The New and Evolving Science of IL-6 in Rheumatoid Arthritis

The Contributions of IL-6 to Bone Resorption in RA

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Dear Colleagues,

This is a very exciting time in the field of rheumatoid arthritis (RA). The more we understand about RA pathogenesis from basic and clinical research, the more equipped we are to understand this disease. We now know that cytokines play many key roles in the inflammation that drives RA. One such example is interleukin-6 (IL-6), a multifunctional cytokine that contributes to chronic inflammation in patients with RA.

Regeneron Pharmaceuticals and Sanofi Genzyme are excited to bring you additional educational material describing some of the fundamental immunology as well as clinical pathology we see in RA patients through a series of scientific monographs entitled The New and Evolving Science of IL-6 in Rheumatoid Arthritis. In the first installment, we reviewed the signaling mechanisms of IL-6 that allow it to have widespread effects in RA. In this second installment, we will focus on the contributions of the IL-6 pathway to bone resorption, both at the joint and more systemically, in RA.

We hope you find this latest installment informative and engaging.

Sincerely,

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Dr Calabrese and Dr Choy were provided honoraria by Sanofi Genzyme and Regeneron Pharmaceuticals.  
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Rheumatoid arthritis (RA) is a chronic, progressive autoimmune disease characterized by debilitating articular and systemic manifestations. Bone resorption represents both types of manifestations; reduced bone mineral density and focal erosions are commonly found at inflamed joints, but also occur systemically at distal locations. Articular bone resorption and cartilage degradation arising from the chronic synovitis associated with RA leads to structural damage that ultimately can impair function. Systemic bone loss contributes to the higher risk of fracture associated with RA. Patients with RA in the General Practice Research Database (>30,000 RA patients) had a 1.5-fold increase in clinical fracture risk compared with control patients. In addition, the FRAX® tool—which measures risk of fracture and was developed by the World Health Organization (WHO) based on population-based cohorts—assigns an approximately 30% increase in risk of major osteoporotic fracture (hip, spine, wrist, humerus) and a 40% increased risk of hip fracture to patients with RA as a clinical risk factor.

The skeleton is a dynamic organ in which mineralized bone is continuously resorbed by osteoclasts and new bone is formed by osteoblasts. This process, known as bone remodeling, is normally highly regulated to ensure bone homeostasis. In pathological conditions such as RA, this homeostasis is disrupted, resulting in uncoordinated osteoclast formation and a skewing towards bone resorption.

It has been established that RA and other inflammatory diseases are driven by a complex network of cytokines, including tumor necrosis factor-α (TNF-α), interleukins (IL)-1, 4, 6, 12, 13, and 17, and interferons. IL-6 is a multifunctional cytokine that performs many diverse functions, including vital pro-inflammatory roles, in response to infection or injury. Persistently elevated IL-6 signaling may play a role in disrupting homeostasis in multiple physiologic processes, which can contribute to pathologic conditions observed in autoimmunity and chronic inflammation conditions such as RA. Elevated IL-6 signaling plays an important role in RA, and may contribute to both articular and systemic manifestations of the disease.

IL-6 is one of the most abundant cytokines in the serum and synovial fluid of patients with RA, and correlates with both disease activity and articular destruction.

The signaling features of IL-6 allow it to interact with a broad range of cells and tissues such as: immune cells, fibroblast-like synoviocytes (FLS), hematopoietic stem cells, hepatocytes, adipocytes, endothelial cells, and pancreatic islets. IL-6 can signal through both a membrane-bound receptor and soluble receptor. The latter differentiates IL-6 signaling from other cytokines such as TNF-α and IL-1, which are also implicated in driving inflammation in RA.

This monograph will describe how the broad cell and tissue distribution of IL-6 signaling allows for its contributions to increased articular and systemic bone resorption.
Distinct patterns of bone remodeling are found in RA, which can be broadly categorized as periarticular osteopenia, focal joint erosions, and systemic osteoporosis. Periarticular osteopenia refers to reduced bone mineral density at the interface of bone and cartilage in diarthrodial joints such as the knee, wrist, and small joints of the hands and feet. The presence of periarticular bone loss has been shown to have high predictive value with respect to the subsequent development of marginal joint erosions of the hand.

Focal joint erosions are found at sites where the inflamed synovial lining, otherwise known as the pannus, comes in contact with the bone surface (Figure 1). These erosions are often localized to the joint margins, in which the bone is organized into a plate-like structure of compact cortical bone. However, protrusions of the pannus may also cause erosion in deeper subchondral regions, which are comprised of a network of cancellous trabecular bone. These subchondral regions of bone erosion may extend through the calcified cartilage that interfaces with bone. This provides the invading inflammatory tissue with access to the articular cartilage, allowing for degradation, and contributing to the joint space narrowing commonly seen in RA patients. Anatomical alterations leading...
to interactions between the synovium and the bone marrow may facilitate the spreading of bone marrow inflammation (osteitis) commonly observed by magnetic resonance imaging (MRI) in patients with RA. Histological examination of biopsies from patients with RA and in vivo models of arthritis show that in both joint margin and subchondral cases, erosions are lined with resorption lacunae, or cavities, containing mono- and multinucleated cells with phenotypic features of osteoclasts. Interestingly, juxta-articular bone loss at sites removed from the inflamed synovium is common in RA, and frequently precedes the development of marginal joint erosions. The degree of generalized bone loss occurring early in the course of RA is also associated with disease activity.

Systemic osteoporosis refers to the systemic reduced bone mineral density (BMD) associated with RA. The prevalence of BMD loss in the overall RA patient population has been reported as between 20% and 56%. Patients with RA who have reduced BMD are at an increased risk for fracture.

Mechanisms of Bone Loss in RA

As mentioned, in RA there is a marked increase in proliferation, or hyperplasia, of cells of the synovial intimal lining, or pannus, which include FLS, osteoclasts, and macrophages. As a result, the lining increases from a depth of 1 to 2 cells to a depth of 10 to 20 cells. RA synovium induces local articular bone resorption through the production of proteins/molecules with the ability to recruit osteoclast precursors and induce their differentiation and activation into bone-resorbing osteoclasts. These include pro-inflammatory cytokines such as IL-6 and TNF-α, chemokines, and pro-osteoclastogenic soluble mediators such as macrophage colony-stimulating factor (M-CSF).

Reciprocal signaling between osteoclasts and osteoblasts regulates the balance between bone generation and resorption, and is largely driven by two different proteins—RANKL and osteoprotegerin (OPG). Osteoclasts are generated from precursor cells that are usually of the monocyte-macrophage lineage. Interactions between receptor activator of the nuclear factor kappa B (RANK) and its ligand (RANKL) are essential in osteoclastogenesis. RANK on monocytes bind to RANKL, initiating osteoclast differentiation. Under physiological conditions, the main source of RANKL is osteoblasts. However, cells of the synovium, such as immune cells and FLS, are the main source of RANKL in pathological conditions such as RA. In addition, a recent study found that C-reactive protein (CRP) stimulated RANKL production in monocytes, and it induced osteoclast differentiation from monocytes and bone resorption in the absence of RANKL. OPG inhibits osteoclast function by binding directly to RANKL to block the RANKL-RANK interaction. By blocking this interaction, OPG attenuates osteoclast differentiation.

Anti-citrullinated protein antibodies (ACPAs) have been shown to be an independent predictor of the development of bone
erosions in patients with RA. ACPAs can be detected years before clinical disease onset of RA, suggesting a role in driving disease progression. Interestingly, a recent study found significant bone loss in healthy individuals with ACPA compared with patients who were negative for ACPA. The fact that bone damage was observed in these patients prior to any signs of inflammation provocatively suggests that autoantibodies may be directly involved in driving bone loss.

The other implication of these findings is that bone loss may be important for priming of the joint for susceptibility to chronic inflammation. Interestingly, the presence of ACPA in patients with RA seems to have little influence on disease activity.
factor (RF) does appear to be associated with higher disease activity, but there exists disagreement on its role in joint erosion.\textsuperscript{40,41,46}

These findings raise the question of how ACPA protein antibodies trigger bone loss. It has been proposed that ACPAs bind directly to citrullinated vimentin expressed on the surface of cells of osteoclast cell lineage (Figure 4).\textsuperscript{47} These interactions can then stimulate osteoclast precursors to differentiate into mature active osteoclasts which lead to increased bone resorption.\textsuperscript{47} A recent study by Hecht et al demonstrates that rheumatoid factor may cooperate with ACPAs to enhance bone erosion.\textsuperscript{49}

Another pathological underpinning of bone resorption in RA is the virtual absence of bone repair in articular focal erosions.\textsuperscript{2} This appears to arise through production of Dickkopf-1 (DKK-1)—the inhibitor of the wingless (Wnt)-signaling pathway that plays a critical role in osteoblast-mediated bone formation—by cells of the synovial lining.\textsuperscript{48} Synovial fibroblasts, endothelial cells, and chondrocytes all express DKK-1.\textsuperscript{2}

**Figure 4.** Autoantibodies can directly impact bone resorption. Autoantibodies produced by plasma cells can bind to citrullinated vimentin on the surface of osteoclast precursors, which stimulates them to undergo differentiation. Osteoclast differentiation is also facilitated by proinflammatory cytokines which cause T cells and synovial fibroblasts to express RANKL and M-CSF. Figure adapted from Kleyer A, Schett G. Curr Opin Rheumatol. 2014;26:80-84.
Pro-inflammatory cytokines like TNF-α, interleukin (IL)-1, IL-6 and IL-17, are effective triggers of osteoclast differentiation and bone resorption. Inflammatory cytokines either directly trigger osteoclast differentiation or support it indirectly by increasing the expression of RANKL.7,48

**Studies in mice demonstrate a role for IL-6 in bone resorption**

A number of genetic studies in mice have demonstrated a role for IL-6 in bone metabolism (Table 1). Transgenic mice engineered to overexpress IL-6 showed increases in osteoclast number and activity leading to impaired skeletal growth at the prepubertal stage but decreased osteoclast formation at the adult stage.49,50 A significant reduction of 3-D trabecular microarchitecture was also observed in these transgenic animals.49 IL-6 knockout mice with experimental arthritis showed significantly decreased osteoclastogenic activity and impaired osteoclast recruitment to inflammatory sites.51 Under physiological conditions, IL-6 deficiency resulted in no detectable change in osteoclast number.52 However, IL-6 knockout mice were protected against ovariectomy-induced bone loss.52

### Table 1. Contributions of IL-6 to Bone Metabolism

<table>
<thead>
<tr>
<th>Effect on Bone Metabolism</th>
<th>Supported By</th>
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<tr>
<td>With sIL-6R, induces expression of RANKL on osteoblasts, leading to osteoclast differentiation</td>
<td>In vitro mouse cell culture model</td>
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<tr>
<td>In the presence of sIL-6R, induces expression on FLS</td>
<td>In vitro human cell culture model</td>
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<tr>
<td>Enhances Th17 cell differentiation to secrete IL-17, which stimulates osteoclastogenesis</td>
<td>In vitro mouse cell culture model</td>
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<tr>
<td>Supports RANKL–independent osteoclast formation</td>
<td>In vitro mouse cell culture model</td>
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<tr>
<td>In prepubertal mice, enhances osteoclastogenesis</td>
<td>Transgenic mice overexpressing IL-6</td>
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<tr>
<td>Important for maintaining 3-D trabecular microarchitecture</td>
<td>Transgenic mice overexpressing IL-6</td>
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<tr>
<td>Induces CRP expression in hepatocytes, leading to increased RANKL expression and increased osteoclast differentiation</td>
<td>In vitro human cell culture model, In vivo studies correlating IL-6 and CRP levels in RA patients</td>
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<tr>
<td>Induces differentiation of B cells into plasma cells that secrete Dickkopf-related protein 1 (DKK-1), which inhibits osteoblast formation</td>
<td>Mouse models and in vitro mouse cell culture model</td>
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<tr>
<td>With sIL-6R, directly inhibits osteoblast differentiation</td>
<td>In vitro mouse cell culture model</td>
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<tr>
<td>Protects against ovariectomy-induced bone loss</td>
<td>IL-6 knockout mouse model</td>
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<tr>
<td>Enhances osteoblast differentiation in vitro</td>
<td>In vitro mouse and human cell culture model</td>
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<td>In the absence of other support cells, IL-6 by itself directly suppresses the differentiation and facilitates the proliferation of osteoclast progenitors</td>
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<tr>
<td>Suppresses TNF-α-induced expression of Dkk-1 by FLS</td>
<td>In vitro human cell culture model</td>
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**Molecular mechanisms governing bone resorption mediated by IL-6**

Roles for IL-6 in promoting both bone resorption and formation have also been demonstrated in a number of in vitro studies (Table 1). These apparently opposing functions may be explained from differences in the cell types and experimental conditions used across the in vitro studies. In vivo, complex cytokine networks exist, with multiple interconnected signaling pathways. IL-6 affects a broad range of cells and can alter the expression of other important mediators of bone metabolism such as IL-1 and TNF-α. In the context of chronic inflammation states like that found in RA, elevated levels of these cytokines likely conspire to increase bone resorption.

It has been proposed that IL-6 signaling components determine whether bone resorption or formation activities of IL-6 are more heavily weighted, and when elevated, can shift the balance from bone formation to resorption. Under steady-state conditions, IL-6 is proposed to suppress osteoclast function, and therefore prevent bone resorption. However, under inflammatory conditions, increased expression of the soluble IL-6 receptor is thought to induce expression of RANKL on osteoblasts and fibroblasts, leading to increased osteoclast activation and proliferation, and ultimately greater bone resorption.

**Effects of elevated IL-6 signaling on bone metabolism in RA**

In the clinical evaluation of synovial fluid from patients with RA, it was determined that the ratio of RANKL to OPG reflects osteoclast function, and a higher ratio of RANKL to OPG is correlated to osteoclast hyperactivity and bone resorption in joints in patients with RA.

**Elevated IL-6 levels are associated with generalized bone mineral density loss.**

The prevalence of BMD loss in the overall RA patient population has been reported as between 19.6% and 56%. The disruption of homeostasis caused by elevated IL-6 signaling, and the resultant increase in bone resorption, can lead to overall BMD loss, bone weakening, cartilage destruction, and an increased susceptibility to fracture.

BMD loss is especially prevalent in postmenopausal women with RA; generalized BMD loss occurs in >50% of this population compared with ~15% in postmenopausal women without RA. Studies have shown that estrogen blocks the synthesis of IL-6 by bone-forming osteoblasts and may also interfere with expression of IL-6 receptors. The serum level of sIL-6R correlates with BMD loss in postmenopausal women who have RA. Elevated serum levels of sIL-6R have also been shown to be the main predictor of BMD loss in a study of postmenopausal women with RA, independent of well-known risk factors of generalized bone loss such as age, disease duration, low body mass index, and cumulative glucocorticoid dose.
Conclusions

The chronic synovitis associated with RA can ultimately lead to disruption of the integrity and functional properties of joint tissues. Progressive bone loss also occurs systemically in RA, and it is associated with an increased risk of fractures. In RA, the balance of bone formation and resorption is skewed in favor of resorption, through increases in the number and activity of osteoclasts relative to osteoblasts. The inflamed synovium acts as a reservoir in RA; it provides the environment for the immune cells and cytokines that enhance osteoclastogenesis. These cells and cytokines can upregulate expression of RANKL directly, or indirectly by stimulating release of proinflammatory cytokines that also influence RANKL expression. IL-6 can stimulate expression of RANKL in a broad array of cells such as osteoblasts and FLS. IL-6 also enhances osteoclast activity through other indirect mechanisms including affecting expression of other cytokines involved in mediating joint damage. Continued research on the many functions of IL-6 may further delineate the pathological origins and underpinnings of bone loss in RA.
References

differentiation through the SHP2/MEK2 and SHP2/Akt2 pathways in vitro.


