IL-6 is a critical cytokine for inflammatory bone loss in rheumatoid arthritis

"*Since IL-6 is recognized as an osteotropic cytokine contributing to bone formation and resorption, it clearly could also play an important role in the altered bone remodeling associated with RA.*"

KEYWORDS: bone erosions ■ IL-6 ■ osteoblasts ■ osteoclasts ■ rheumatoid arthritis

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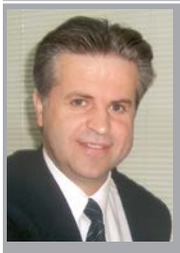

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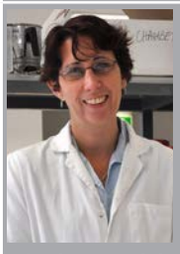

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Among the cytokines implicated in inflammatory bone loss, most attention has focused on TNF-α but the acquisition of neutralizing antibodies that target the IL-6 receptor (IL-6R) has focused new interest on the potential roles of IL-6.

Rheumatoid arthritis (RA) is characterized by persistent synovitis and joint destruction. Within 5 years, up to 50% of patients will have joint damage, which contributes to irreversible functional disability [1]. RA is also associated with systemic osteoporosis and increased bone fragility. The increased fracture risk is more prominent at the hip and spine and is associated with RA severity, disease duration and glucocorticoid use [2].

Ultimately, the disordered dynamics of osteoclast and osteoblast numbers and function dictate inflammation-induced bone loss in RA. Osteoclastogenesis, which is fueled by receptor activator for nuclear factor-κB ligand (RANKL), IL-6, TNF-α and IL-17 (among other cytokines), leads to increased osteoclast numbers and bone destruction. Osteoclasts that are located both at the synovial–bone interface and within the bone marrow adjacent to inflamed synovium are responsible for focal bone erosions [3,4]. At the same time, the capacity of osteoblasts to form properly mineralized bone is compromised at these sites, contributing to a net bone loss [5].

IL-6 is a member of a cytokine family that shares the same receptor subunit, gp130, in their transmembrane receptor complex. The cell-specific effects of IL-6 are controlled by selective expression of the membrane-bound IL-6R on certain cell types. When IL-6 interacts with cell-anchored IL-6Rs, it forms a receptor complex that contains gp130 homodimers, thus activating the JAK/STAT signaling pathway [6]. Of special interest to arthritis pathophysiology, IL-6 also exerts many effects via a pivotal 'trans-signaling' mechanism, whereby soluble IL-6 receptor (sIL-6R) complexes interact directly with cell-bound gp130 [7–9].

Relative to other proinflammatory cytokines, IL-6 and sIL-6R are expressed abundantly in synovial membrane, joint fluids and serum of patients with RA, contributing both to pannus development and local and systemic manifestations of synovitis [10]. Importantly, IL-6 levels in the synovial fluid correlate with the severity of joint damage and the number of erosions in RA patients [11]. Since IL-6 is recognized as an osteotropic cytokine contributing to bone formation and resorption, it clearly could also play an important role in the altered bone remodeling associated with RA.

Effect of IL-6 *in vitro*

In vitro studies show that IL-6 trans-signaling promotes osteoclastogenesis by increasing RANKL expression in osteoblasts and T cells [12,13], this effect is dependent on the JAK/STAT-3 signaling pathway [14]. IL-6 trans-signaling also increases RANKL expression by synovial fibroblasts to support osteoclastogenesis in the inflamed joint. Of note, however, under some circumstances IL-6 can actually inhibit osteoclastogenesis from human $CD14^+$ precursors *in vitro*, diverting them to a macrophage differentiation fate [15]. The *in vitro* action of IL-6 on osteoblasts is also controversial. Osteoblasts are among the cells that express the IL-6R but the low level of IL-6R expression is limiting and sIL-6R is critical for IL-6 to act on osteoblasts [16]. IL-6 promotes osteoblast differentiation via the activation of the JAK/STAT pathway [17] and increases the release of pro-osteoblastic cytokines in the bone microenvironment [18]. However, IL-6 has also been shown to inhibit osteoblast precursor cell proliferation

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and induce apoptosis at the late stage of the differentiation process [16,19]. Therefore, the precise effects of IL-6 on osteoblasts are complex and dependent on the stage of cellular differentiation.

Effect of IL-6 *in vivo*

Animal models highlight the deleterious effect of IL-6 on bone. Transgenic mice overexpressing IL-6 show increased bone turnover with reduced osteoblast and increased osteoclast numbers, resulting in osteopenia [20]. Conversely, although mice lacking IL-6 do not exhibit any bone phenotype, these mice are partially protected from ovariectomy-induced osteoporosis, suggesting that IL-6 contributes to estrogen-induced bone loss [21]. If administered at the time of arthritis induction, anti-IL-6R antibody abolishes the inflammatory response in a mice collagen-induced arthritis model but not if given later in the disease process [22]. The critical function of IL-6 was shown in antigen-induced arthritis, which was less severe in IL-6-deficient mice, with reduced inflammation, fewer osteoclasts and bone erosions [23]. Finally, intra-articular injection of soluble gp130 (which blocks IL-6 trans-signaling) at the time of antigen-induced arthritis induction was sufficient to inhibit inflammation and joint destruction [24].

> *"...in the context of TNF-α-driven inflammation, IL-6 could exert a direct effect on osteoclastogenesis, which is independent of inflammation."*

Animal models have also elucidated interactions between TNF-α and IL-6. As TNF-α induces IL-6 production by stromal and osteoblastic cells, the bone protective effect of IL-6 blockade could be related to a direct effect on bone cells or reduced inflammation. Interestingly, TNF-α-dependent synovitis in the human TNF-α transgenic mice is not blocked by anti-IL-6R treatment but this treatment is able to reduce bone erosion. This result indicates that, in the context of TNF-α-driven inflammation, IL-6 could exert a direct effect on osteoclastogenesis, which is independent of inflammation [25].

Clinical studies

Clinicians now have access to tocilizumab, a humanized monoclonal antibody targeting the IL-6R and recent publications have demonstrated its efficacy in RA [26]. Only the Study of Active Controlled Monotherapy Used for Rheumatoid Arthritis, an IL-6 Inhibitor (SAMURAI) has evaluated radiographic progression as a primary outcome of IL-6R blockade. In this study, patients with RA (<5 years) who failed disease-modifying antirheumatic drug (DMARD) treatment were treated with tocilizimab monotherapy versus standard DMARD. At 52 weeks, patients treated with tocilizimab showed less radiographic progression (mean Sharp score change: 2.3; 95% CI: 1.5–3.2) than the DMARD group (mean Sharp score change: 6.1; 95% CI: 4.2–8.0; $p < 0.01$). In this study, tocilizumab was more effective in reducing radiological progression in patients presenting with risk factors for rapid progression than in low-risk patients [27]. Garnero *et al.* recently reported that in the Pivotal Trial in Methotrexate Inadequate Responders (OPTION) study, in which tocilizumab was added to methotrexate in patients who failed to respond to methotrexate therapy alone, tocilizimab was able to significantly reduce the serum bone degradation marker CTX-I compared with the placebo [28]. These preliminary data provide support the bone-sparing effects of IL-6 blockade in RA. However, further studies are needed to confirm a potential beneficial effect of tocilizumab on systemic bone loss.

Emerging therapy

On the clinical horizon, drugs that target the JAK/STAT signal transduction pathway offer a new approach for the treatment of inflammatory bone loss. A JAK-3 selective inhibitor [29,30] and, more recently, a JAK-1/2 selective inhibitor [31] (which potentially targets IL-6 activity) exhibit promising results in both preclinical and clinical studies. The effect of this class of drugs on focal and systemic bone loss associated with RA warrants further investigation in both animal models and human RA.

Conclusion & future perspective

While IL-6 is a pre-eminent cytokine associated with bone loss in RA, the direct effects of IL-6 on bone cells in the context of arthritis need to be precisely determined. Targeting IL-6 trans-signaling or the JAK/STAT signaling pathway subserving the IL-6R is a rational strategy to address inflammatory bone loss in RA, especially for patients who are refractory to TNF inhibition. Studies that compare TNF inhibition head-to-head with IL-6 inhibition will inform the relative efficacy of these two anticytokine strategies. Finally, prospective studies of the long-term effects of IL-6 blockade in RA are essential to confirm beneficial effects on focal and systemic bone loss.

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Bibliography

1 Gabriel SE: The epidemiology of rheumatoid arthritis. *Rheum. Dis. Clin. North Am.* 27(2), 269–281 (2001).

2 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.* 54(10), 3104–3112 (2006).

3 Gravallese EM, Harada Y, Wang JT, Gorn AH, Thornhill TS, Goldring SR: Identification of cell types responsible for bone resorption in rheumatoid arthritis and juvenile rheumatoid arthritis. *Am. J. Pathol.* 152(4), 943–951 (1998).

4 Romas E, Bakharevski O, Hards DK *et al.*: Expression of osteoclast differentiation factor at sites of bone erosion in collagen-induced arthritis. *Arthritis Rheum.* 43(4), 821–826 (2000).

5 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.* 24(9), 1572–1585 (2009).

6 Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F: Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochem. J.* 374(Pt 1), 1–20 (2003).

7 Nowell MA, Williams AS, Carty SA *et al.*: Therapeutic targeting of IL-6 trans signaling counteracts STAT3 control of experimental inflammatory arthritis. *J. Immunol.* 182(1), 613–622 (2009).

8 Jones GW, McLoughlin RM, Hammond VJ *et al.*: Loss of $CD4^+$ T cell IL-6R expression during inflammation underlines a role for IL-6 trans signaling in the local maintenance of Th17 cells. *J. Immunol.* 184(4), 2130–2139 (2010).

9 Rose-John S, Scheller J, Elson G, Jones SA: Interleukin-6 biology is coordinated by membrane-bound and soluble receptors: role in inflammation and cancer. *J. Leukoc. Biol.* 80(2), 227–236 (2006).

10 Houssiau FA, Devogelaer JP, Van Damme J, De Deuxchaisnes CN, Van Snick J: Interleukin-6 in synovial fluid and serum of patients with rheumatoid arthritis and other inflammatory arthritides. *Arthritis Rheum.* 31(6), 784–788 (1988).

11 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.* 11(1), 88–95 (1996).

12 Palmqvist P, Persson E, Conaway HH, Lerner UH: IL-6, leukemia inhibitory factor, and oncostatin M stimulate bone resorption and regulate the expression of receptor activator of NF-κB ligand, osteoprotegerin, and receptor activator of NF-κB in mouse calvariae. *J. Immunol.* 169(6), 3353–3362 (2002).

13 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.* 54(1), 158–168 (2006).

14 O'Brien CA, Lin SC, Bellido T, Manolagas SC: Expression levels of gp130 in bone marrow stromal cells determine the magnitude of osteoclastogenic signals generated by IL-6-type cytokines. *J. Cell. Biochem.* 79(4), 532–541 (2000).

15 Duplomb L, Baud'huin M, Charrier C *et al.*: Interleukin-6 inhibits receptor activator of nuclear factor-κB ligand-induced osteoclastogenesis by diverting cells into the macrophage lineage: key role of Serine727 phosphorylation of signal transducer and activator of transcription 3. *Endocrinology* 149(7), 3688–3697 (2008).

16 Li Y, Backesjo CM, Haldosen LA, Lindgren U: IL-6 receptor expression and IL-6 effects change during osteoblast differentiation. *Cytokine* 43(2), 165–173 (2008).

17 Bellido T, Borba VZ, Roberson P, Manolagas SC: Activation of the Janus kinase/STAT (signal transducer and activator of transcription) signal transduction pathway by interleukin-6-type cytokines promotes osteoblast differentiation. *Endocrinology* 138(9), 3666–3676 (1997).

18 Taguchi Y, Yamamoto M, Yamate T *et al.*: Interleukin-6-type cytokines stimulate mesenchymal progenitor differentiation toward the osteoblastic lineage. *Proc. Assoc. Am. Physicians* 110(6), 559–574 (1998).

19 Malaval L, Gupta AK, Liu F, Delmas PD, Aubin JE: LIF, but not IL-6, regulates osteoprogenitor differentiation in rat calvaria cell cultures: modulation by dexamethasone. *J. Bone Miner. Res.* 13(2), 175–184 (1998).

20 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.* 54(11), 3551–3563 (2006).

21 Poli V, Balena R, Fattori E *et al.*: Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. *EMBO J.* 13(5), 1189–1196 (1994).

22 Takagi N, Mihara M, Moriya Y *et al.*: Blockage of interleukin-6 receptor ameliorates joint disease in murine collagen-induced arthritis. *Arthritis Rheum.* 41(12), 2117–2121 (1998).

23 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* 95(14), 8222–8226 (1998).

24 Nowell MA, Richards PJ, Horiuchi S *et al.*: Soluble IL-6 receptor governs IL-6 activity in experimental arthritis: blockade of arthritis severity by soluble glycoprotein 130. *J. Immunol.* 171(6), 3202–3209 (2003).

25 Axmann R, Bohm C, Kronke G, Zwerina J, Smolen J, Schett G: Inhibition of interleukin-6 receptor directly blocks osteoclast formation *in vitro* and *in vivo*. *Arthritis Rheum.* 60(9), 2747–2756 (2009).

26 Singh JA, Beg S, Lopez-Olivo MA: Tocilizumab for rheumatoid arthritis. *Cochrane Database Syst. Rev.* 7, CD008331 (2010).

27 Hashimoto J, Garnero P, Van Der Heijde D *et al.*: Humanized anti-interleukin-6-receptor antibody (tocilizumab) monotherapy is more effective in slowing radiographic progression in patients with rheumatoid arthritis at high baseline risk for structural damage evaluated

with levels of biomarkers, radiography, and BMI: data from the SAMURAI study. *Mod. Rheumatol.* (2010) (Epub ahead of print).

28 Garnero P, Thompson E, Woodworth T, Smolen JS: Rapid and sustained improvement in bone and cartilage turnover markers with the anti-interleukin-6 receptor inhibitor tocilizumab plus methotrexate in rheumatoid arthritis patients with an inadequate response to methotrexate: results from a substudy of the multicenter double-blind, placebo-controlled trial of tocilizumab in inadequate responders to methotrexate alone. *Arthritis Rheum.* 62(1), 33–43 (2010).

29 Lin TH, Hegen M, Quadros E *et al.*: Selective functional inhibition of JAK-3 is sufficient for efficacy in collagen-induced arthritis in mice. *Arthritis Rheum.* 62(8), 2283–2293 (2010).

30 Kremer JM, Bloom BJ, Breedveld FC *et al.*: The safety and efficacy of a JAK inhibitor in patients with active rheumatoid arthritis: results of a double-blind, placebo-controlled Phase IIa trial of three dosage levels of CP-690,550 versus placebo. *Arthritis Rheum.* 60(7), 1895–1905 (2009).

31 Fridman JS, Scherle PA, Collins R *et al.*: Selective inhibition of JAK1 and JAK2 is efficacious in rodent models of arthritis: preclinical characterization of INCB028050. *J. Immunol.* 184(9), 5298–5307 (2010).