of anti-TNF agents, owing to differences in study design, patient population, and outcome measures.

Regrettably, combination of anakinra and etanercept in RA patients did not significantly improve clinical efficacy and was associated with a high rate of infections. For example, neutralization of TNF inhibits production of IL-1, IL-6, and GM-CSF in synovial cells derived from RA patients; neutralization of IL-1, however, does not decrease the production of TNF. These experiments suggest that TNF may be the primary inducer of IL-1 and other proinflammatory cytokines, thus representing the crucial mediator in a cascade that leads to diminished synthetic activity by articular chondrocytes and enzymatic destruction of the matrix.

The clinical efficacy and potency of TNF and IL-1-neutralizing biologic reagents, in not only alleviating RA symptoms, but also in retarding the progression of the disease, may have profound implications for other cytokine/receptor systems that potentiate RA synovitis.
including interferon-α, interferon-β, IL-6, and IL-17. Current studies of RA-derived tissues and arthritic mouse models have identified other potential therapeutic targets such as IL-15, an IL-2-like cytokine, and IL-18, an IL-1-like cytokine, as important mediators in the proinflammatory response underlying RA pathogenesis. Evidence from these studies shows elevated concentrations of both cytokines in RA synovial fluids, as well as a synergistic effect of IL-15 and IL-18 to induce TNF expression in vitro. In contrast, experiments, however, it has been shown that TNF can similarly upregulate IL-15 and IL-18 synthesis, illustrating the complexity in establishing cytokine hierarchy during RA inflammatory responses. Although the details of this hierarchy remain incomplete, TNF is clearly central to the pathogenesis of inflammatory osteolysis and acts as the primary trigger in the early phases of synovitis.

CONCLUSIONS
The erosive processes leading to joint destruction in RA arise from a complex network of cytokines that mediate signaling among lymphocytes, fibroblasts, chondrocytes, and synovial cells. Advances in understanding the role of cytokines in the inflammatory process have led to newer DMARD that have the potential to dramatically improve the treatment of this chronic disease. IL-1Ra primarily affects secretion of metalloproteinases, degradation of proteoglycans, and osteoclast activation, thereby reducing damage to cartilage and bone. Clinically, this manifests as a reduction in radiographic disease progression with only modest improvements in inflammation. Etanercept and infliximab neutralize the effects of TNF, which may play more of a pivotal role in blocking synovial inflammation than IL-1Ra. By virtue of blocking critical upstream activation events in the cytokine cascade, these agents can produce dramatic improvements in both inflammation and radiographic damage.

Further, these agents improve quality-of-life assessments in patients with RA. Additional studies comparing biologic agents as monotherapy and in combination with conventional DMARD and other biologic response modifiers will be necessary to determine the most effective and safe treatment regimen for long-term treatment of rheumatoid synovitis.

CHRISTOPHER T. RITCHLIN, MD,
Associate Professor of Medicine,
Clinical Director, Allergy, Immunology and Rheumatology Unit,
University of Rochester School of Medicine and Dentistry,
Rochester, New York, USA

Address reprint requests to Dr. C.T. Ritchlin, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Room G-6427J, Rochester, NY 14642-0001.
E-mail: Christopher_Ritchlin@urmc.rochester.edu

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