Review

Direct and Indirect Effects of IL-17A on RANKL-Induced Osteoclastogenesis

Masao Maeno¹,², Hideki Tanaka¹,², Fan Zhang³, Satoshi Kitami¹, Kumiko Nakai⁴ and Takayuki Kawato¹,²

¹) Department of Oral Health Sciences, Nihon University School of Dentistry, Tokyo, Japan
²) Division of Functional Morphology, Dental Research Center, Nihon University School of Dentistry, Tokyo, Japan
³) Department of Orthodontics, Shandong University School of Dentistry, Jinan, Shandong Province, China
⁴) Division of Oral Health Sciences, Nihon University Graduate School of Dentistry, Tokyo, Japan

(Accepted for publication, May 10, 2013)

Abstract: Inflammation-mediated bone loss is a major feature of various bone diseases, including chronic periodontitis, rheumatoid arthritis, and osteoarthritis, and is due to an imbalance in bone remodeling that favors resorption. This imbalance is caused by increased cytokine levels in the inflamed tissue. Interleukin (IL)-17 is secreted primarily by activated Th17 cells, and IL-17s and IL-17 receptors play an important role in many autoimmune and inflammatory diseases. The aim of this paper is to review the differences between the indirect effect of IL-17A via osteoblasts and the direct effect of IL-17A on differentiation of osteoclast precursors into osteoclasts and the function of mature osteoclasts in the presence of receptor activator of nuclear factor κB ligand (RANKL). IL-17A stimulates the production of bone resorption-related inflammatory cytokines through an autocrine mechanism involving celecoxib-blocked prostaglandins (PGs), mainly PGE₂, in osteoblasts. Furthermore, IL-17A induces the differentiation of osteoclast precursors into osteoclasts and the function of mature osteoclasts via PGE₂ in osteoblasts. On direct effect of IL-17 to osteoclast precursors in the absence of RANKL, IL-17 induces the differentiation as well as indirect effect via osteoblasts. However, the differentiation and function of osteoclasts are suppressed by stimulating osteoclast precursors directly with IL-17A in the presence of RANKL. In conclusion, the effect of IL-17A on RANKL-induced osteoclastogenesis may conflict with the direct action on osteoclast precursors and the indirect action through osteoblasts.

Key words: IL-17, IL-17 receptor, RAW264.7 cells, RANKL, Osteoclastogenesis

Introduction

Cytokines are important messenger molecules in cell-to-cell communication and are involved in various aspects of the immune system, such as homeostasis maintenance and mediating and resolving pathologic conditions. Inflammation-mediated bone loss is a major feature of various bone diseases, including chronic periodontitis, rheumatoid arthritis, and osteoarthritis, and is due to an imbalance in bone remodeling that favors resorption. This imbalance is caused by increased cytokines and mediators in the inflamed tissue.

Interleukin (IL)-17 is secreted primarily by activated Th17 cells²,³, and IL-17s and IL-17 receptors play an important role in many autoimmune and inflammatory diseases⁴. IL-17 induces the production of extracellular matrix proteins in osteoblasts⁵. On the other hand, several studies have indicated that IL-17 is a pro-inflammatory cytokine crucial for osteoclastic bone resorption in the presence of osteoblasts⁶-⁸. In cartilage, IL-17 activates chondrocytes and macrophages to release degradative enzymes, which probably contributes to the cartilage destruction seen in rheumatoid arthritis at the interface of cartilage⁹. Our recent study showed that IL-17F stimulates cartilage degradation by increasing the expression of collagenases and stromelysin, and decreasing the expression of their inhibitors, type II collagen, and proteoglycans, and the plasminogen/plasmin pathway in chondrocytes¹⁰,¹¹. IL-17 has been shown to promote osteoclast differentiation indirectly through the induction of IL-1, tumor necrosis factor (TNF)-α, and receptor activator of nuclear factor κB ligand (RANKL) expression⁷,¹². Regarding the indirect effect of IL-17 on osteoclastogenesis, many studies have reported that IL-17A promotes the differentiation of osteoclast precursors into osteoclasts and the function through osteoblasts¹²-¹⁸. Regarding the direct effect of IL-17, IL-17 was reported to induce the differentiation of human osteoclast precursors⁹ and human CD11b-positive cells into osteoclasts in the absence of osteoblasts or exogenous RANKL¹⁰. In contrast, our report indicates that IL-17A, in the presence of RANKL, suppresses the expression of bone resorption-related proteases and osteoclast differentiation in the murine monocyte/macrophage cell line RAW264.7¹⁹.
The aim of this paper is to review the differences between the indirect effect of IL-17A via osteoblasts and the direct effect of IL-17A via osteoclast precursors on osteoclastogenesis in the presence of RANKL.

Molecules Related to Osteoclastogenesis

RANKL, RANK and OPG

RANKL, a member of the TNF family of cytokines, plays a key role in bone resorption. RANKL is expressed by osteoclastogenesis-supporting cells, including synovial fibroblasts, T lymphocytes, and osteoblasts in response to osteoclastogenic factors, such as 1, 25-dihydroxyvitamin D₃, prostaglandin (PG)E₂, parathyroid hormone, and several pro-inflammatory cytokines, including IL-1, IL-6, IL-17, and TNF-α; this is a crucial determinant of the level of bone resorption in vitro. RANKL functions both as a membrane-anchored molecule and as a soluble molecule²²-²⁵. Both forms bind to RANK, the receptor of RANKL, on monocytes, and osteoclastogenesis is induced via intracellular NFκB signaling and the activation protein (AP)-1 transcription factor family⁹⁰. RANK is expressed not only in monocytes and osteoclast precursors, but also in mature osteoclasts²⁷. In contrast, osteoprotegerin (OPG), the physiological inhibitor of RANKL, is a decoy receptor that binds RANKL. OPG is produced by various cells, such as osteoblasts and fibroblasts. OPG expression in osteoblasts is increased by 1,25-dihydroxyvitamin D₃, IL-1, TNF-α, bone morphogenetic protein-2, transforming growth factor-β, 17-estradiol, and the Wnt signaling pathway. Its expression is decreased by PGE₂, glucocorticoids, and insulin-like growth factor-1²⁸.

M-CSF

Osteoblasts produce macrophage colony-stimulating factor (M-CSF), which is required for the survival of cells in the macrophage-osteoclast lineage²⁹,³⁰. Most definitive studies demonstrating a role for M-CSF in osteoclast recruitment have been performed in the osteopetrotic (op/op) mouse. Mice homozygous for this mutation have a severe deficiency of osteoclasts and mononuclear phagocytes³¹, and are completely devoid of serum and tissue M-CSF activity. This deficiency results from a single base pair insertion in the coding region of the M-CSF gene, resulting in the production of defective M-CSF. Treatment of mutant mice with M-CSF corrects the defect in bone remodeling³². In addition, osteoblasts derived from op/op mice do not support osteoclast development in vitro³³, but exogenous M-CSF induces osteoclast formation in op/op hematopoietic cells³¹, suggesting that the defect in osteoclast recruitment in these animals is not in osteoclast progenitor cells. Additional studies supporting the role of M-CSF in osteoclast recruitment include a report demonstrating that M-CSF is the most effective CSF in stimulating osteoclast formation in normal murine bone marrow³⁴ and a study showing that M-CSF stimulates bone resorption in an
organ culture system designed to examine osteoclast formation. On the other hand, M-CSF was reported to be one of the indispensable factors of differentiation from osteoclast precursors to preosteoclasts through the RANK-RANKL signaling system.

**Carbonic anhydrase II, cathepsin K and MMP-9**

Mature osteoclasts secrete hydrogen ions and proteinases, such as cathepsin K and matrix metalloproteinase (MMP)-9 from a ruffled border that dissolve the inorganic and organic components of bone. Hydrogen ions are produced via carbonic anhydrase II (CA II) from CO₂ and H₂O in the cytoplasm and are secreted extracellularly by a vacuolar H⁺-ATPase. This results in the secretion of HCl into the resorptive microenvironment, thus producing a pH equal to approximately 4.5. This acidic milieu first mobilizes bone minerals; subsequently, the demineralized organic component of bone is degraded by lysosomal protease, cathepsin K, and MMP-9. Cathepsin K and MMP-9 are efficient collagenases that cleave both collagen type I and II.

Our previous study indicated that the production of CA II, cathepsin K, and MMP-9 in the murine monocyte/macrophage cell line RAW264.7 is not induced by M-CSF, but by RANKL in the presence of IL-1ß.

**Effects of IL-17A on RANKL-Induced Osteoclastogenesis**

**IL-17 family and its receptor**

IL-17, initially referred to as CTLA8, is a cloned cytokine produced exclusively by activated Th17 cells. IL-17 shares little or no homology with other interleukins, and at least six members of the family are in the human and mouse genomes: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F. The IL-17s are similar in size of 150-180 amino acids and secretory proteins, and display the greatest similarity among their C-terminal 70 amino acids. IL-17A and IL-17F share 44% amino acid identity, whereas other members of the IL-17 family share a more limited 15-27% amino acid identity, suggesting that IL-17A and IL-17F form a distinct subgroup within the IL-17 family. IL-17B through IL-17E are less related, sharing only 16-30% amino acid identity at the primary sequence level, and map to different chromosomes. However, differences in the function of each IL-17 subtype are not clear. IL-17 receptors (IL-17Rs) have been found on several cells and tissues and consist of five subtypes: IL-17RA, IL-17RB, IL-17RC, IL-17RD, and IL-17RE.

Our report indicated that IL-17R is found in all types in MC3T3-E1 cells from a mouse calvarial cell line. A previous study revealed that IL-17RA and IL-17RC interact together for an optimal response and form a complex to mediate the functions of IL-17A and IL-17F homodimers, as well as IL-17F/IL-17A heterodimers. In mice, the existence of at least two types of receptors (IL-17RA and IL-17RC) has been reported. Our study indicated that IL-17RA and IL-17RC expression is highest during the differentiation of RAW264.7 cells into osteoclasts. In this study, IL-17RA expression was found to be induced by the addition of IL-17A, whereas IL-17RC is not affected by IL-17A. In addition, the expression of IL-17RB, IL-17RD, and IL-17RE was not detected.

**Indirect effect of IL-17A on osteoclasts via osteoblasts**

Many studies have reported that IL-17A indirectly promotes osteoclastogenesis through osteoblasts. Furthermore, IL-17 was indicated to stimulate RANKL expression in the culture of osteoblasts and bone marrow cells. RANKL expression is also induced by PGE₂ in osteoblasts stimulated by IL-17, which is inhibited by NS398, a selective cyclooxygenase (COX)-2 inhibitor. Thus, IL-17 indirectly induces osteoclastogenesis via osteoblasts by this mechanism. IL-17 has also been shown to stimulate osteoclast differentiation by inducing the expression of RANKL via a mechanism involving the production of PGE₂ in coculture of bone marrow cells and osteoblasts in vitro. Our report indicated that IL-17A not only stimulates the expression of inflammatory cytokines such as IL-1α, IL-8, IL-11, and TNF-α via PGE₂ production in osteoblasts, but also induces the differentiation of osteoclast precursors into osteoclasts by increasing the expression of RANKL and M-CSF, and decreasing OPG expression via PGE₂ production in osteoblasts. In addition, we indicated that IL-17A induces the function by increasing cathepsin K and MMP-9 expression of mature osteoclasts via PGE₂ produced by osteoblasts.

**Direct effect of IL-17A on osteoclasts in the presence of RANKL**

Previous studies provide evidence for a direct effect of IL-17 on osteoclast differentiation. In vitro, IL-17 upregulates RANK
expression on human osteoclast precursors to sensitize them to RANKL. Another study demonstrated that IL-17 induces osteoclastogenesis in cultures of human CD11b-positive cells in the absence of osteoblasts or exogenous RANKL; this is blocked by the application of OPG or infliximab, suggesting a RANKL-and TNF-dependent mechanism. In contrast, our previous study indicated that the differentiation of osteoclast precursors into osteoclasts is suppressed at high concentrations of IL-17A in the presence of RANKL (Fig. 4). Furthermore, IL-17A in the presence of RANKL suppresses the hydrolysis of matrix proteins during bone resorption by decreasing the production of cathepsin K and MMP-9 in mature osteoclasts (Fig. 4). Thus, the effect of IL-17A in the presence of RANKL may conflict with the direct action on osteoclast precursors and the indirect action through osteoblasts.

Acknowledgments

Our recent studies were supported by a grant from the Ministry of Education, Culture, Sports, Science and Technology, Japan, to promote multidisciplinary research projects; a Grant-in-Aid for Scientific Research (C) (No. 24592842) and Grant-in-Aid for Young Scientists (B) (No. 24792009) from the Japanese Society for the Promotion of Science; the Strategic Research Base Development Program for Private Universities, subsidized by MEXT, 2010 (S1001024); and the Promotion and Mutual Aid Corporation for Private Schools of Japan. The present study was also supported by the Sato fund, Nihon University School of Dentistry.

References

8. Chan FK. Three is better than one: Pre-ligand receptor assembly in the regulation of TNF receptor signaling. Cytokine 37: 101-107, 2007
Effects of IL-17A on RANKL-Induced Osteoclastogenesis

Masao Maeno et al.


32. Takahashi N, Udagawa N, Akatsu T, Tanaka H, Isogai Y and Suda T. Deficiency of osteoclasts in osteopetrotic mice is due to a defect in the local microenvironment provided by osteoblastic cells. Endocrinology 128: 1792-1796, 1991


