Pro-inflammatory Cytokines Modulating Osteoclast Differentiation and Function

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In general, bone homeostasis is maintained through the balance between bone formation and resorption. Disruption in this balance results in bone-related diseases such as osteopetrosis, osteoporosis, and rheumatoid arthritis. Often, enhanced osteoclastogenesis is followed by accelerated bone resorption that is induced by pro-inflammatory cytokines in osteoporosis or rheumatoid arthritis, and leads to bone destruction. In this review study, factors involved in osteoclast differentiation and function are discussed, and how the prevention of such factors is effective in ameliorating bone loss in osteoporosis or rheumatoid arthritis.

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Key Words. Bone and bones, Osteoclasts, Rheumatoid arthritis, Cytokines

INTRODUCTION

Bone tissue maintains homeostasis through a process called bone remodeling, in which bone resorption by osteoclasts and bone formation by osteoblasts are continuously repeated [1]. The balance between resorption and formation provides a stable rate of bone remodeling. Imbalances due to an ectopic increase or decrease in either process results in diseases, including osteoporosis, osteopetrosis, osteopenia, rheumatoid arthritis (RA), and other bone related diseases [2,3].

Osteoclasts are originated from hematopoietic lineages. Initially, hematopoietic stem cells differentiate into macrophages in the presence of macrophage-colony stimulating factor (M-CSF) and then further differentiate into osteoclasts in the presence of both M-CSF and receptor activator of nuclear factor kappa-B ligand (RANKL) [4]. Therefore, M-CSF and RANKL are indispensable factors for osteoclast differentiation. Once an osteoclast is fully differentiated, it secretes proteases such as cathepsin K and matrix metalloproteinase (MMP) to degrade bone matrix [5].

RA, a chronic autoimmune disease characterized by bone destruction, erosion, and inflammation in synovial joints, can result in osteopenia and osteoporosis in severe cases [6-8]. Several studies suggest that irregular osteoclast activation is involved in bone destruction of RA. In addition, pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, IL-6, IL-17, and IL-23 secreted by various immune and non-immune cells including T cells, B cells, macrophages, and fibroblast-like synoviocytes are reported to be pro-osteoclastogenic by supporting additional RANKL expression directly or indirectly [9-11]. It has been suggested that accumulations of pro-inflammatory cytokines and osteoclastogenic factors in the synovium induce abnormal osteoclastogenesis in RA [12]. This abnormally enhanced osteoclastogenesis apparently results in bone destruction and implies an importance of osteoclast differentiation in RA. Therefore, osteoclast is a target of pro-inflammatory cytokines and an effector in initiating bone destruction and joint erosion in RA, which suggests that osteoclastogenesis should be carefully controlled to maintain normal bone remodeling or to reduce the progression of ectopic
bone resorption and erosion in RA. In this review, the connection between RA and enhanced osteoclastogenesis will be approached by providing an overview of pro-inflammatory cytokine-mediated osteoclastogenesis in RA.

### MAIN SUBJECTS

**Tumor necrosis factor alpha**

TNF-α is a cytokine that is produced during inflammation in RA and is able to induce osteoclastogenesis, either through the upregulation of M-CSF and RANKL expression in osteoblasts and osteocytes [13,14] or independent of RANKL-RANK signaling (Table 1) [15].

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Effects on osteoclasts</th>
<th>Effects on osteoblasts</th>
<th>Effects of antibody/blockade</th>
<th>Sources</th>
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<tr>
<td>TNF-α</td>
<td>Enhances osteoclastogenesis independent of RANKL-RANK signaling pathway</td>
<td>Upregulates M-CSF and RANKL in osteoblasts and osteocytes</td>
<td>Inhibitor utilization alleviates synovial inflammation and joint erosion</td>
<td>Macrophages</td>
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<td></td>
<td>Exhibits pro-osteoclastogenic effects synergistically with IL-1</td>
<td>Inhibits osteoclastogenesis and nodule formation</td>
<td>Prevents systemic bone loss</td>
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<tr>
<td>IL-1</td>
<td>Enhances osteoclastogenesis and prevents osteoclast apoptosis by upregulating RANKL expression and stimulating M-CSF production</td>
<td>Stimulates RANKL production</td>
<td>Decreases cartilage damage and bone resorption</td>
<td>Macrophages</td>
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<tr>
<td>IL-6</td>
<td>Dual roles on osteoclasts; induces bone resorption Inhibits RANKL signaling in osteoclast progenitors in the absence of supporting cells</td>
<td>Induces RANKL synthesis in osteoblasts</td>
<td>Suppresses RANKL-stimulated osteoclast differentiation and bone resorption</td>
<td>T cells</td>
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<td>Enhances osteoclastogenesis and bone resorption</td>
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<td>IL-17</td>
<td>Supports osteoclastogenesis dependent of RANKL-RANK signaling pathway</td>
<td>Upregulates RANKL expression</td>
<td>Reduces bone destruction and joint erosion</td>
<td>Th 17 cells</td>
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<td>IL-18</td>
<td>Suppresses osteoclastogenesis but involved in bone destruction Stimulates osteoclastogenesis by upregulating RANKL production from T cells</td>
<td>Increases OPG expression in osteoblasts</td>
<td>Decreases inflammation and cartilage degradation Inhibits TNF-α, IL-6, and IFN-gamma secretion</td>
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<td>IL-23</td>
<td>Enhances osteoclastogenesis</td>
<td>Direct effect of IL-23 on osteoblasts is unknown</td>
<td>Suppresses osteoclastogenesis, inflammation, and bone destruction</td>
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addition, TNF-α is known to inhibit osteoblastogenesis and nodule formation by downregulating Runx2/Cbfa-1 and Osx (Table 1) [16]. Furthermore, TNF-α induces Dkk-1, a Wnt antagonist, to inhibit osteoblastogenesis [17]. Meanwhile, TNF-α and IL-1 synergistically induce osteoclastogenesis with RANKL [18]. Collectively, these pro-osteoclastogenic and anti-osteoblastogenic effects of TNF-α result in elevated osteoclastogenesis and bone resorption, leading to bone destruction.

Such a destructive effect on bone and the inverse correlation between bone mineral density and serum TNF-α levels led to the utilization of TNF-α inhibitors such as infliximab, adalimumab, and golimumab for treatment of RA [19]. TNF-α inhibitor utilization resulted in alleviated synovial inflammation and joint erosion (Table 1) [20]. Furthermore, it has been reported that TNF-α inhibitors prevented systemic bone loss by regulating osteoclastogenesis, whereas little effect was observed on osteoblastogenesis [21].

**Interleukin-1**

IL-1 is another pro-inflammatory cytokine produced by macrophages in inflammatory diseases, which upregulates RANKL expression and results in enhanced osteoclastogenesis and bone resorption activity (Table 1) [22]. IL-1 also stimulates M-CSF production to suppresses apoptosis of osteoclasts [23]. Furthermore, IL-1 mediates TNF-α-induced osteoclastogenesis; therefore, IL-1 is able to stimulate osteoclastogenesis independent or dependent of the RANKL signaling pathway [24]. In addition to stimulation of osteoclastogenesis, IL-1 upregulates MMPs and stimulates synovial fibroblasts and chondrocytes to secrete MMPs and cathepsins [25]. These MMPs and cathepsins contribute to cartilage degradation, joint destruction, and bone resorption [22].

The importance of IL-1 also has been observed using IL-1-deficient mice. These mice exhibited reduced osteoclastogenesis, which led to increased bone density, trabecular bone mass, and cortical thickness as well as decreased cartilage damage [26]. Therefore, these studies suggest that IL-1 blockade might provide a therapeutic effect. Although TNF-α inhibitors exhibit beneficial effects, IL-1 blockade could be also important, because TNF-α inhibitors are able to reduce inflammation but, unable to avoid cartilage damage [27]. However, it has been reported that that antagonism of IL-1β with neutralizing antibodies exhibited decreased cartilage damage and bone resorption (Table 1) [28]. Therefore, these findings suggest an importance of IL-1 blockade as a therapeutic treatment.

**Interleukin-6**

IL-6, produced by synovial fibroblasts, T cells, and activated macrophages, is responsible for synovial inflammation [29]. Similar to TNF-α and IL-1, IL-6 is also able to promote RANKL synthesis in osteoblasts [30] and development of T helper 17 (Th 17), which provides an extra supplement of RANKL via induction of RANKL expression in fibroblast like synoviocytes (Table 1) [31]. Although IL-6 is osteoclastogenic, it has dual roles in osteoclastogenesis. IL-6 induces bone resorption and inhibits RANK signaling in osteoclast progenitors when the additional supporting cells are missing (Table 1) [32]. Signal transducer and activator of transcription 3 (STAT3) plays a critical role in the stimulation of RANKL expression. It has been reported that inflammatory cytokines such as TNF-α, IL-1, and IL-6 activate STAT3 in murine osteoblasts either in a direct or indirect manner. Furthermore IL-6 is known to stimulate RANKL expression in osteoblasts through STAT3 activation, and STAT3 is associated with inflammation and joint destruction (Table 1) [33]. Therefore, manipulation of STAT3 could be an important therapeutic target in RA.

The critical role of IL-6 was verified using IL-6 transgenic and knockout mice. In IL-6 transgenic mice, it was observed that osteoclastogenesis was enhanced, which further resulted in damaged skeletal growth in young mice in the prepubertal stage, however, osteoclast formation was decreased at the adult stage [34]. In IL-6 knockout mice, decreased osteoclast activity and recruitment to inflammatory sites were observed [31]. Furthermore, the absence of IL-6 provided protection from ovariectomy-induced bone loss and it has been reported that IL-6 is associated with accelerated bone turnover [35]. Indeed, IL-6 receptor antagonists have been shown to diminish the bone turnover [36] and the IL-6 antagonist tocolizumab suppresses RANKL-stimulated osteoclast differentiation and bone resorption (Table 1). Moreover, it alleviated joint destruction in RA patients [37]. Therefore, these studies suggest that IL-6 induced bone loss in inflammatory diseases could be due to ectopically accelerated bone turnover, and usage of IL-6 blockade or anti IL-6 is able to improve the accelerated bone turnover by suppressing osteoclast differentiation and bone resorption.
Interleukin-17

IL-17 is a pro-inflammatory cytokine produced by Th 17 cells, and its family consists of six members: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F [38]. IL-17 receptors are expressed in a variety of cells including osteoblasts, osteoclasts, chondrocytes, and synoviocytes, suggesting that IL-17 is able to influence several cells [39]. Stimulation of its ligand results in activation of nuclear factor-κB and mitogen-activated protein kinases, which suggests a possibility that IL-17 is involved in osteoclastogenesis [40]. Indeed, IL-17 is known to support osteoclastogenesis dependent of the RANKL-RANK signaling pathway. For example, IL-17 enhances the sensitivity of osteoclast precursors to RANKL by increasing RANK expression on the precursors (Table 1) [41]. Furthermore, IL-17 upregulates RANKL expression in several cells including osteoblasts, synovial cells, and mesenchymal cells, thereby increasing the RANKL/osteoprotegerin ratio and enhancing osteoclastogenesis, ultimately resulting in bone destruction (Table 1) [42]. In addition to the induction of RANKL expression, IL-17 induces the expression and secretion of pro-inflammatory cytokines including IL-1, TNF-α, and IL-6 [43]. Such pro-inflammatory cytokines further enhance synovial inflammation by activating macrophages, lymphocytes, and neutrophils [44]. For example, it has been reported that recombinant IL-17 activity is synergistically increased with other pro-inflammatory cytokines and ultimately results in joint inflammation and damage [43]. Such involvement of IL-17 in bone resorption, and cartilage and joint erosion led to the utilization of anti-IL-17 in RA. Antibody neutralization exhibited reduced bone destruction in collagen-induced arthritis [45]. Moreover, soluble IL-17 receptor resulted in reduced joint erosion and inflammation by downregulating the expression of RANKL and other inflammatory cytokines (Table 1) [9]. According to Genovese et al. [46] utilization of anti-IL-17 monoclonal antibody ameliorated RA symptoms in RA patients. Furthermore, a human antibody of IL-17A treatment improved inflammation in synovial joints and clinical scores in RA patients [47]. Collectively these studies suggest that IL-17 blockade may be a putative therapeutic for the prevention of joint inflammation and destruction.

Interleukin-23 and Interleukin-18

In addition to the aforementioned major pro-inflammatory cytokines, there are other cytokines involved in osteoclast differentiation such as IL-18 and IL-23. IL-23 stimulates IL-17 synthesis to form an axis in order to control inflammation [48]. Furthermore, systemic IL-23 exposure has been shown to cause chronic arthritis and bone loss, which in turn resulted in enhanced osteoclast differentiation; whereas, IL-23 knockout mice exhibited reduced osteoclast maturation [49]. In addition, it has been reported that IL-23 upregulates RANKL expression to induce osteoclast differentiation (Table 1) [50]. On the contrary, anti-IL-23 antibody treatment suppressed osteoclast differentiation, inflammation, bone destruction, and ultimately ameliorated collagen-induced arthritis (Table 1) [51]. Although IL-18 has been reported to suppress osteoclastogenesis, several studies have indicated that IL-18 is involved in bone destruction [52], wherein, IL-18 stimulates osteoclast differentiation by upregulating RANKL production from T cells in RA.
synovitis (Table 1). IL-18 is able to induce expression of both soluble and membrane bound RANKL [53]. It has been observed that its effect on osteoclastic formation was similar to IL-1 \( \beta \) but not as effective as TNF- \( \alpha \). Although there are contradictory roles for the cytokines, they still have the potential to induce osteoclast differentiation in RA and cause bone destruction.

**CONCLUSION**

Under physiological conditions, bone homeostasis is maintained by a strictly controlled balance between bone resorption and formation. However, under pathological conditions, resorption is relatively preferred over formation, ultimately resulting in bone destruction. Such enhanced bone resorption or osteoclastogenesis is induced by pro-inflammatory cytokines such as TNF- \( \alpha \), IL-1, IL-6, and IL-17 produced by various immune cells. These pro-inflammatory cytokines either directly or indirectly stimulate osteoclastogenesis by increasing RANKL expression; they can also induce osteoclastogenesis dependent or independent of the RANKL signaling pathway (Figure 1). Therefore, the investigation and understanding of pro-inflammatory cytokine signaling and their antibody or blockade usages may provide a better ability to ameliorate bone loss.

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**CONFLICT OF INTEREST**

No potential conflict of interest relevant to this article was reported.

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