Korean J Physiol Pharmacol
Vol 6: 269–274, October, 2002

Effects of Interleukin-1 β and Tumor Necrosis Factor-α on the Release of Collagenase and Gelatinase from Osteoblasts

Jong-Gab Eun, Dong-Heon Baek[1], and Se-Won Kim

Departments of Dental Pharmacology, [1]Oral Microbiology and Immunology, School of Dentistry, Dankook University, Cheonan 330–714, Korea

A large number of factors such as osteotropic hormones, cytokines, or growth factors are related to the bone remodeling which is characterized by the coupling of osteoclast-mediated bone resorption and osteoblast-mediated bone formation. Recent investigations have indicated that cytokines such as interleukin-1 β (IL-1 β) and tumor necrosis factor-α (TNF-α) play a potential role in the bone resorption associated with a variety of pathological conditions such as inflammatory osteolytic disease. Collagen is the most abundant protein of the extracellular matrix of bone, and the participation of collagenase in bone resorption has been widely investigated. In this study, effects of IL-1 β and TNF-α on the release of collagenase from osteoblastic cells were measured. The gelatinase activity was also measured by gel substrate analysis (zymography) after electrophoresis of conditioned media of osteoblastic cell culture. IL-1 β increased the collagenase activity in ROS17/2.8 and HOS cell culture. TNF-α also increased the collagenase activity of osteoblastic cells. When two kinds of cytokines were treated simultaneously in the culture of osteoblastic cells, synergistic increase of collagenase activity was seen in ROS17/2.8 cells.

IL-1 β and TNF-α significantly increased the collagenase activity after 6 hour treatment in the osteoblastic cell culture, and there was no additional increase according to the culture period. Osteoblastic cells released the gelatinase and molecular weight of this enzyme was measured about 70 KDa as assessed by zymogram. IL-1 β and TNF-α showed increase of the gelatinase activity produced by ROS17/2.8 and HOS cells. Taken together, this study suggested that IL-1 β and TNF-α can modulate bone metabolism, at least in part, by increased release of collagenase and gelatinase from osteoblasts.

Key Words: Interleukin-1 β, Tumor necrosis factor-α, Osteoblast, Collagenase, Gelatinase

INTRODUCTION

Osteoblasts and osteoclasts are responsible for strict bone maintenance with a balance between bone formation and resorption by interacting with each other. A variety of hormones, local factors, and interaction between osteoblasts and osteoclasts regulate the balance and timing of bone formation and resorption (Ducy et al, 2000; Teitelbaum, 2000). The breakdown of bone is mediated by osteoclasts which are unique multinucleated cells, and bone which is removed by osteoclasts is replaced by osteoblasts. In recent years, there has been a marked increase in our understanding of the cytokines which are generated in the bone microenvironment and modulate bone cell formation and activity. These cytokines may be generated by immune cells such as lymphocytes and monocytes in the marrow cavity, or by other bone cells, particularly cells of osteoblast lineage. They include interleukin-1 (IL-1) α and β, tumor necrosis factor-α (TNF-α), lymphotoxin, interleukin-6, interferon-γ, transforming growth factor-β (TGF-β), and the newly described IL-1 receptor antagonists (Bertolini *et al*, 1986; Tatakis, 1993; Suda *et al*, 1997; Rifas, 1999).

The term IL-1 was introduced in 1979 to describe the molecule(s) that appear to be responsible for a multitude of in vitro biologic activities associated with the immune system (Aarden et al, 1979). Historically 3 biologic activities were initially related to the inflammation-associated breakdown of connective tissues: mononuclear cell factor (Dayer et al, 1977), catabolin (Saklatvala, 1981), and osteoclast activating factor (Horton et al, 1972). First, mononuclear cell factor (Mizel et al, 1981), then catabolin (Saklatvala et al, 1984), and osteoclast activating factor were identified as IL-1 (Dewhirst et al, 1985). The effects of IL-1 on bone metabolism and the detection of IL-1 in tissue fluids, extracts, or conditioned media have implicated IL-1 in several pathologic conditions of bone. IL-1 is considered as a principal mediator of the localized osteolytic lesions (Mundy, 1991) and the hypercalcemia associated with various tumor (Mundy, 1990).

TNF is a proinflammatory cytokine which is produced mainly by macrophages and lymphocytes. TNF-α mediates phenomena such as leukocyte recruitment and activation, synovial macrophage and fibroblast proliferation, increased

Corresponding to: Se-Won Kim, Department of Dental Pharmacology, School of Dentistry, Dankook University, San 7-1, Shinbu-dong, Cheonan, Choongnam 330-714, Korea. (Tel) 82-41-550-1936, (Fax) 82-41-552-7648, E-mail: doban@dku.edu

ABBREVIATIONS: IL-1 β, Interleukin-1 β; TNF-α, Tumor necrosis factor-α; MMP, Matrix metalloproteinase.

prostaglandin and matrix degrading matrix metalloproteinase (MMP) activity, as well as bone and cartilage destruction. TNF-α is one of the most potent osteoclastogenic cytokines produced in inflammation. The functional role of TNF-α in the activation of osteoclast has been demonstrated by Garrett et al (1987), in that human myeloma cells may produce TNF-α and that monoclonal antibody directed against TNF-α inhibits the bone-resorbing potential of myeloma cells in vitro. Recently, IL-1β and TNF-α concomitantly increased osteoprotegerin expression by human osteoblasts (Hofbauer et al, 1999). These previous reports suggested that proinflammatory cytokines, such as TNF-α and IL-1β, play a important role in bone metabolisms.

Bone resorption involves the removal of both the mineral and organic matrix components of bone. The mineral phase is solubilized by acidification of the sub-osteoclastic lacuna, thus allowing dissolution of hydroxyapatite (Vaes, 1988). However, the mechanism for degradation of organic matrix remains controversial. Cysteine proteinases, particularly cathepsins, and metalloproteinases such as interstitial collagenase (MMP-1) are capable of degrading native type I collagen (Maciewicz & Etherington, 1988). Another member of the metalloproteinase family, type IV collagenase (gelatinase), is produced by bone cells (Lorenzo et al, 1992). Although the actual role for gelatinase in the bone remodeling process is unknown, it is believed to contribute the final degradation of collagen. This study was undertaken to investigate the possible role of IL-1β and TNF-α on the production and secretion of collagenase and gelatinase from osteoblastic cells.

METHODS

Cell culture

Two well-characterized osteoblastic cell lines, ROS17/2.8 and HOS, were used in this study. ROS17/2.8 and HOS cells were cultured with Dulbecco's modified Eagle medium (DMEM, BioWhittaker) containing 10% fetal bovine serum (FBS, Gibco). Cells were cultured in 75 cm^2 culture flask and maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2. When reached confluency, cells were subcultured at a ratio of 1 : 10 by suspending cells with trypsin and EDTA solution. Each cell line was replated into 35 mm culture dish at 1×10^4 cells/ml and used for each experiment.

Preparation of collagenase assay plate

Acid soluble collagen (Sigma, USA) from calf skin was solubilized with 0.2 % acetic acid (1.4 mg/ml) and mixed with same volume of neutralizing buffer (100 mM Tris-HCl, 200 mM NaCl, 0.04% NaN_3, pH 7. 8). Fifty μl (3.5 μg/ml) of collagen solution was added into the each well of 96-well plate (Nunc, USA). The assay plate was coated for 40 hours at 30°C (16 hours at wet condition, 24 hours at dry condition) and washed with reagent grade water and dried at room temperature.

Spectrophotometric collagenase assay

Cells were washed two times with phosphate buffered saline (PBS) and cultured for 24 hours with serum-free media. Media was replaced with fresh media or media containing various concentrations of cytokines, and cultured for additional 36 hours. In another experiments, cells were treated with 5 ng/ml IL-1β or 25 ng/ml TNF-α and cultured for indicated period. The treatment condition is outlined below (Table 1). After culture, 50 μl of culture media was loaded into each well of collagenase assay plate and 200 μl of collagenase assay buffer (50 mM Tris · HCl pH 7.5, 100 mM NaCl, 10 mM $CaCl_2$, 0.02% NaN_3) was added. The assay plate was incubated for 1 hour at 35°C and washed twice with assay buffer and twice with reagent grade water. The plate was stained with 100 μl of staining solution (0.25% coomassie blue R-250, 10% acetic acid, 50% methanol) for 25 minutes at 25°C, and washed three-times with reagent grade water and dried at room temperature. The O.D. value at 590 nm was measured with spectrophotometer (SLT 400, SFC).

Table 1. The treatment conditions of cytokines

A. IL-1β	
Concentration (ng/ml) for 36 hr	Duration (hr) at 5 ng/ml
0.008	6
0.04	12
0.2	24
1.0	48
5.0	
B. TNF-α	
Concentration (ng/ml) for 36 hr	Duration (hr) at 25 ng/ml
0.04	6
0.2	12
1.0	24
5.0	48
25.0	
C. Mixed treatment	
Concentration	IL-1β 5 ng/ml+TNF-α 25 ng/ml
Duration	36 hr

Type IV collagenase/gelatinase assay (zymography)

Cells were grown to confluence in 24-well culture plate. Media was replaced with serum-free media containing various concentrations of cytokines and cultured for additional 24 hours. After culture, conditioned media was concentrated about 5-fold with a 30,000 molecular weight cut-off device (Centricon^{TM}, Amicon). Gelatin-degrading activity was assessed by SDS-PAGE with a zymogram gel containing 0.1% gelatin (NOVEX) using a mini-gel apparatus. After electrophoresis, the enzyme was renatured by soaking the gel in renaturing buffer (2.5% Triton X-100) for 30 min at room temperature and then in developing buffer (50 mM Tris-HCl, pH 8.3, 0.2 M NaCl, 6.7 mM $CaCl_2$, and 0.02% Brij 35) for 30 min. After overnight incubation at 37°C in fresh developing buffer, the gel was stained with 0.5% coomassie blue R250 and destained in 10% methanol /10% acetic acid. Enzyme activity appeared as clear band in a dark blue background. Molecular weight standards

were run on the same gels.

Statistics

All data were expressed as Mean±S.E. *Student's t*-test for unpaired data was used to compare the control and the experimental groups. P value of less than 0.05 were considered to indicate statistically significant differences.

RESULTS

Effects of IL-1β and TNF-α on the collagenase activity

Collagenase activity was detected in the conditioned media of osteoblastic cell culture. IL-1β increased the collagenase activity of ROS17/2.8 cell culture. Statistically significant increase was measured in the 0.008~5 ng/ml concentration of IL-1β treatment (Table 2). In the HOS cell culture, IL-1β also increased the collagenase activity. However, there was no correlation between enzyme activity and concentration of IL-1β, and statistically significant increase was observed in the 0.008, 0.2 and 1 ng/ml IL-1β treatment (Table 2). TNF-α dose-dependently increased the collagenase activity of osteoblastic cells, and statistically significant increase was observed at 0.04~25 ng/ml in ROS17/2.8, and 0.2~25 ng/ml in HOS cell culture (Table 3). When two kinds of cytokines were treated simultaneously in the culture of osteoblastic cells, synergistic increase of collagenase activity was observed in ROS17/2.8 cell culture (Table 4). IL-1β and TNF-α increased the collagenase activity after 6 hour treatment in the osteoblastic cell culture, and there was no additional increase according to the culture period (Fig. 1). The same

Table 2. Effect of IL-1β on the collagenase activity of osteoblastic cell culture

Treatment (IL-1β, ng/ml)	Amount of remaining collagen (% of control)	
	ROS17/2.8	HOS
0	100.0±2.51	100.0±2.51
0.008	82.5±6.73*	77.3±1.36**
0.04	80.4±2.69**	98.0±5.08
0.2	80.4±2.19**	82.4±6.80*
1	73.5±2.31**	83.5±4.65**
5	75.6±0.90**	92.9±4.34

Osteoblastic cells were treated with various concentrations of IL-1β for 36 hours. Conditioned media were incubated with collagenase assay plate. Amount of remaining collagen was measured at 590 nm with spectrophotometer after staining with coomassie blue R-250. Values are Mean±S.E. (n=4). *P<0.05, **P<0.01, significantly different from control.

Table 3. Effect of TNF-α on the collagenase activity of osteoblastic cell culture

Treatment (TNF-α, ng/ml)	Amount of remaining collagen (% of control)	
	ROS17/2.8	HOS
0	100.0±2.51	100.0±2.51
0.04	91.8±0.98*	89.4±8.89
0.2	93.8±0.48*	88.5±1.91**
1	90.5±2.39*	82.6±3.34**
5	88.3±4.61*	79.8±1.69**
25	82.9±4.42**	75.7±1.01**

Osteoblastic cells were treated with various concentrations of TNF-α for 36 hours. Values are Mean±S.E (n=4). *P<0.05, **P<0.01, significantly different from control.

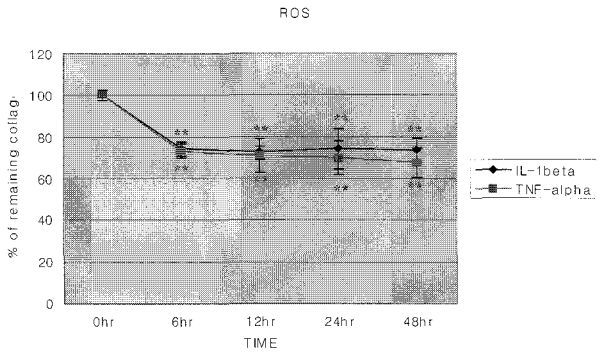

Fig. 1. Effects of IL-1β and TNF-α on the collagenase activity of ROS17/2.8 cell culture. ROS17/2.8 cells were treated with IL-1β (5 ng/ml) or TNF-α (25 ng/ml) for indicated culture period. Values are Mean±S.E. (n=4). **P<0.01, significantly different from control.

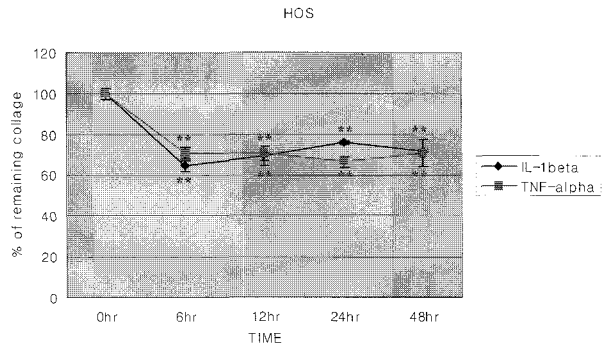

Fig. 2. Effects of IL-1β and TNF-α on the collagenase activity of HOS cell culture. HOS cells were treated with IL-1β (5 ng/ml) or TNF-α (25 ng/ml) for indicated culture period. Values are Mean±S.E. (n=4). **P<0.01, significantly different from control.

Table 4. Effect of combined treatment of IL-1β and TNF-α on the collagenase activity of osteoblastic cell culture

Treatment	Amount of remaining collagen (% of control)	
	ROS17/2.8	HOS
Control	100.0±2.70	100.0±2.70
IL-1β, 5 ng/ml	75.6±0.90**	92.9±4.34
TNF-α, 25 ng/ml	82.9±4.42**	75.7±1.01**
IL-1β (5 ng/ml) + TNF-α (25 ng/ml)	65.4±3.90**§§	77.9±7.60**

Osteoblastic cells were treated with IL-1β (5 ng/ml) and/or TNF-α (25 ng/ml) for 36 hours. Values are Mean±S.E. (n=4). **P<0.01, significantly different from control, §§P<0.01, significantly different from IL-1β or TNF-α treatment.

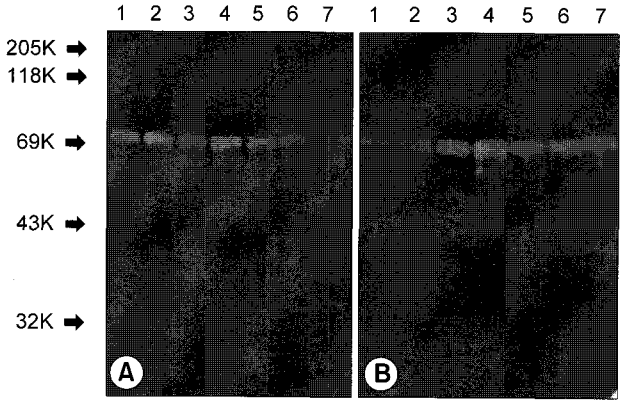

Fig. 3. Zymogram of concentrated conditioned media obtained from ROS17/2.8 (A) and HOS (B) cell culture. Concentrated conditioned media after culture with various concentrations of IL-1 β or TNF- α was resolved in 10% zymogram gel containing 1 mg/ml gelatin. Numerals are molecular weight standard. Lane 1, Control; Lane 2-4, IL-1 β, 0.008, 0.2, 5 ng/ml; Lane 5-7, TNF- α, 0.04, 1, 25 ng/ml.

results were observed in the HOS cell culture, and maximum increase of collagenase activity was observed after 6 hour treatment of cytokines (Fig. 2).

Effects of IL-1 β and TNF- α on the gelatinase activity

Osteoblastic cells released the collagenolytic enzyme and molecular weight of this enzyme was measured about 70 KDa as assessed by zymogram. IL-1 β and TNF- α increased the gelatinase activity produced by ROS17/2.8 and HOS cells (Fig. 3).

DISCUSSION

IL-1 is a potent multifunctional cytokine that appears to be a central regulator of the inflammatory and the immune responses. It is now known that there are 2 genetically- and biochemically-distinct IL-1 molecules, IL-1 α and IL-1 β (Lomedico et al, 1986). In this study, effect of IL-1 β on the release of collagenase and gelatinase from two kinds of osteoblastic cell lines were observed. Collagenase activity was detected in the conditioned media of osteoblastic cell culture. IL-1 β increased the collagenase activity of ROS17/2.8 and HOS cell culture (Table 2). Bone resorption involves the removal of both the mineral and organic matrix components of bone. The production of protons is probably necessary for the demineralization of bone and for providing an optimal environment for lysosomal enzymes to exert their enzymatic activity. Also, bone resorption involves bone matrix breakdown through the action of several metalloproteinases (Delaisse et al, 1988; Sakamoto & Sakamoto, 1988). Collagen is the most abundant protein of the extracellular matrix of bone, and collagenase is the primary enzyme affecting collagen degradation (Sakamoto & Sakamoto, 1988). Most evidence to date indicates that osteoblasts may secrete interstitial collagenase in response to stimulators of bone resorption (Heath *et al*, 1984).

Some investigators have suggested that IL-1 could not stimulate collagenase activity (Partridge et al, 1987) or induce osteoclast-independent collagenolysis (Jilka & Hamilton, 1985). A study by Rifas et al (1989) confirmed the lack of osteoblastic collagenase activity even after stimulation by IL-1 or other bone resorbing factors in human. However, numerous studies have reported that IL-1 could increase procollagenase levels extracted from mouse calvarial cultures (Delaisse et al, 1988), and stimulate collagenase production in rat osteoblastic cells (Shen et al, 1988). Also, Shingu et al (1993) reported that in the chondrocyte supernatant, collagenase activity/tissue inhibitor of metalloproteinase ratio was significantly elevated by treatment of IL-1 β. Our results are in accordance with these reports and IL-1-induced osteoblastic collagenase synthesis may thus contribute to the IL-1-stimulated bone resorption. Production of IL-1 by cells is considered to be a response to injury, since IL-1 does not seem to be constitutively produced. Gowen & coworkers (1983) were the first to describe the bone resorbing activity of highly purified IL-1. Soon thereafter the same group reported that IL-1 had the ability to resorb cartilage (Gowen et al, 1984). Several in vivo studies confirmed the bone resorbing activity of IL-1 (Konig et al, 1988; Nguyen et al, 1991). Increased production and release of collagenase from osteoblasts may one of the important pathways of bone resorption induced by IL-1 β.

In another experiment, TNF- α also increased the collagenase activity of osteoblastic cells (Table 3). TNF- α has been shown to be involved in tumor-induced bone resorption (Johnson et al, 1989; Mundy, 1991) and nontumor-induced osteopenia (Bertolini et al, 1986; Kimble et al, 1995). And along with IL-1 β, TNF- α is secreted in higher amounts from mononuclear cells of postmenopausal women than premenopausal women (Ralston et al, 1990; Cohen-Solal et al, 1993). Meikle et al (1989) have suggested that gingival fibroblasts secreted collagenase and secretion of this enzyme was increased by TNF- α treatment. They proposed the hypothesis that connective tissue destruction during the inflammatory disease may be initiated by cytokines such as IL-1 β and TNF- α. Also, Callaghan et al (1996) have reported the important role of TNF- α in the autocrine regulation of collagenase gene expression by monocyte/macrophage cell line, U937. Our results are in accordance with these reports and TNF- α may also modulate bone metabolism via activation and secretion of collagenase.

When two kinds of cytokines were treated simultaneously in the culture of osteoblastic cells, synergistic increase of collagenase activity was seen in ROS17/2.8 cells (Table 4). In stimulating bone resorption, IL-1 acts synergistically with other mediators such as parathyroid hormone, TNF, and TGF- α. Our results also suggested the synergistic effects of two cytokines on the collagenase production and these results are in accordance with the report of Meyer et al (1990) which demonstrated that combination of IL-1 β and TNF- α showed marked synergistic effects on PGE and collagenase production by human synovial fibroblasts. After culture of osteoblastic cells in the presence of cytokines for various culture period, collagenase activity in the culture media was measured. IL-1 β and TNF- α significantly increased the collagenase activity in ROS17/2.8 cells after 6 hour treatment, and there was no additional increase of enzyme activity according to the culture period (Fig. 1). The same results were observed in the HOS cell culture, and maximum increase of collagenase activity was observed after 6 hour treatment of cytokines (Fig. 2).

Also, osteoblastic cells released the gelatinolytic enzyme

with molecular weight of about 70 KDa as assessed by zymogram (Fig. 3). In addition to collagenase, another member of the metalloproteinase family, type IV collagenase (gelatinase), is produced by bone cells (Lorenzo et al, 1992). Although the actual role for gelatinase in the bone remodeling process is unknown, it is believed to contribute the final degradation of collagen by osteoclasts. In this study, because of proteolytic activation of latent gelatinase and shifting molecular weights of activated forms in concentrated conditioned media samples, it was not possible to conclude that this enzyme represented gelatinase A or gelatinase B by the zymogram method. Recent Western blot analysis with specific anti-gelatinase A and B antisera indicated that the main activity produced by osteoblast is gelatinase A (data not shown). IL-1 β and TNF- α increased the gelatinase activity produced by ROS17/2.8 and HOS cells (Fig. 3).

Taken together, this study suggested that cytokines such as IL-1 β and TNF - α can modulate bone metabolism, at least in part, by release of collagenase and gelatinase from osteoblasts. The precise mechanisms by which interactions between cytokines and bone cells will require further detailed study.

REFERENCES

Aarden LA, Brunner TK, Cerottini JC. Revised nomenclature for antigen-nonspecific T cell proliferation and helper factors. *J Immunol* 123: 2928–2929, 1979

Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR. Stimulation of bone resorption and inhibition of bone formation in vitro by human tumor necrosis factors. *Nature* 319: 516–518, 1986

Callaghan MM, Lovis RM, Rammohan C, Lu Y, Pope RM. Autocrine regulation of collagenase gene expression by TNF-alpha in U937 cells. *J Leukoc Biol* 59: 125–132, 1996

Cohen-Solal ME, Graulet AM, Denne MA, Gueris J, Baylink D, de Vernejoul MC. Peripheral monocyte culture supernatants of menopausal women can induce bone resorption: involvement of cytokines. *J Clin Endocrinol Metab* 77: 1648–1653, 1993

Dayer JM, Robinson DR, Krane SM. Prostaglandin production by rheumatoid synovial cells: Stimulation by a factor from human mononuclear cells. *J Exp Med* 145: 1399–1404, 1977

Delaisse JM, Eeckhout Y, Vaes G. Bone resorbing agents affect the production and distribution of procollagenase as well as the activity of collagenase in bone tissue. *Endocrinology* 123: 264–276, 1988

Dewhirst FE, Stashenko PP, Mole JE, Tsurumachi T. Purification and partial sequence of human osteoclast-activating factor: identity with interleukin 1 beta. *J Immunol* 135: 2562–2568, 1985

Ducy P, Schinke T, Karsenty G. The osteoblasts: A sophisticated fibroblast under central surveillance. *Science* 289: 1501–1504, 2000

Garrett R, Durie BGM, Nedwin GB, Gillespie A, Bringman T, Sabatini M, Bertolini DR, Mundy GR. Production of lymphotoxin, a bone-resorbing cytokine by cultured human myeloma cells. *N Eng J Med* 317: 526–532, 1987

Gowen M, Wood DD, Ihrie EJ, McGuire MKB, Russell RGG. An interleukin 1 like factor stimulates bone resorption in vitro. *Nature* 306: 378–380, 1983

Gowen M, Wood DD, Ihrie EJ, Meats JE, Russell RG. Stimulation by human interleukin-1 of cartilage breakdown and production of collagenase and proteoglycanase by human chondrocytes but not by human osteoblasts in vitro. *Biochim Biophys Acta* 797: 186–193, 1984

Heath JK, Atkinson SJ, Meikle MC, Reynolds JJ. Mouse osteoblasts synthesize collagenase in response to bone resorbing agents. *Biochim Biophys Acta* 802: 151–154, 1984

Hofbauer LC, Lacey DL, Dunstan CR, Spelsberg TC, Riggs BL, Khosla S. Interleukin-1 β and tumor necrosis factor- α but not interleukin-6, stimulate osteoprotegerin ligand gene expression in human ostoblastic cells. *Bone* 25: 255–259, 1999

Horton JE, Raisz LG, Simmons HA, Oppenheim JJ, Mergenhagen SE. Bone resorbing activity in supernatant fluid from human cultured peripheral blood leukocytes. *Science* 177: 793–795, 1972

Jilka RL, Hamilton JW. Evidence for two pathways for stimulation of collagenolysis in bone. *Calcif Tissue Int* 37: 300–306, 1985

Johnson RA, Boyce BF, Mundy GR, Roodman GD. Tumors producing human tumor necrosis factor induced hypercalcemia and osteoclastic bone resorption in nude mice. *Endocrinology* 124: 1424–1427, 1989

Kimble RB, Matayoshi AB, Vannice JL, Kung V, Williams C, Pacifici R. Simultaneous block of interleukin-1 and tumor necrosis factor is required to completely prevent bone loss in the early postovariectomy period. *Endocrinology* 136: 3054–3061, 1995

Konig A, Muhlbauer RC, Fleisch H. Tumor necrosis factor α and interleukin-1 stimulate bone resorption in vivo as measured by urinary [^{3}H] tetracycline excretion from prelabeled mice. *J Bone Miner Res* 3: 621–627, 1988

Lomedico PT, Kilian PL, Gubler U, Stern AS, Chizzonite R. Molecular biology of interleukin-1. *Cold Spring Harbor Symp Quant Biol* 51: 631–639, 1986

Lorenzo JA, Pilbeam CC, Kalinowski JF, Hibbs MS. Production of both 92- and 72-KDa gelatinase by bone cells. *Matrix* 12: 282–290, 1992

Maciewicz RA, Etherington DJ. A comparison of four cathepsins (B, L, N and S) with collagenolytic activity from rabbit spleen. *Biochem J* 256: 433–440, 1988

Meikle MC, Atkinson SJ, Ward RV, Murphy G, Reynolds JJ. Gingival fibroblasts degrade type I collagen films when stimulated with tumor necrosis factor and interleukin 1: evidence that breakdown is mediated by metalloproteinases. *J Periodontal Res* 24: 207–213, 1989

Meyer FA, Yaron I, Yaron M. Synergistic, additive, and antagonistic effects of interleukin-1 beta, tumor necrosis factor alpha, and gamma-interferon on prostaglandin E, hyaluronic acid, and collagenase production by cultured synovial fibroblasts. *Arthritis Rheum* 33: 1518–1525, 1990

Mizel SB, Dayer JM, Krane SM, Mergenhagen SE. Stimulation of rheumatoid synovial cell collagenase and prostaglandin production by partially purified lymphocyte-activating factor (interleukin-1) *Proc Natl Acad Sci USA* 78: 2474–2477, 1981

Mundy GR. Incidence and pathophysiology of hypercalcemia. *Calcif Tissue Int* 46 (Suppl. 1): S3–S10, 1990

Mundy GR. Mechanisms of osteolytic bone destruction. *Bone* 12 (Suppl. 1): S1–S6, 1991

Nguyen L, Dewhirst FE, Hauschka PV, Stashenko P. Interleukin-1 β stimulates bone resorption and inhibits bone formation in vivo. *Lymphokine Cytokine Res* 10: 15–21, 1991

Partridge NC, Jeffrey JJ, Ehlich LS, Teitelbaum SL, Fliszar C, Welgus HG, Kahn AJ. Hormonal regulation of the production of collagenase inhibitor activity by rat osteogenic sarcoma cells. *Endocrinology* 120: 1956–1962, 1987

Ralston SH, Russel RG, Gowen M. Estrogen inhibits release of tumor necrosis factor from peripheral blood mononuclear cells in postmenopausal women. *J Bone Min Res* 5: 983–988, 1990

Rifas L. Bone and cytokines: Beyond IL-1, IL-6 and TNF- α. *Calcif Tissue Int* 64: 1–7, 1999

Rifas L, Halstead LR, Peck WA, Avioli LV, Welgus HG. Human osteoblasts in vitro secrete tissue inhibitor of metalloproteinases and gelatinase but not interstitial collagenase as major cellular products. *J Clin Invest* 84: 686–694, 1989

Sakamoto S, Sakamoto M. Degradative processes of connective tissue proteins with special emphasis on collagenolysis and bone resorption. *Mol Aspects Med* 10: 299–428, 1988

Saklatvala J. Characterization of catabolin, the major product of synovium that induces resorption of cartilage proteoglycan in vitro. *Biochem J* 199: 705–714, 1981

Saklatvala J, Pilsworth LMC, Sarsfield SJ, Gavrilovic J, Heath JK.

Pig catabolin is a form of interleukin 1. Cartilage and bone resorb, fibroblasts make prostaglandin and collagenase, and thymocyte proliferation is augmented in response to one protein. *Biochem J* 224: 461−466, 1984.

Shen V, Kohler G, Jeffrey JJ, Peck WA. Bone-resorbing agents promote and interferon-gamma inhibits bone cell collagenase production. *J Bone Miner Res* 3: 657−666, 1988.

Shingu M, Nagai Y, Isayama T, Naono T, Nobunaga M, Nagai Y. The effects of cytokines on metalloproteinase inhibitors (TIMP) and collagenase production by human chondrocytes and TIMP production by synovial cells and endothelial cells. *Clin Exp Immunol* 94: 145−149, 1993

Suda T, Nakamura I, Jimi E, Takahashi N. Regulation of osteoclast function. *J Bone Min Res* 12: 869−879, 1997

Tatakis DN. Interleukin-1 and bone metabolism: A review. *J Periodontol* 64: 416−431, 1993

Teitelbaum SL. Bone resorption by osteoclasts. *Science* 289: 1504−1508, 2000

Vaes G. Cellular biology and biochemical mechanism of bone resorption. A review of recent developments on the formation, activation and mode of action of osteoclasts. *Clin Orthop Rel Res* 231: 239−271, 1988