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Original

Interleukin-6 and soluble interleukin-6 receptor suppress osteoclastic differentiation by inducing PGE_2 production in chondrocytes

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Abstract: This study examined how interleukin-6 (IL-6) and soluble IL-6 receptor (sIL-6r) influence osteoclastic differentiation through the function of chondrocytes. Chondrocytes were cultured with or without IL-6 and/or sIL-6r in the presence or absence of NS398, a specific inhibitor of cyclooxygenase (COX)-2, for up to 28 days. Chondrocytes were also cultured with or without IL-6 and sIL-6r for 28 days, and the conditioned medium from cells cultured without IL-6 and sIL-6r was used to induce differentiation of RAW264.7 cells into osteoclast precursors. Osteoclastic differentiation was assessed by tartrate-resistant acid phosphatase (TRAP) staining. Expression of osteoprotegerin (OPG), receptor activator of NF-κB ligand (RANKL), COX-2, and prostaglandin E_2 (PGE_2) increased in cells exposed to IL-6 and sIL-6r, whereas expression of macrophage colony-stimulating factor (M-CSF) and bone resorption-related enzymes decreased. NS398 blocked the stimulatory/suppressive effects of IL-6 and sIL-6r on the expression of OPG, RANKL, and M-CSF. Fewer TRAP-positive multinucleated cells were detected after treatment with conditioned medium from IL-6- and sIL-6r-treated chondrocytes than after treatment with conditioned medium from untreated chondrocytes. These results suggest that IL-6 and sIL-6r interfere with osteoclast function through the involvement of chondrocytes. Specifically, they appear to suppress the differentiation of osteoclast precursors into osteoclasts by inducing chondrocytic PGE_2 production, which, in turn, increases OPG secretion and decreases M-CSF secretion by chondrocytes. (J Oral Sci 53, 87-96, 2011)

Keywords: interleukin-6; chondrocytes; osteoclast precursors; osteoprotegerin; prostaglandin E_2.

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Introduction

Inflammation-mediated bone loss is a major feature of various bone diseases, including chronic periodontitis, rheumatoid arthritis, and osteoarthritis. It is caused by an imbalance in bone remodeling that favors resorption. This imbalance results from increases in the levels of cytokines and mediators, such as interleukin (IL)-1, IL-6, tumor necrosis factor-α, and prostaglandin E_2 (PGE_2), in inflammatory tissues (1).

IL-6 contributes to the development of arthritis and is present at high concentrations in the serum and synovial fluid of patients with chronic rheumatoid arthritis and temporomandibular joint (TMJ) osteoarthritis (2-4). Soluble IL-6 receptor (sIL-6r) is also elevated in the serum and synovial fluid of rheumatoid arthritis patients (5,6). Elevated concentrations of IL-6 and sIL-6r in the serum and synovial fluid of patients with arthritis have been implicated in the severe joint tissue destruction that characterizes this group of conditions (7). Additionally, IL-6 is closely associated with the expression of receptor activator of NF-κB (RANK) ligand (RANKL) and osteoprotegerin (OPG) in osteoblasts (8).

RANKL, macrophage colony-stimulating factor (M-CSF), and OPG are key regulators of osteoclastogenesis

(9-12). RANKL is highly expressed on osteoblasts/stroma cells and on the primitive mesenchymal cells surrounding the cartilaginous anlagen, ie, proliferating and hypertrophying chondrocytes (13-15). Osteoclast precursors recognize RANKL via RANK expressed on their cell surfaces and differentiate into mononuclear perfusion osteoclasts (POCs) in the presence of M-CSF. RANKL is also involved in mononuclear POC survival and fusion and in mature osteoclast activation (9,14). In contrast, OPG functions as a soluble decoy receptor that competes with RANK to bind RANKL. Consequently, OPG is an effective inhibitor of osteoclast maturation and activation (9,14,16). Recently, Watanabe et al. (15) reported that RANKL, M-CSF, and OPG were expressed in chondrocytes derived from normal human femoral cartilage.

PGE_2 has been detected in TMJ synovial fluid from patients with internal derangement of the joint (17-19). Additionally, plasma levels of PGE_2 reflected general inflammatory activity in such patients (20). PGE_2 is produced when the phospholipids found in cell membranes release arachidonic acid, which is converted by cyclooxygenases (COXs) into prostaglandin H_2, which is subsequently converted into PGE_2 by PGE synthetase. There are two types of COX that regulate this process: COX-1, which is expressed constitutively, and COX-2, which is induced in inflammation (21).

In a previous study that examined the effects of cytokines on osteoclastic differentiation, Watanabe et al. (15) demonstrated that IL-1β suppressed the formation of osteoclast-like cells by increasing OPG production and decreasing M-CSF production in chondrocytes. Their data suggested that OPG induction may be achieved through an autocrine mechanism involving celecoxib-related prostaglandins, primarily PGE_2. However, the mechanism by which IL-6 and sIL-6r influence osteoclastic differentiation through effects on chondrocytes was not explored. In the present study, the effects of IL-6 and sIL-6r on the expression of OPG, RANKL, M-CSF, and COXs, and production of PGE_2, in human chondrocytes were examined in the presence and absence of NS398, a specific inhibitor of COX-2. Additionally, the indirect effects of IL-6 and sIL-6r on osteoclast differentiation and the expression of bone resorption-related enzymes, such as carbonic anhydrase II (CA II), matrix metalloproteinase-9 (MMP-9), and cathepsin K, in osteoclasts were evaluated using RAW264.7 cells as osteoclast precursors.

Materials and Methods

Cell culture

Chondrocytes derived from normal human femoral cartilage were obtained from Cell Applications (San Diego, CA, USA). Cells were maintained in Dulbecco's modified Eagle's medium/nutrient mixture F-12 (DMEM/F-12; Gibco-BRL, Rockville, MD, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; HyClone Laboratories, Logan, UT, USA), 1% (v/v) insulin-transferrin-selenium-X supplement (Invitrogen, Carlsbad, CA, USA), and 1% (v/v) penicillin-streptomycin solution (Sigma Chemical, St. Louis, MO, USA) at 37°C in a humidified atmosphere of 95% air / 5% CO_2. They were subsequently seeded in 100-mm tissue culture dishes at a density of 5×10^6 cells/cm^2. After an overnight incubation, the cells were cultured for up to 28 days in DMEM/F-12 containing 10% FBS with or without 50 ng/ml IL-6 and/or 30 ng/ml sIL-6r (R&D Systems, Minneapolis, MN, USA) in the presence or absence of 1 μM NS398. They were examined daily using a light microscope. The concentrations of IL-6 and sIL-6r used in this study were identical to those used by Namba et al. (22), in which the effect of IL-6 and/or sIL-6r on the expression of cartilage matrix proteins in human chondrocytes was examined. The concentration of NS398 used in this study was identical to that used by Ouellet and Percival (23), in which the time-dependency of its COX isoform selectivity was examined.

Real-time PCR

Total RNA was isolated on culture days 1, 3, 5, 7, 14, 21, and/or 28 using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA). Amounts of RNA were equalized using a human β-actin competitive PCR kit (Takara Bio, Shiga, Japan). The mRNA was used as the template for cDNA synthesis, which was performed using an RNA PCR kit (GeneAmp; Perkin Elmer, Branchburg, NJ, USA). The cDNA mixtures were diluted 5-fold in sterile distilled water and subjected to real-time PCR analysis using SYBR Green I dye (Bio Whittaker Molecular Applications, Rockland, ME, USA). Reaction mixtures (25 μl) contained 2 μl of mRNA, 1 × R-PCR buffer, 1.5 mM dNTP mixture, 1 × SYBR Green I, 15 mM $MgCl_2$, 0.25 U ExTaq (R-PCR version; Takara Bio), and sense and antisense primers (0.2 μM; Table 1). Primers were designed using Primer3 software (Whitehead Institute for Biomedical Research, Cambridge, MA, USA). Assays were performed using a SmartCycler II system (Cepheid, Sunnyvale, CA, USA) and analyzed using the SmartCycler software (ver. 2.0d). Amplification was achieved through 45 cycles of 95°C for 3 s and 60°C for 20 s. Measurements were made at the end of the 60°C annealing step. All real-time PCR reactions were performed in triplicate. Target gene expression levels were normalized to the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) signal.

Table 1 Real-time PCR primers used in the experiments

Target	Primer sequence (5'-3')	GenBank Acc.
OPG	AGCTGCAGTACGTCAAGCAGGA TTTGCAAACTGTATTTCGCTCTGG	NM_002546.3
RANKL	TGCCAGTGGGAGATGTTAGAC CCTTCAATTGCGCTAGATGAC	NM_03012.2
M-CSF	TAGCCACATGATTGGGAGTGGA CTCAAATGTAATTTGGCACGAGGTC	NM_172212.1
COX-1	ACCTTGAAGGAGTCAGGCATGAG TGTTCGGTGTCCAGTTCCAATA	U63846
COX-2	TTTCTACCAGAAGGGCAGGAT TATCACAGGCTTCCATTGACC	NM_000963
CA ll	CATTACTGTCAGCGACGAGCA GACGCCAGTTGTCCACCATC	NM_009801
MMP-9	GCCCTGGAACTCACACGACA TTGGAAACTCACACGCCAGAAG	NM_013599
cathepsin K	CAGCAGAACGGAGGCATTGA CCTTTGCCGTGGCGTTATAC	NM_007802
GAPDH	GCACCGTCAAGGCTGAGAAC ATGGTGGTGAAGACGCCAGT	NM_002046

Enzyme-linked immunosorbent assay (ELISA)

Chondrocytes were incubated in DMEM/F-12 supplemented with 10% (v/v) FBS with or without 50 ng/ml IL-6 and/or 30 ng/ml sIL-6r in the presence or absence of 1 μM NS398 for 1 or 28 days. The culture medium was then replaced with DMEM/F-12 containing 2% (v/v) FBS and 50 ng/ml IL-6 and/or 30 ng/ml sIL-6r, and the cells were cultured for 24 h at 37°C in a humidified atmosphere containing 5% CO_2.

Protein levels of OPG, RANKL, and M-CSF in the culture medium were measured using a commercially available ELISA kit (R&D Systems) according to the manufacturer's instructions. Amounts of PGE_2 in the culture medium were determined using a commercially available ELISA kit (Assay Designs, Ann Arbor, MI, USA) according to the manufacturer's instructions. All samples were analyzed in triplicate.

Conditioned medium

Chondrocytes were cultured in DMEM/F-12 supplemented with 10% (v/v) FBS with or without 50 ng/ml IL-6 and 30 ng/ml sIL-6r for 28 days. The culture medium was replaced with DMEM/F-12 containing 2% (v/v) FBS without IL-6 and sIL-6r, and the cells were incubated for 24 h at 37°C in a humidified atmosphere containing 5% CO_2. Samples of culture medium were then collected and diluted 5-fold in DMEM/F-12 supplemented with 2% (v/v) FBS (without IL-6 or sIL-6R) and 50 ng/ml soluble RANKL (Wako Pure Chemical, Osaka, Japan) and then used as conditioned medium. The RANKL concentration used was equivalent to that used in a study examining the expression in RAW264.7 cells of enzymes involved in bone resorption (24).

Tartrate-resistant acid phosphatase (TRAP) staining

RAW264.7 cells were plated in 96-well plates at a density of 1.25×10^4 cells/cm^2 and left overnight to settle. Conditioned medium containing 50 ng/ml soluble RANKL was then applied to the cells for up to 7 days. Cells were then fixed and stained on culture days 3, 5, and 7 using a TRAP staining kit (Takara Bio) according to the manufacturer's instructions. Numbers of osteoclast-like cells per well were counted. TRAP-positive cells with more than 3 nuclei were considered to be osteoclast-like cells.

Statistical analyses

All experiments were performed in triplicate. Each value represents the mean ± standard deviation (SD). The significance of differences was determined using Bonferroni's modification of the Student *t*-test. A P value less than 0.05 was considered significant.

Results

Effects of IL-6 and sIL-6r on the expression of OPG, RANKL, and M-CSF

Chondrocytes were cultured with or without 50 ng/ml IL-6 and/or 30 ng/ml sIL-6r for up to 28 days. OPG, RANKL, and M-CSF mRNA and protein levels were

measured by real-time PCR and ELISA, respectively, on culture days 1, 7, 14, 21, and/or 28 (Fig. 1). In the presence of both IL-6 and sIL-6r, the expression of OPG and RANKL mRNAs increased significantly (1.9- to 2.5-fold and 2.9- to 39.0-fold, respectively) as compared with expression in control cells at the indicated time points (Fig. 1a, c). In contrast, expression of M-CSF mRNA was significantly lower than that in control cells (Fig. 1e). When levels of expression were compared, OPG mRNA had the highest level and RANKL mRNA had the lowest level. Secretion of OPG and RANKL increased significantly (1.5- to 2.4-fold and 1.4- to 1.8-fold, respectively) as compared with that in control cells at the indicated time points (Fig. 1b, d). In contrast, secretion of M-CSF was significantly lower than in control cells (Fig. 1f). When levels of secreted protein were compared, OPG had the highest level and RANKL had the lowest level.

Indirect effects of IL-6 and sIL-6r on TRAP staining of osteoclast-like cells

The indirect effects of IL-6 and sIL-6r on the formation of osteoclast-like cells were examined by staining for TRAP, an osteoclast differentiation marker. RAW264.3 cells were cultured with or without 20% conditioned medium (supplemented with 50 ng/ml soluble RANKL) from untreated chondrocytes or chondrocytes treated for up to 7 days with 50 ng/ml IL-6 and 30 ng/ml sIL-6r. Cells were stained on culture days 3, 5, and 7 and examined using light microscopy. TRAP staining of osteoclast-like cells was weaker after treatment with conditioned medium from chondrocytes treated with IL-6 and sIL-6r than after treatment with conditioned medium from untreated chondrocytes (Fig. 2). Significantly fewer TRAP-positive multinucleated cells were detected on culture day 5 after treatment with conditioned medium from chondrocytes treated with IL-6 and sIL-6r than after treatment with conditioned medium from untreated chondrocytes (Fig. 3).

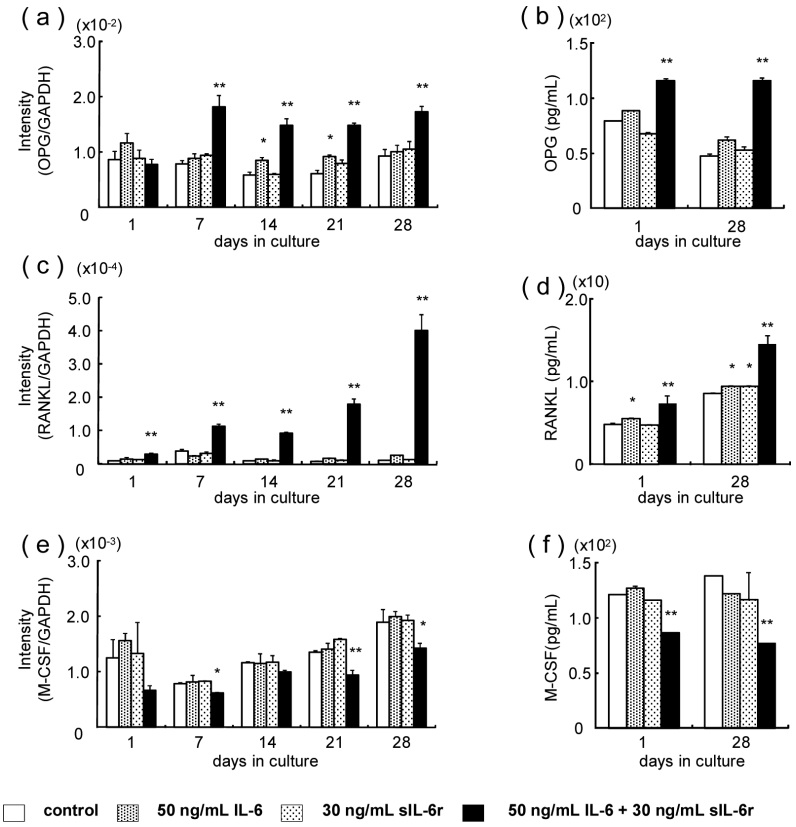

Fig. 1 Effects of IL-6 and/or sIL-6r on OPG, RANKL, and M-CSF expression. Chondrocytes were cultured with or without 50 ng/ml IL-6 and/or 30 ng/ml sIL-6r for up to 28 days. Expression of OPG, RANKL, and M-CSF mRNA and protein levels was measured by real-time PCR (a, c, e) and ELISA (b, d, f), respectively, on culture days 1, 7, 14, 21, and/or 28. Data represent the mean ± SD of 3 separate experiments. $*P < 0.05$, $**P < 0.01$, vs. control.

Indirect effects of IL-6 and sIL-6r on the expression of CA II, MMP-9, and cathepsin K

RAW264.3 cells were cultured with or without 20% conditioned medium (supplemented with 50 ng/ml soluble RANKL) from chondrocytes treated for up to 7 days with 50 ng/ml IL-6 and 30 ng/ml sIL-6r. Expression of CA II, MMP-9, and cathepsin K mRNAs in RAW264.7 cells on culture days 3, 5, and 7 was measured using real-time PCR (Fig. 4). Expression of CA II, MMP-9, and cathepsin K mRNAs at the indicated time points was significantly lower (0.1- to 0.6-fold, 0.2- to 0.4-fold, and 0.1- to 0.2-

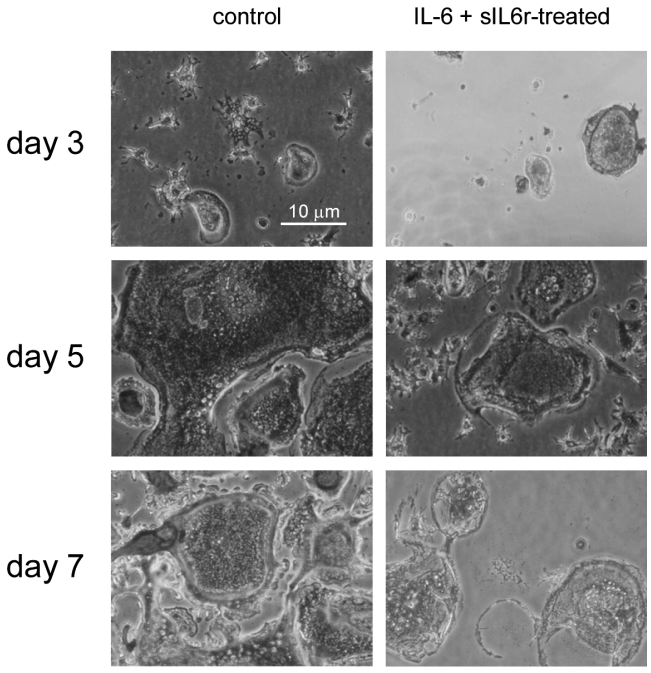

Fig. 2 Indirect effects of IL-6 and sIL-6r on TRAP staining in osteoclast-like cells.
RAW264.3 cells were cultured for 7 days with or without 20% conditioned medium (supplemented with 50 ng/ml soluble RANKL) from chondrocytes treated with 50 ng/ml IL-6 and 30 ng/ml sIL-6r or untreated control chondrocytes. Cells were stained on culture days 3, 5, and 7 and examined by light microscopy.

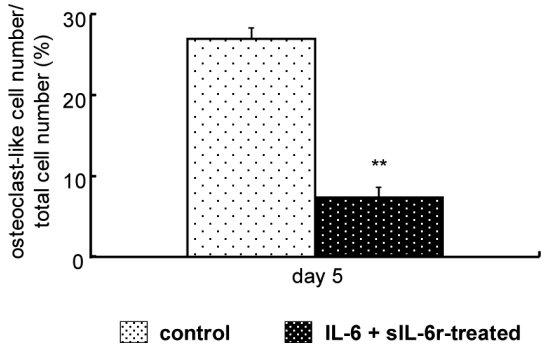

Fig. 3 Number of TRAP-positive multinucleated cells.
RAW264.3 cells were cultured with or without 20% conditioned medium (supplemented with 50 ng/ml soluble RANKL) from chondrocytes treated with 50 ng/ml IL-6 and 30 ng/ml sIL-6r or untreated control chondrocytes. The number of TRAP-positive cells with more than 3 nuclei was counted under a light microscope on culture day 5. Percentages of cells that were TRAP-positive and multinucleated are shown. Data represent the mean ± SD of 4 separate experiments. $^{**}P < 0.01$, vs. control.

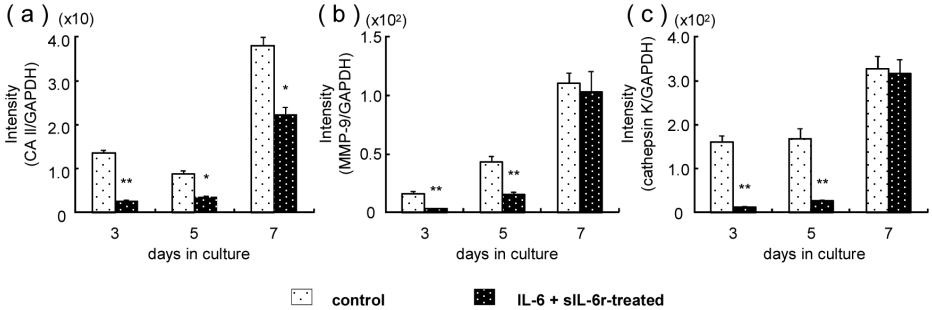

Fig. 4 Indirect effects of IL-6 and sIL-6r on the expression of CA II, MMP-9, and cathepsin K.
RAW264.3 cells were cultured for up to 7 days with or without 20% conditioned medium (supplemented with 50 ng/ml soluble RANKL) from chondrocytes treated with 50 ng/ml IL-6 and 30 ng/ml sIL-6r or untreated control chondrocytes. Expression of CA II (a), MMP-9 (b), and cathepsin K (c) mRNAs was determined by real-time PCR on culture days 3, 5, and 7. Data represent the mean ± SD of 3 separate experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, vs. control.

fold, respectively) after treatment with conditioned medium from chondrocytes treated with IL-6 and sIL-6r than after treatment with conditioned medium from untreated chondrocytes.

Effects of IL-6, sIL-6r, and NS398 on PGE_2 production

Chondrocytes were cultured with or without 50 ng/ml IL-6 and/or 30 ng/ml sIL-6r in the presence or absence of 1 μM NS398 for up to 28 days. PGE_2 production on culture days 1, 7, 14, 21, and 28 was measured by ELISA (Fig. 5).

PGE_2 production at the indicated time points was significantly higher (2.0- to 6.5-fold) in the presence of both IL-6 and sIL-6r than in their absence (Fig. 5a). NS398 blocked the stimulatory effects of IL-6 and sIL-6r on PGE_2 production on culture day 28 (Fig. 5b). Production of PGE_2 by cells treated with IL-6, sIL-6r, and NS398 was similar to that of untreated control cells.

Effects of IL-6 and/or sIL-6r on COX expression

Chondrocytes were cultured with or without 50 ng/ml IL-6 and/or 30 ng/ml sIL-6r for up to 28 days. Expression of COX-1 and COX-2 mRNAs on culture days 1, 7, 14, 21, and 28 was measured using real-time PCR (Fig. 6).

Expression of COX-2 mRNA at the indicated time points was significantly higher (2.4- to 6.4-fold) in cells treated with IL-6 and sIL-6r than in untreated control cells (Fig. 6b). In contrast, the expression of COX-1 was not affected by treatment with IL-6 and sIL-6r (Fig. 6a).

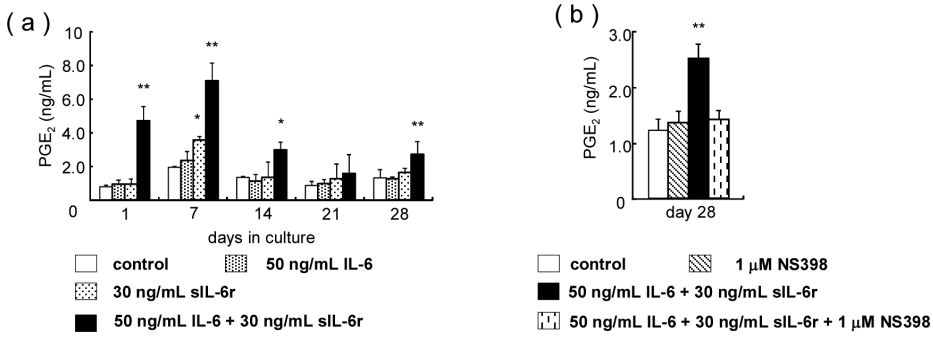

Fig. 5 Effects of IL-6, sIL-6r, and NS398 on PGE2 expression.
(a) Chondrocytes were cultured with or without 50 ng/ml IL-6 and/or 30 ng/ml sIL-6r for up to 28 days. PGE2 production was measured by ELISA on culture days 1, 7, 14, 21, and 28. (b) Cells were cultured with or without 50 ng/ml IL-6 and 30 ng/ml sIL-6r in the presence or absence of 1 μM NS398. PGE_2 production was measured by ELISA on culture day 28. Data represent the mean ± SD of 3 separate experiments. $*P < 0.05$, $**P < 0.01$, vs. control.

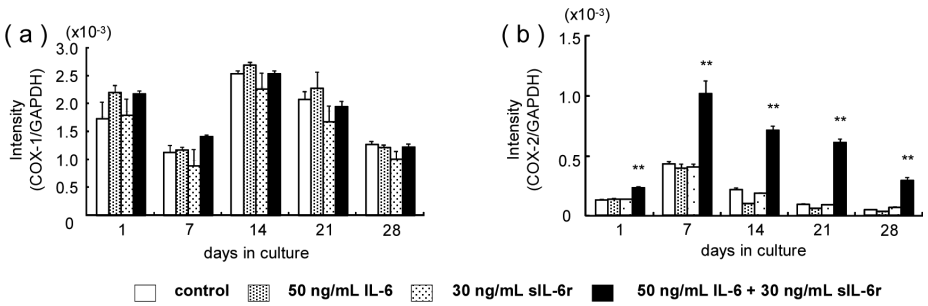

Fig. 6 Effects of IL-6 and/or sIL-6r on COX-1 and COX-2 expression.
Chondrocytes were cultured with or without 50 ng/ml IL-6 and/or 30 ng/ml sIL-6r for up to 28 days. Expression of COX-1 (a) and COX-2 (b) mRNAs was measured using real-time PCR on culture days 1, 7, 14, 21, and 28. Data represent the mean ± SD of 3 separate experiments. $**P < 0.01$, vs. control.

Effects of IL-6, sIL-6r, and NS398 on the expression of OPG, RANKL, and M-CSF

Chondrocytes were cultured with or without 50 ng/ml IL-6 and/or 30 ng/ml sIL-6r in the presence or absence of 1 μM NS398. Expression of OPG, RANKL, and M-CSF mRNAs on culture day 28 was measured using real-time PCR (Fig. 7). NS398 blocked both the stimulatory effects of IL-6 and sIL-6r on OPG and RANKL mRNA expression (Fig. 7a, b) and the suppressive effects of IL-6 and sIL-6r on M-CSF mRNA expression (Fig. 7c). OPG, RANKL, and M-CSF mRNA expression in cells treated with IL-6, sIL-6r, and NS398 was similar to that in control cells.

Discussion

This study was performed to clarify the effects of IL-6, sIL-6r, and NS398, a specific inhibitor of COX-2 (23), on the expression of OPG, RANKL, and M-CSF in human chondrocytes and the indirect effects of IL-6 and sIL-6r on the generation of osteoclast-like cells from RAW264.7 cells.

The chondrocytes used in this study showed strong expression of type II collagen and aggrecan, a typical phenotype of proliferating, hypertrophic chondrocytes, but expression of type X collagen, a marker of hypertrophic chondrocytes, was very low (data not shown). Thus, the chondrocytes used in this study showed only some of the characteristics of proliferating chondrocytes. In designing these experiments, it was necessary to determine an appropriate length of time for culture. In many previous studies of the expression of OPG, RANKL, and M-CSF in chondrocytes, the culture periods were brief (25-29). However, because TMJ osteoarthrosis is typically chronically progressive, the expression of mRNA and protein levels in chondrocytes was examined over a much longer period of time, 28 days.

TMJ osteoarthrosis results from an imbalance between anabolic and catabolic processes that are predominantly controlled by chondrocytes (30,31). Cytokines released at sites of inflammation and infection can impair the normal turnover of bone and cartilage, resulting in their pathological destruction or accumulation (32). Additionally, concentrations of inflammatory cytokines and mediators, such as IL-1, IL-6, and PGE_2, are increased in the synovial fluid of patients with TMJ osteoarthrosis (3,33). Recently, Namba et al. (22) reported that IL-6 and sIL-6r suppress differentiation of chondrocytes and induce expression of cartilage matrix proteins. On the other hand, Watanabe et al. (15) reported that IL-1β suppressed the formation of osteoclasts by increasing OPG production via an autocrine mechanism involving the production of celecoxib-related prostaglandins, primarily PGE_2, by chondrocytes. Thus, the present study focused on osteoclastic differentiation mediated by RANK/RANKL/OPG, through which chondrocytes communicate with osteoclast precursors. This study showed that the expression of OPG and RANKL in chondrocytes increased significantly in cells cultured with IL-6 and sIL-6r, whereas that of M-CSF decreased. Additionally, it was shown that OPG mRNA expression and protein secretion were significantly higher than RANKL mRNA expression and protein secretion (Fig. 1). In addition, IL-6 and sIL-6r indirectly suppressed osteoclastic differentiation through the function of chondrocytes (Figs. 2 and 3). Palmqvist et al. (34) reported that IL-6 and sIL-6r up-regulated OPG and RANKL in

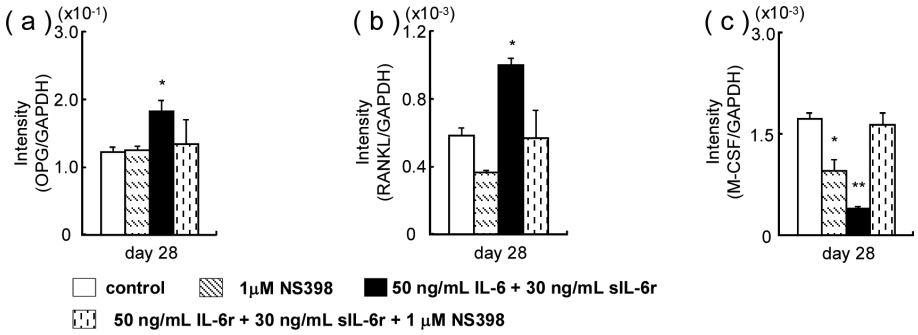

Fig. 7 Effects of IL-6, sIL-6r, and/or NS398 on the expression of OPG, RANKL, and M-CSF.
Chondrocytes were cultured with or without 50 ng/ml IL-6 and/or 30 ng/ml sIL-6r in the presence or absence of 1 μM NS398. Expression of OPG (a), RANKL (b), and M-CSF (c) mRNAs was measured using real-time PCR on culture day 28. Data represent the mean ± SD of 3 separate experiments. *$P < 0.05$, **$P < 0.01$, vs. control.

neonatal mouse calvarial bones. Additionally, M-CSF is known to be an indispensable factor for the differentiation of osteoclast precursors into mononuclear POCs under the control of the RANK/RANKL/OPG system (9). Thus, the results of the present study suggest that IL-6 and sIL-6r suppress osteoclastic differentiation by increasing OPG production and decreasing M-CSF production, respectively, in chondrocytes.

Mature osteoclasts secrete proteinases (24) and release hydrogen ions (35). The proteinases cathepsin K and MMP-9 are secreted from the ruffled borders of osteoclasts to dissolve the organic components of bone. Hydrogen ions are generated from CO_2 and H_2O through the action of CA II (24) and pumped by H^+-ATPases in osteoclasts into the extracellular space, where they dissolve inorganic bone constituents (35). In this study, the expression of CA II, MMP-9, and cathepsin K in RAW264.7 cells decreased significantly after treatment with conditioned medium from chondrocytes treated with IL-6 and sIL-6r as compared with that after treatment with conditioned medium from untreated control chondrocytes (Fig. 4). These results suggest that IL-6 and sIL-6r indirectly suppress the function of mature osteoclasts by reducing the expression of bone resorption-related enzymes through the function of chondrocytes.

PGE_2 is a naturally occurring inflammatory mediator. Watanabe et al. (36) reported that human chondrocytes express PG receptors, including the EP1, EP2, and EP4 receptors, and that IL-1β stimulated expression of EP4 receptor via an autocrine mechanism involving PGE_2 production. The present study showed that IL-6 and sIL-6r stimulated PGE_2 production in human chondrocytes, a response that was blocked by NS398 (Fig. 5). Additionally, IL-6 and sIL-6r significantly increased the expression of COX-2, but not COX-1 (Fig. 6). These results suggest that IL-6 and sIL-6r induce PGE_2 production in chondrocytes by increasing COX-2 expression. To confirm the role of PGE_2 in regulating the expression of OPG, RANKL, and M-CSF, the effects of IL-6, sIL-6r, and NS398 on chondrocytes were examined. NS398 blocked the stimulatory effects of IL-6 and sIL-6r on the chondrocytic expression of OPG, RANKL, and M-CSF, which suggests that IL-6 and sIL-6r modulate the expression of OPG, RANKL, and M-CSF via an autocrine mechanism involving the induction of PGE_2 production in chondrocytes.

In bone, IL-6 stimulates osteoclastic differentiation and osteoclast activity in association with sIL-6r (34). However, IL-6 exhibits quite different effects in cartilage. Shingu et al. (37) reported that IL-6 promoted the production of tissue inhibitor of metalloproteinases (TIMPs), but not MMPs, in cartilage. Namba et al. (22) reported that IL-6 and sIL-6r induce repair of arthrodial cartilage by increas ing the expression of cartilage matrix proteins, bone morphogenetic protein (BMP)-7, and BMP-7 receptors in chondrocytes. These previous findings and those of the present study indicate that IL-6 and sIL-6r may protect cartilage and that their action in TMJ osteoarthrosis differs between bone and cartilage.

In conclusion, the present findings suggest that IL-6 and sIL-6r suppress both (1) differentiation of osteoclast precursors into osteoclasts by increasing OPG expression and decreasing M-CSF expression via an autocrine mechanism involving the induction of PGE_2 production in chondrocytes and (2) the function of mature osteoclasts by decreasing their expression of CA II, MMP-9, and cathepsin K through the involvement of chondrocytes.

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