

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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# Introduction

Rheumatoid arthritis (RA) is a chronic, progressive autoimmune disease characterized by debilitating articular and systemic manifestations.<sup>1</sup> 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.<sup>2,3</sup> Systemic bone loss contributes to the higher risk of fracture associated with RA.<sup>2</sup> 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.<sup>4</sup> In addition, the FRAX<sup>®</sup> 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.<sup>5,6</sup>

The skeleton is a dynamic organ in which mineralized bone is continuously resorbed by osteoclasts and new bone is formed by osteoblasts.<sup>7</sup> 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.<sup>7</sup>

It has been established that RA and other inflammatory diseases are driven by a complex network of cytokines, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukins (IL)-1, 4, 6, 12, 13, and 17, and interferons.<sup>1,8</sup> IL-6 is a multifunctional cytokine that performs many diverse functions, including vital pro-inflammatory roles, in response to infection or injury.<sup>1,8,9</sup> 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.<sup>10,11</sup> Elevated IL-6 signaling plays an important role in RA, and may contribute to both articular and systemic manifestations of the disease.<sup>1,12-14</sup> 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.<sup>1,15</sup>

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.<sup>8,16-20</sup> IL-6 can signal through both a membrane-bound receptor and soluble receptor.<sup>1</sup> The latter differentiates IL-6 signaling from other cytokines such as TNF- $\alpha$  and IL-1, which are also implicated in driving inflammation in RA.<sup>21,22</sup>

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.

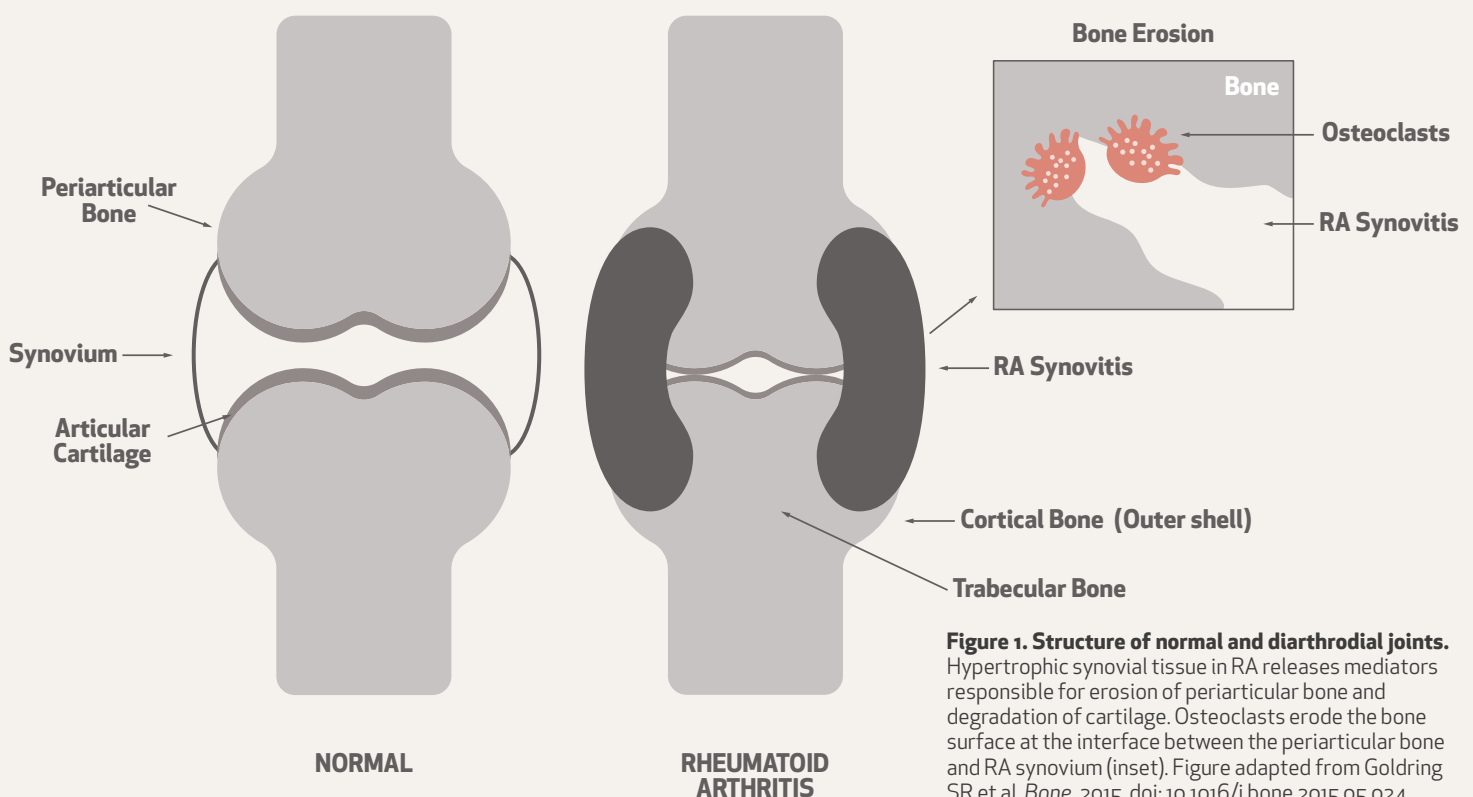
# Patterns of Pathologic Bone Remodeling Observed in RA

Distinct patterns of bone remodeling are found in RA, which can be broadly categorized as periarticular osteopenia, focal joint erosions, and systemic osteoporosis.<sup>2</sup> 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.<sup>23</sup>

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.<sup>23-25</sup>

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**).<sup>2</sup> These erosions are often localized to the joint margins, in which the bone is organized into a plate-like structure of compact cortical bone.<sup>2</sup> However, protrusions of the pannus may also cause erosion in deeper subchondral regions, which are comprised of a network of cancellous trabecular bone.<sup>7</sup> These subchondral regions of bone erosion may extend through the calcified cartilage that interfaces with bone.<sup>2</sup> 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.<sup>26</sup> Anatomical alterations leading



**Figure 1. Structure of normal and diarthrodial joints.** Hypertrophic synovial tissue in RA releases mediators responsible for erosion of periarticular bone and degradation of cartilage. Osteoclasts erode the bone surface at the interface between the periarticular bone and RA synovium (inset). Figure adapted from Goldring SR et al. *Bone*. 2015. doi:10.1016/j.bone.2015.05.024 [Epub ahead of print].

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.<sup>27</sup> 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.<sup>2,28,29</sup> 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.<sup>2</sup> The degree of generalized bone loss occurring early in the course of RA is also associated with disease activity.<sup>30</sup>

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%.<sup>31-35</sup> Patients with RA who have reduced BMD are at an increased risk for fracture.<sup>31</sup>

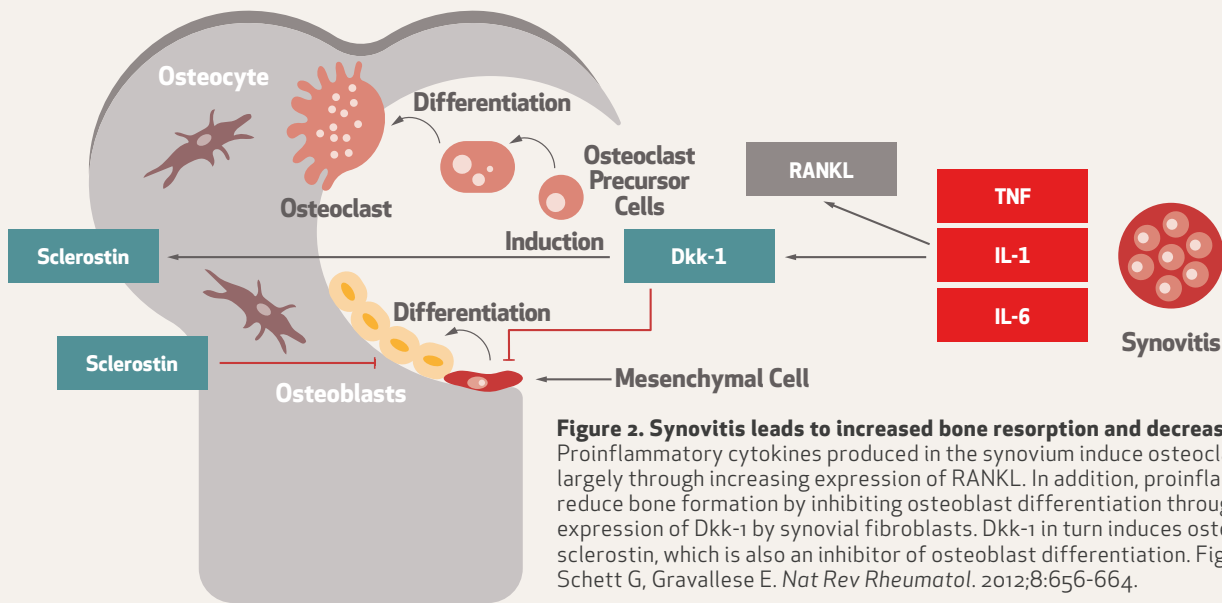
## Mechanisms of Bone Loss in RA

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.<sup>36</sup> 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.<sup>2,7</sup> These include pro-inflammatory cytokines such as IL-6 and TNF- $\alpha$ , chemokines, and pro-osteoclastogenic soluble mediators such as macrophage colony-stimulating factor (M-CSF).<sup>7</sup>

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).<sup>2</sup> Osteoclasts are generated from precursor cells that are usually of the monocyte-macrophage lineage.<sup>7</sup> Interactions between receptor activator of the nuclear factor kappa B (RANK) and its ligand (RANKL) are essential in osteoclastogenesis.<sup>2,7</sup> RANK on monocytes bind to RANKL, initiating osteoclast differentiation (**Figure 2**). Under physiological conditions, the main source of RANKL is osteoblasts.<sup>7</sup> However, cells of the synovium, such as immune cells and FLS, are the main source of RANKL in pathological conditions such as RA (**Figure 3**).<sup>7</sup> 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.<sup>37</sup> OPG inhibits osteoclast function by binding directly to RANKL to block the RANKL-RANK interaction. By blocking this interaction, OPG attenuates osteoclast differentiation.

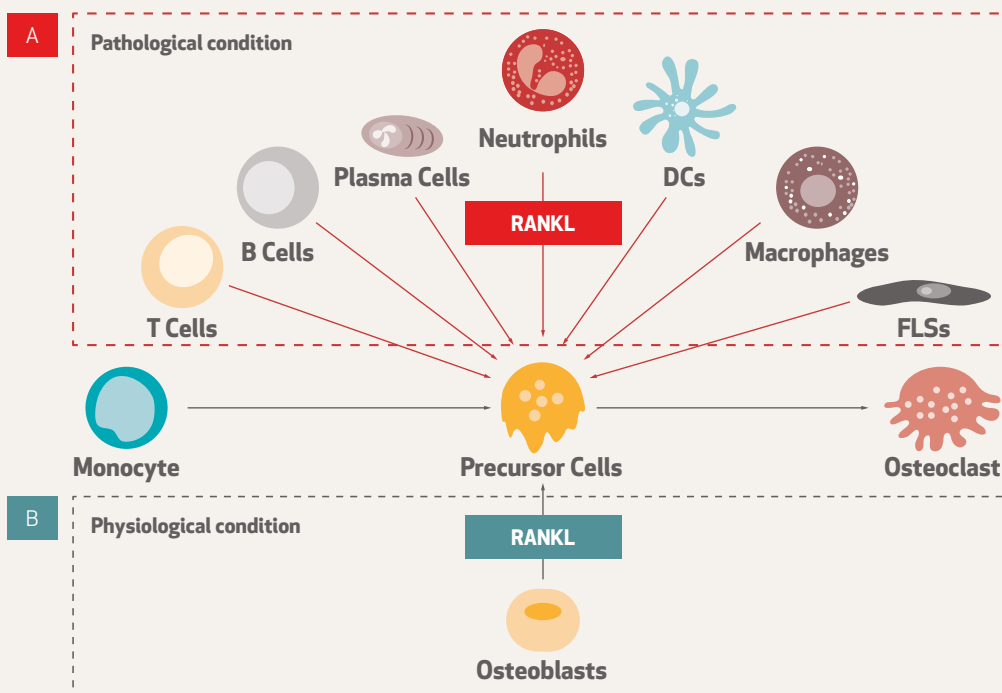
Anti-citrullinated protein antibodies (ACPAs) have been shown to be an independent



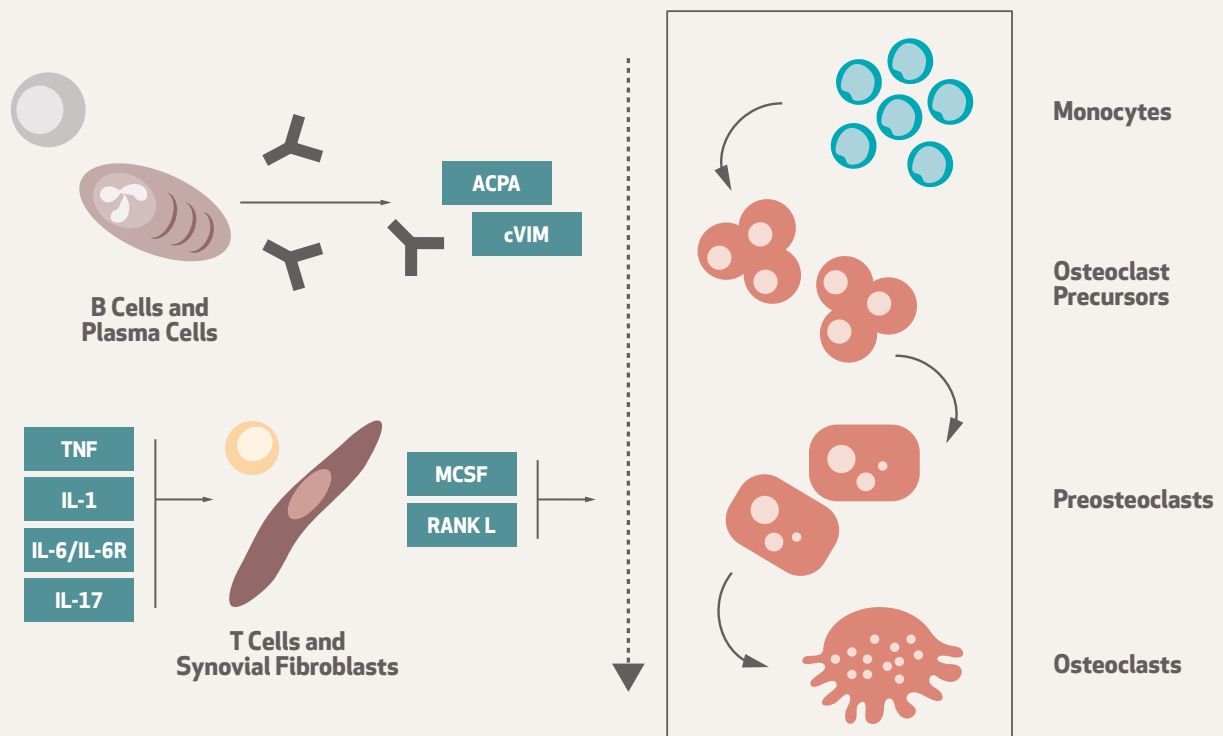
**Figure 2. Synovitis leads to increased bone resorption and decreased bone formation.** Proinflammatory cytokines produced in the synovium induce osteoclastogenesis largely through increasing expression of RANKL. In addition, proinflammatory cytokines reduce bone formation by inhibiting osteoblast differentiation through increasing the expression of Dkk-1 by synovial fibroblasts. Dkk-1 in turn induces osteocytes to express sclerostin, which is also an inhibitor of osteoblast differentiation. Figure adapted from Schett G, Gravallese E. *Nat Rev Rheumatol.* 2012;8:656-664.

predictor of the development of bone erosions in patients with RA.<sup>38-41</sup> ACPAs can be detected years before clinical disease onset of RA, suggesting a role in driving disease progression.<sup>42</sup> Interestingly, a recent study found significant bone loss in healthy individuals with ACPA compared with patients who were negative for ACPA.<sup>43</sup> 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.<sup>44</sup> The other implication of these findings is that bone loss may be important for priming of the joint for susceptibility to chronic inflammation.<sup>44</sup> Interestingly, the presence of ACPA in patients with RA seems to have little influence on disease activity.<sup>45</sup> In contrast,



**Figure 3. RANKL is aberrantly expressed in RA.** Monocytes are largely differentiated into mature osteoclasts through RANKL binding. Under normal conditions, osteoblasts are the predominate source of RANKL. In RA and other pathologic conditions, RANKL is expressed by a variety of cell types which normally do not, under physiologic conditions. Figure adapted from Jung SM et al. *J Immunol Res.* 2014;2014:263625.



**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.

the presence of the autoantibody rheumatoid factor (RF) does appear to be associated with higher disease activity, but there exists disagreement on its role in joint erosion.<sup>40,41,46</sup>

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**).<sup>47</sup> These interactions can then stimulate osteoclast precursors to differentiate into mature active osteoclasts which lead to increased bone resorption.<sup>47</sup>

A recent study by Hecht et al demonstrates that rheumatoid factor may cooperate with ACPAs to enhance bone erosion.<sup>40</sup>

Another pathological underpinning of bone resorption in RA is the virtual absence of bone repair in articular focal erosions.<sup>2</sup> 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.<sup>48</sup> Synovial fibroblasts, endothelial cells, and chondrocytes all express DKK-1.<sup>2</sup>



# Contributions of IL-6 to Bone Remodeling

Pro-inflammatory cytokines like TNF- $\alpha$ , 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.<sup>7,48</sup>

## 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.<sup>49,50</sup> A significant reduction of 3-D trabecular microarchitecture was also observed in these transgenic animals.<sup>49</sup> IL-6 knockout mice with experimental arthritis showed significantly decreased osteoclastogenic activity and impaired osteoclast recruitment to inflammatory sites.<sup>51</sup> Under physiological conditions, IL-6 deficiency resulted in no detectable change in osteoclast number.<sup>52</sup> However, IL-6 knockout mice were protected against ovariectomy-induced bone loss.<sup>52</sup>

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

	Effect on Bone Metabolism	Supported By
Bone Resorption	With sIL-6R, induces expression of RANKL on osteoblasts, leading to osteoclast differentiation <sup>53</sup>	In vitro mouse cell culture model
	In the presence of sIL-6R, induces expression on FLS <sup>54</sup>	In vitro human cell culture model
	Enhances Th17 cell differentiation to secrete IL-17, <sup>55,56</sup> which stimulates osteoclastogenesis <sup>57</sup>	In vitro mouse cell culture model
	Supports RANKL-independent osteoclast formation <sup>58</sup>	In vitro mouse cell culture model
	In prepubertal mice, enhances osteoclastogenesis <sup>49</sup>	Transgenic mice overexpressing IL-6
	Important for maintaining 3-D trabecular microarchitecture <sup>49</sup>	Transgenic mice overexpressing IL-6
	Induces CRP expression in hepatocytes, <sup>59</sup> leading to increased RANKL expression and increased osteoclast differentiation <sup>37</sup>	In vitro human cell culture model In vivo studies correlating IL-6 and CRP levels in RA patients
	Induces differentiation of B cells into plasma cells <sup>60</sup> that secrete Dickkopf-related protein 1 (DKK-1), which inhibits osteoblast formation <sup>48</sup>	Mouse models and in vitro mouse cell culture model
With sIL-6R, directly inhibits osteoblast differentiation <sup>61</sup>	In vitro mouse cell culture model	
Bone Formation	Protects against ovariectomy-induced bone loss <sup>52</sup>	IL-6 knockout mouse model
	Enhances osteoblast differentiation in vitro <sup>62,63</sup>	In vitro mouse and human cell culture model
	In the absence of other support cells, IL-6 by itself directly suppresses the differentiation and facilitates the proliferation of osteoclast progenitors <sup>64</sup>	In vitro mouse cell culture model
	Suppresses TNF- $\alpha$ -induced expression of Dkk-1 by FLS <sup>65</sup>	In vitro human cell culture model

## 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.<sup>66</sup> 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- $\alpha$ . 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.<sup>64</sup> Under steady-state conditions, IL-6 is proposed to suppress osteoclast function, and therefore prevent bone resorption.<sup>64</sup> 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.<sup>64</sup>

## 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.<sup>67</sup>

### **Elevated IL-6 levels are associated with generalized bone mineral density loss.**<sup>31,68,69</sup>

The prevalence of BMD loss in the overall RA patient population has been reported as between 19.6% and 56%.<sup>31-35</sup> 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.<sup>49</sup>

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.<sup>31,70</sup> 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.<sup>68</sup> 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.<sup>68</sup>

# Conclusions

The chronic synovitis associated with RA can ultimately lead to disruption of the integrity and functional properties of joint tissues.<sup>2</sup> Progressive bone loss also occurs systemically in RA, and it is associated with an increased risk of fractures.<sup>2</sup> 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.<sup>2,7</sup> The inflamed synovium acts as a reservoir in RA; it provides the environment for the immune cells and cytokines that enhance osteoclastogenesis.<sup>2,7</sup> These cells and cytokines can upregulate expression of RANKL directly, or indirectly by stimulating release of proinflammatory cytokines that also influence RANKL expression.<sup>2,7</sup> IL-6 can stimulate expression of RANKL in a broad array of cells such as osteoblasts and FLS.<sup>53,54</sup> IL-6 also enhances osteoclast activity through other indirect mechanisms including affecting expression of other cytokines involved in mediating joint damage.<sup>37,55,56,58</sup> Continued research on the many functions of IL-6 may further delineate the pathological origins and underpinnings of bone loss in RA.

# References

1. Dayer JM, Choy E. Therapeutic targets in rheumatoid arthritis: the interleukin-6 receptor. *Rheumatology*. 2010;49(1):15-24.
2. Goldring SR. Inflammatory signaling induced bone loss. *Bone*. 2015; doi:10.1016/j.bone.2015.05.024.
3. Koevoets R, Dirven L, Klarenbeek NB, et al. Insights in the relationship of joint space narrowing versus erosive joint damage and physical functioning of patients with RA. *Ann Rheum Dis*. 2013;72(6):870-874.
4. van Staa TP, Geusens P, Bijlsma JW, Leufkens HG, Cooper C. Clinical assessment of the long-term risk of fracture in patients with rheumatoid arthritis. *Arthritis Rheum*. 2006;54(10):3104-3112.
5. Deal C. Bone loss in rheumatoid arthritis: systemic, periarticular, and focal. *Curr Rheumatol Rep*. 2012;14(3):231-237.
6. Kanis JA, Johnell O, Oden A, Johansson H, McCloskey E. FRAX and the assessment of fracture probability in men and women from the UK. *Osteoporos Int*. 2008;19(4):385-397.
7. Jung SM, Kim KW, Yang CW, Park SH, Ju JH. Cytokine-mediated bone destruction in rheumatoid arthritis. *J Immunol Res*. 2014;2014:263625.
8. McInnes IB, Schett G. Cytokines in the pathogenesis of rheumatoid arthritis. *Nat Rev Immunol*. 2007;7(6):429-442.
9. Calabrese LH, Rose-John S. IL-6 biology: implications for clinical targeting in rheumatic disease. *Nat Rev Rheumatol*. 2014;10(12):720-727.
10. Janeway CJ, Travers P, Walport M, Shlomchik M. *Immunobiology: The Immune System in Health and Disease*. 5th ed. New York: Garland Science; 2001.
11. O'Shea J, Sims J, Siegel R, Farber J. Cytokines. In: Hochberg M, Silman A, Smolen J, Weinblatt M, Weisman M, eds. *Rheumatology*. 5th ed. Philadelphia, PA: Mosby Elsevier; 2001.
12. Choy EH, Kavanaugh AF, Jones SA. The problem of choice: current biologic agents and future prospects in RA. *Nat Rev Rheumatol*. 2013;9(3):154-163.
13. Liang KP, Myasoedova E, Crowson CS, et al. Increased prevalence of diastolic dysfunction in rheumatoid arthritis. *Ann Rheum Dis*. 2010;69(9):1665-1670.
14. Rho YH, Chung CP, Oeser A, et al. Inflammatory mediators and premature coronary atherosclerosis in rheumatoid arthritis. *Arthritis Rheum*. 2009;61(11):1580-1585.
15. 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 Min Res*. 1996;11(1):88-95.
16. Bode JG, Albrecht U, Haussinger D, Heinrich PC, Schaper F. Hepatic acute phase proteins--regulation by IL-6- and IL-1-type cytokines involving STAT3 and its crosstalk with NF-kappaB-dependent signaling. *Eur J Cell Biol*. 2012;91(6-7):496-505.
17. Campbell IL, Cutri A, Wilson A, Harrison LC. Evidence for IL-6 production by and effects on the pancreatic beta-cell. *J Immunol*. 1989;143(4):1188-1191.
18. Ito A, Itoh Y, Sasaguri Y, Morimatsu M, Mori Y. Effects of interleukin-6 on the metabolism of connective tissue components in rheumatoid synovial fibroblasts. *Arthritis Rheum*. 1992;35(10):1197-1201.
19. Jansen JH, Kluin-Nelemans JC, Van Damme J, Wientjens GJ, Willemze R, Fibbe WE. Interleukin 6 is a permissive factor for monocytic colony formation by human hematopoietic progenitor cells. *J Exp Med*. 1992;175(4):1151-1154.
20. Okada A, Yamasaki S, Koga T, et al. Adipogenesis of the mesenchymal stromal cells and bone oedema in rheumatoid arthritis. *Clin Exp Rheumatol*. 2012;30(3):332-337.
21. Colmegna I, Ohata BR, Menard HA. Current understanding of rheumatoid arthritis therapy. *Clin Pharmacol Ther*. 2012;91(4):607-620.
22. Sha Y, Markovic-Plese S. A role of IL-1R1 signaling in the differentiation of Th17 cells and the development of autoimmune diseases. *Self Nonself*. 2011;2(1):35-42.
23. Goldring SR. Periarticular bone changes in rheumatoid arthritis: pathophysiological implications and clinical utility. *Ann Rheum Dis*. 2009;68(3):297-299.
24. Hoff M, Haugeberg G, Odegard S, et al. Cortical hand bone loss after 1 year in early rheumatoid arthritis predicts radiographic hand joint damage at 5-year and 10-year follow-up. *Ann Rheum Dis*. 2009;68(3):324-329.
25. Stewart A, Mackenzie LM, Black AJ, Reid DM. Predicting erosive disease in rheumatoid arthritis. A longitudinal study of changes in bone density using digital X-ray radiogrammetry: a pilot study. *Rheumatology*. 2004;43(12):1561-1564.
26. Smolen JS, van der Heijde DM, Aletaha D, et al. Progression of radiographic joint damage in rheumatoid arthritis: independence of erosions and joint space narrowing. *Ann Rheum Dis*. 2009;68(10):1535-1540.
27. McQueen FM. Bone marrow edema and osteitis in rheumatoid arthritis: the imaging perspective. *Arthritis Res Ther*. 2012;14(5):224.
28. 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 USA*. 1998;95(14):8222-8226.
29. Pope RM. Apoptosis as a therapeutic tool in rheumatoid arthritis. *Nat Rev Immunol*. 2002;2(7):527-535.
30. Gough AK, Lilley J, Eyre S, Holder RL, Emery P. Generalised bone loss in patients with early rheumatoid arthritis. *Lancet*. 1994;344(8914):23-27.
31. Forsblad D'Elia H, Larsen A, Waltbrand E, et al. Radiographic joint destruction in postmenopausal rheumatoid arthritis is strongly associated with generalised osteoporosis. *Ann Rheum Dis*. 2003;62(7):617-623.
32. Haugeberg G, Orstavik RE, Uhlig T, Falch JA, Halse JJ, Kvien TK. Clinical decision rules in rheumatoid arthritis: do they identify patients at high risk for osteoporosis? Testing clinical criteria in a population based cohort of patients with rheumatoid arthritis recruited from the Oslo Rheumatoid Arthritis Register. *Ann Rheum Dis*. 2002;61(12):1085-1089.
33. Xu S, Wang Y, Lu J, Xu J. Osteoprotegerin and RANKL in the pathogenesis of rheumatoid arthritis-induced osteoporosis. *Rheumatol Int*. 2012;32(11):3397-3403.
34. Gonzalez-Lopez L, Gamez-Nava JI, Vega-Lopez A, et al. Performance of risk indices for identifying low bone mineral density and osteoporosis in Mexican Mestizo women with rheumatoid arthritis. *J Rheumatol*. 2012;39(2):247-253.
35. Mobini M, Kashi Z, Ghobadifar A. Prevalence and associated factors of osteoporosis in female patients with rheumatoid arthritis. *Caspian J Intern Med*. 2012;3(3):447-450.
36. Bartok B, Firestein GS. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. *Immunol Rev*. 2010;233(1):233-255.
37. Kim KW, Kim BM, Moon HW, Lee SH, Kim HR. Role of C-reactive protein in osteoclastogenesis in rheumatoid arthritis. *Arthritis Res Ther*. 2015;17:41.
38. Klareskog L, Catrina AI, Paget S. Rheumatoid arthritis. *Lancet*. 2009;373(9664):659-672.
39. Meyer O, Labarre C, Dougados M, et al. Anticitrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five year radiographic damage. *Ann Rheum Dis*. 2003;62(2):120-126.
40. Hecht C, Englbrecht M, Rech J, et al. Additive effect of anti-citrullinated protein antibodies and rheumatoid factor on bone erosions in patients with RA. *Ann Rheum Dis*. 2014.
41. van Steenberg HW, Ajeganova S, Forslind K, Svensson B, van der Helm-van Mil AH. The effects of rheumatoid factor and anticitrullinated peptide antibodies on bone erosions in rheumatoid arthritis. *Ann Rheum Dis*. 2015;74(1):e3.
42. Rantapää-Dahlqvist S, de Jong BA, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum*. 2003;48(10):2741-2749.
43. Kleyer A, Finzel S, Rech J, et al. Bone loss before the clinical onset of rheumatoid arthritis in subjects with anticitrullinated protein antibodies. *Ann Rheum Dis*. 2014;73(5):854-860.

44. Kleyer A, Schett G. Arthritis and bone loss: a hen and egg story. *Curr Opin Rheumatol*. 2014;26(1):80-84.
45. Aletaha D, Alasti F, Smolen J. Rheumatoid factor, not ACPA, is associated with disease activity in rheumatoid arthritis. *Arthritis Rheum*. 2014;66(10):5464-5465.
46. Hecht C, Schett G, Finzel S. The impact of rheumatoid factor and ACPA on bone erosion in rheumatoid arthritis. *Ann Rheum Dis*. 2015;74(1):e4.
47. Harre U, Georgess D, Bang H, et al. Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. *J Clin Invest*. 2012;122(5):1791-1802.
48. Diarra D, Stolina M, Polzer K, et al. Dickkopf-1 is a master regulator of joint remodeling. *Nat Med*. 2007;13(2):156-163.
49. De Benedetti F, Rucci N, Del Fattore A, et al. Impaired skeletal development in interleukin-6-transgenic mice: a model for the impact of chronic inflammation on the growing skeletal system. *Arthritis Rheum*. 2006;54(11):3551-3563.
50. Kitamura H, Kawata H, Takahashi F, Higuchi Y, Furuichi T, Ohkawa H. Bone marrow neutrophilia and suppressed bone turnover in human interleukin-6 transgenic mice. A cellular relationship among hematopoietic cells, osteoblasts, and osteoclasts mediated by stromal cells in bone marrow. *Am J Pathol*. 1995;147(6):1682-1692.
51. Wong PK, Quinn JM, Sims NA, van Nieuwenhuijze A, Campbell IK, Wicks IP. Interleukin-6 modulates production of T lymphocyte-derived cytokines in antigen-induced arthritis and drives inflammation-induced osteoclastogenesis. *Arthritis Rheum*. 2006;54(1):158-168.
52. Poli V, Balena R, Fattori E, et al. Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. *EMBO J*. 1994;13(5):1189-1196.
53. O'Brien CA, Gubrij I, Lin SC, Saylor RL, Manolagas SC. STAT3 activation in stromal/osteoblastic cells is required for induction of the receptor activator of NF-kappaB ligand and stimulation of osteoclastogenesis by gp130-utilizing cytokines or interleukin-1 but not 1,25-dihydroxyvitamin D3 or parathyroid hormone. *J Biol Chem*. 1999;274(27):19301-19308.
54. Hashizume M, Hayakawa N, Mihara M. IL-6 trans-signaling directly induces RANKL on fibroblast-like synovial cells and is involved in RANKL induction by TNF-alpha and IL-17. *Rheumatology*. 2008;47(11):1635-1640.
55. Ogura H, Murakami M, Okuyama Y, et al. Interleukin-17 promotes autoimmunity by triggering a positive-feedback loop via interleukin-6 induction. *Immunity*. 2008;29(4):628-636.
56. Sato K, Suematsu A, Okamoto K, et al. Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. *J Exp Med*. 2006;203(12):2673-2682.
57. Nakashima T, Kobayashi Y, Yamasaki S, et al. Protein expression and functional difference of membrane-bound and soluble receptor activator of NF-kappaB ligand: modulation of the expression by osteotropic factors and cytokines. *Biochem Biophys Res Commun*. 2000;275(3):768-775.
58. Kudo O, Sabokbar A, Pocock A, Itonaga I, Fujikawa Y, Athanasou NA. Interleukin-6 and interleukin-11 support human osteoclast formation by a RANKL-independent mechanism. *Bone*. 2003;32(1):1-7.
59. Gauldie J, Richards C, Harnish D, Lansdorp P, Baumann H. Interferon beta 2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proc Natl Acad Sci USA*. 1987;84(20):7251-7255.
60. Muraguchi A, Hirano T, Tang B, et al. The essential role of B cell stimulatory factor 2 (BSF-2/IL-6) for the terminal differentiation of B cells. *J Exp Med*. 1988;167(2):332-344.
61. Kaneshiro S, Ebina K, Shi K, et al. IL-6 negatively regulates osteoblast differentiation through the SHP2/MEK2 and SHP2/Akt2 pathways in vitro. *J Bone Miner Metab*. 2014;32(4):378-392.
62. Erices A, Conget P, Rojas C, Minguell JJ. Gp130 activation by soluble interleukin-6 receptor/interleukin-6 enhances osteoblastic differentiation of human bone marrow-derived mesenchymal stem cells. *Exp Cell Res*. 2002;280(1):24-32.
63. Li Y, Backesjo CM, Haldosen LA, Lindgren U. IL-6 receptor expression and IL-6 effects change during osteoblast differentiation. *Cytokine*. 2008;43(2):165-173.
64. Yoshitake F, Itoh S, Narita H, Ishihara K, Ebisu S. Interleukin-6 directly inhibits osteoclast differentiation by suppressing receptor activator of NF-kappaB signaling pathways. *J Biol Chem*. 2008;283(17):11535-11540.
65. Yeremenko N, Zwerina K, Rigter G, et al. TNF-alpha and IL-6 differentially regulate Dkk-1 in the inflamed arthritic joint. *Arthritis Rheumatol*. 2015; doi:10.1002/art.39183.
66. Schmitz ML, Weber A, Roxlau T, Gaestel M, Kracht M. Signal integration, crosstalk mechanisms and networks in the function of inflammatory cytokines. *Biochim Biophys Acta*. 2011;1813(12):2165-2175.
67. Haynes DR, Crotti TN, Loric M, Bain GI, Atkins GJ, Findlay DM. Osteoprotegerin and receptor activator of nuclear factor kappaB ligand (RANKL) regulate osteoclast formation by cells in the human rheumatoid arthritic joint. *Rheumatology*. 2001;40(6):623-630.
68. Oelzner P, Franke S, Lehmann G, Eidner T, Hein G, Wolf G. The balance between soluble receptors regulating IL-6 trans-signaling is predictive for the RANKL/osteoprotegerin ratio in postmenopausal women with rheumatoid arthritis. *Rheumatol Int*. 2012;32(1):199-206.
69. Giuliani N, Sansoni P, Girasole G, et al. Serum interleukin-6, soluble interleukin-6 receptor and soluble gp130 exhibit different patterns of age- and menopause-related changes. *Exp Gerontol*. 2001;36(3):547-557.
70. Ahlborg HG, Rosengren BE, Jarvinen TL, et al. Prevalence of osteoporosis and incidence of hip fracture in women--secular trends over 30 years. *BMC Musculoskelet Disord*. 2010;11:48.

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