Induction of osteoclast-like cells derived from the synovial lavage fluids of patients with temporomandibular joint disorders

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Summary

Objective: Although biochemical studies have examined the synovial fluid (SF) of patients with temporomandibular joint (TMJ) disorders (TMDs), the details of the molecular mechanism of bone destruction and remodeling remain unknown. In this study, we induced and characterized osteoclast-like cells from the SF of patients with TMD and investigated the participation of these cells in the pathogenesis of TMD.

Methods: We collected SF cells from patients with TMD after a pumping procedure, cultured osteoclast-like cells, and examined their characteristics, including osteoclast markers and bone resorption activities. In addition, we obtained fibroblastic cells from the SF of TMD patients by continuous sub-culturing. Using these fibroblastic cells, we examined fibroblast markers using immunocytochemical staining and analyzed the receptor activator of nuclear-factor-κB ligand (RANKL) mRNA levels. Detection of soluble form of RANKL (sRANKL) in the SF was measured by enzyme-linked immunosorbent assay (ELISA).

Results: Osteoclast-like cells were induced from the SF cells of patients with TMD by adding recombinant human (rh) macrophage colony stimulating factor (M-CSF) and either 1,25-dihydroxy vitamin D₃ [1,25(OH)₂D₃] or prostaglandin E₂ (PGE₂). These multinucleated giant cells were positive for tartrate-resistant acid phosphatase (TRAP) and had the ability to absorb bone. The fibroblastic cells from the SF of TMD patients were positive for fibroblast markers and RANKL mRNA was up-regulated. Detection of sRANKL in SF of patient group was significantly higher than control group.

Conclusion: The results suggest that the joint-infiltrating SF cells from TMD patients play important roles in the pathogenesis of these disorders, which is characterized by progressive bone destruction or remodeling.

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Key words: Temporomandibular joint disorders, Synovial fluid, Joint-infiltrating cells, Osteoclast.

Introduction

Temporomandibular joint (TMJ) disorders (TMDs) represent a cluster of joint and muscle disorders that are characterized by joint sound, pain, and dysfunction of the TMJ. Previous reports have suggested that variable degrees of inflammation exist in certain TMDs.[1,2] Recent advances in arthroscopy and the biochemical analysis of synovial fluids (SF) have provided new insights into the pathophysiological nature of TMDs. Various proteinases,[3,4] peptidases,[5] and inflammatory cytokines, which include interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α)[6–13] have been detected in the SF of TMD patients and have been associated with factors that influence bone destruction.

Osteoarthritis (OA), which is a major TMD with a complex etiology, involves degenerative changes in the articular cartilage or subchondral bone that lead to loss of joint function. Morphological abnormalities, such as flattening, erosion, sclerosis, and chondro-osteophytes, are observed on the articular surface of the mandibular condyle in OA of the TMJ.[14,15] Although biochemical studies have been performed previously on the SF of TMD patients, little is known about the molecular events that occur during the development of TMDs, including the pathogenesis of OA.

On the other hand, several lines of evidence suggest an association between osteoclast formation and bone destruction in arthritis. It has been widely accepted that osteoclasts play a pivotal role in joint destruction or bone remodeling of multiple joints in rheumatoid arthritis (RA) and OA, and significant effort has gone into investigating the precise mechanism underlying this phenomenon. Osteoclasts are multinucleated, bone-resorbing cells that are derived from CD34-positive hematopoietic stem cells.[16–18] The osteoclast progenitors are members of the monocyte/macrophage lineage,[17] which differentiate to become the...
mononuclear precursors of osteoclasts, the so-called pre-
osteoclasts. Mature osteoclasts are generated by
the fusion of these mononuclear preosteoclasts, and their
differentiation occurs in microenvironments that are sup-
ported by physiological stimuli provided by osteoblasts or
stromal cells in the bone marrow.

Recently, we reported that multinucleated bone-resorbing
osteoclast-like cells were generated from cells of the SF in
knee joints from RA and OA patients. We detected tartrate-
resistant acid phosphatase (TRAP)-positive mononuclear
cells, which differentiated into multinucleated bone-resor-
bing giant cells, in the SF of patients with RA or OA. Al-
though the presence of these bone-resorbing cells and their
participation in the destruction of RA and OA joints are well-
known phenomena, the details of bone destruction and re-
modeling in the TMJs of patients with TMD remain un-
known. In the present study, we collected a characterized
osteooclast-like cells from the SF of patients with TMD. In addition, we obtained fibroblastic cells from
the SF of TMD patients by continuous sub-culturing. Using
these fibroblastic cells, we developed a co-culture system
of fibroblastic cells and peripheral blood monocytes, which
we used to investigate whether fibroblastic cells from the
SF are capable of supporting the differentiation of mono-
cytes into osteoclasts. Thus, we considered the participa-
tion of cells obtained from SF of TMJ patients in the pathogenesis of TMD.

Methods

SUBJECTS

SF samples were collected from 13 TMJs of 11 TMD pa-

tients. The control group consisted of SF samples from
seven TMJs of four volunteers who had no symptoms of
TMD. The patients consisted of 11 females with an average
age of 33.8 years (range 17–61 years), while the control
group consisted of two males and two females with an aver-
age age of 27.0 years (range 25–30 years). All of the
TMD patients had received 3 months of conservative treat-
ment without relief of symptoms prior to the intra-articular
pumping and lavage. The conservative treatment modalities
included medication (non-steroidal anti-inflammatory drugs
and muscle relaxants), use of a bite splint, and physical
therapy. The patients were given no medication for at least
2 weeks before the SF sampling was performed.

Full, informed consent for sample aspiration was ob-
ained from each patient, and all of the subsequent proce-
dures were approved by the ethics committee of Kyushu
Dental College.

COLLECTION OF SF SAMPLES AND CELLS

SF samples were collected from the TMJs of patients with
TMD as previously described. Briefly, after the pumping
procedure, by aspiration with a 22-gauge needle under aseptic
conditions, and then washing the superior joint space with physiological saline. The mixture of SF and saline
was gently aspirated and re-injected a total of 10 times.

The SF sample was then collected. As a result, SF was di-
luted by saline, and viscosity of SF was not admitted. The
joint-infiltrating cells from the SF were collected by centrifu-
gation at 1900 × g, the supernatant of SF was separated
and stored at −80 °C until assayed for enzyme-linked immu-
nosorbent assay (ELISA). Erythrocytes in SF were elimi-
nated by lysis with an ammonium chloride solution. The nucleated cells were washed twice with Ca2+– and
Mg2+–free phosphate-buffered saline (PBS) and counted
using a hemocytometer. Dead cells were excluded by stain-
ing with trypan blue.

CULTURING OF CELLS FROM THE SF

The joint-infiltrating cells from the SF samples were cul-
tured in α-minimum essential medium (α-MEM; Gibco
BRL, Gaithersburg, MD) that contained 10% heat-inactivat-
ed fetal calf serum (FCS; Gibco BRL) and 100 U/ml pen-
cillin–streptomycin (Gibco BRL). A total of 100,000 cells
were seeded onto 8-well chamber slides (Lab-Tek Chamber
Slide; Becton Dickinson, Franklin Lakes, NJ), and cultured
in the presence or absence of 20 ng/ml recombinant human
(rh) macrophage colony stimulating factor (M-CSF) (Pepro-
Teck EC, London, UK) and either 10−7 M 1,25-dihydroxy vi-
tamin D3 [1,25(OH)2D3] (Calbiochem, San Diego, CA) or
10−7 M prostaglandin E2 (PGE2) (Cayman Chemical, Ann
Arbor, MI). The cells were maintained at 37 °C in humidified
air that contained 5% CO2, and half of the medium was
changed every 3 days. After 4 weeks, all of the wells
were treated with trypsin/EDTA (Gibco BRL), and the fibro-
blast-like cells were removed. The remaining adherent cells
were cytochemically stained. The fibroblast-like cells which
of no stimulation re-plated, and half of the medium was
changed every 7 days. After four to five passages, these
cells were used for the subsequent experiments.

CYTOCHEMICAL STAINING

At the end of the culture period, the cells were stained for
TRAP using a kit (Sigma Chemical Co., St. Louis, MO), in
accordance with the manufacturer’s instructions. The cells
were also stained with May–Grunwald–Giemsa by incuba-
tion for 5 min with a 1:1 dilution of May–Grunwald solution
(Merck, Darmstadt, Germany) followed by incubation for 10
min with a 1:20 dilution of Giemsa solution (Merck).

BONE RESORPTION ASSAYS

To determine the resorption activities of the TRAP- posi-
tive giant cells, 100,000 cells from the SF were cultured
on dentin slices in 8-well chamber slides. Each well was
filled with 0.3 ml α-MEM that contained 10% FCS, 10−7 M
1,25(OH)2D3, and 20 ng/ml rhM-CSF. After incubation for 4
weeks, the adherent cells on the dentin slices were re-
moved by brushing into distilled water, and the slices
were cleaned by ultrasonication. Resorption pits were
then detected by staining with horseradish peroxidase-conju-
gated wheat germ agglutinin (WGA)-lectin (Sigma), as de-
scribed previously. Briefly, the dentin slices were
incubated with 50 μg/ml horseradish peroxidase-conjugated
WGA-lectin in 0.1 M PBS overnight at room temperature.
After three washes, the peroxidase reaction was developed
for 30 min using 0.05 M Tris buffer (pH 7.6) that contained
0.02% 3,3-diaminobenzidine tetrahydrochloride (DAB; Do-
jindo Laboratories, Kumamoto, Japan) and 0.006% H2O2.
The dentin slices were mounted on aluminum stubs,
sputtered with gold, and examined under the Hitachi S-
4300 scanning electron microscope (Tokyo, Japan). As an
alternative method for the analysis of bone resorption,
the joint-infiltrating cells from the SF were cultured on calcium
phosphate-coated discs (Osteologic; Becton Dickinson) us-
ing the culture conditions described above. After 14 days of
incubation, the discs were washed in 6% NaClO and 5.2%
NaCl to remove the cells, dried, and examined by phase-
contrast microscopy.
SEMI-QUANTITATIVE REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION (RT-PCR)

After the fibroblast-like cells had been subcultured four to five times and treated with 10^{-5} M 1,25(OH)2D3 or 10^{-7} M prostaglandin E2 (PGE2) for 24 h, the receptor activator of nuclear-factor-κB ligand (RANKL) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels were analyzed by RT-PCR. RNA was extracted from the cells using the Total RNA Extraction Miniprep System (Viogene Co., Sunnyvale, CA), as directed by the manufacturer. Briefly, CDNA was synthesized from 2 μg of total RNA in a 30-μl reaction mixture that contained 500 μM dNTPs, 20 U ribonuclease inhibitor (Promega, Madison, WI), and 200 U Superscript-α reverse transcriptase (Invitrogen Life Technology, Carlsbad, CA). The reaction was initially carried out for 7 min at 70°C, then for 60 min at 45°C, followed by 10 min at 70°C, with a final step of cooling to 4°C. The PCR primer sequences were designed and their specificities confirmed using a BLAST search of the NLM non-redundant nucleotide sequence database (National Library of Medicine, Bethesda, MD). Each cycle consisted of denaturation and annealing steps, with a final extension step (72°C for 9 min). The primers used for the amplification of RANKL were as follows: forward, 5′-GGCTCATGGTTAGTCTGGCC-3′; and reverse, 5′-TGACCAATCTTGTTGCTTCC-3′. The reproducibility of the RT-PCR was confirmed by at least three replicates. Individual PCR reactions were carried out using reverse-transcribed RNA, Taq polymerase buffer, and 1 μl each of the sense and antisense primers, in a total volume of 20 μl. The PCR products were electrophoresed in 2% agarose gels and visualized with ethidium bromide.

IMMUNOCYTOCHEMICAL STAINING OF FIBROBLAST-LIKE CELLS

The fibroblast-like cells from the SF after subculture were seeded at 2 × 10^5 cells per well onto 4-well Lab-Tek chamber slides and allowed to attach and spread on the slides for 48 h. The cells were cultured in α-MEM that was supplemented with 10% heat-inactivated FCS and 100 U/ml penicillin–streptomycin (Gibco BRL), and maintained at 37°C in humidified air that contained 5% CO2. After incubation, the cultured medium was removed, and the cells were washed twice with PBS. The cells were fixed in 4% PBS-buffered paraformaldehyde for 10 min and then washed twice in PBS.

The immunostaining procedures were carried out at room temperature using the DAKO LSAB kit (DAKO, Carpinteria, CA) in accordance with the manufacturer’s instructions. The fibroblast-like cells were stained immunocytochemically with anti-human prolyl 4-hydroxylase (DAKO), anti-human vimentin (COSMO Bio Co., Ltd, Tokyo, Japan), anti-human macrophage marker (YLEM, Roma, Italy), and anti-human dendritic cell (Serotec Ltd, Oxford, UK) murine monoclonal antibodies.

CO-CULTURE OF FIBROBLAST-LIKE CELLS AND PERIPHERAL BLOOD MONOCYTES

The fibroblast-like cells from the SF were subcultured in α-MEM that was supplemented with 10% FCS. The cells were seeded at 5 × 10^4 cells per well into 24-well multi-well dishes and the cultures reached confluence after incubation for 3 days. Peripheral blood monocytes were collected as plastic-adherent cells, as described previously. Briefly, peripheral blood was obtained from healthy donors, and mononuclear cells were separated by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density-gradient (1077 g/ml) centrifugation (30 min at 400 × g) and suspended in medium. The cell suspensions were placed into 6-well multi-well dishes and incubated for 2 h at 37°C in a humidified 5% CO2 atmosphere. Thereafter, the non-adherent cells were removed by repeated washing and the adherent cells were collected by vigorous pipetting. More than 98% of the recovered adherent cells were monocytes, based on their morphology and CD14 expression, as assessed by flow cytometry using FACSscan (Becton Dickinson) (data not shown). The isolated monocytes were added at 5 × 10^6 cells per well to the fibroblast-like cells in 24-well multi-well dishes. The cells were co-cultured for 4 weeks in α-MEM that contained 10% FCS in the presence of 20 ng/ml rhM-CSF and either 10^{-7} M 1,25(OH)2D3 or 10^{-7} M PGE2. At the end of this incubation period, all of the wells were treated with trypsin/EDTA, and the fibroblast-like cells were removed. The remaining adherent cells were examined for the cytochemical and functional characteristics of osteoclasts, as described above.

To evaluate the effect of osteoprotegerin (OPG) on this co-culture system, rhOPG (PEPRO TECH EC Ltd, London, UK) was added to the cultures at a concentration of 10 or 100 ng/ml throughout the culture period.

MEASUREMENT OF THE sRANKL CONTENT AND TOTAL PROTEIN

Detection of soluble form of RANKL (sRANKL) was measured by an ELISA using the specific antibodies and ELISA amplification system (Invitrogen Life Technology, Carlsbad, CA). Ninety-six-well plates were coated overnight at 4°C with anti-human polyclonal RANKL antibody (1:300 dilution; Santa Cruz Biotechnology, CA). Unbound antibodies were washed away using 0.2% Tween 20 (Sigma) in 0.1 M PBS. Then, the plates were blocked with 1% bovine serum albumin (BSA) in 0.1 M PBS for 1 h at room temperature. After washing, the samples were added and incubated for 1 h at room temperature. After washing, the detecting biotinylated goat IgG anti-human RANKL antibody (1:300 dilution; Zymed Laboratories, South San Francisco, CA) was added and the plates were incubated for 1 h at 37°C. Next, alkaline phosphatase–avidin (Zymed Laboratories) was added and incubated for 30 min at room temperature. The substrate was added and incubated for 15 min and stopped with 0.3 M H2SO4. The optical density was measured at 495 nm using a micro plate reader (Bio-Rad, Herts, UK). Protein assay was performed using bicinchoninic acid (BCA) assay method (Pierce, Rockford, IL). Each detectable sRANKL concentration (pg/ml) shown was calculated per 1 mg of SF total protein as previously described.

STATISTICAL ANALYSIS

The results are presented as the means ± SD. Statistical analysis was performed using the non-parametric Mann–Whitney U test. P values of <0.05 were considered to be statistically significant.

Results

QUANTIFICATION OF JOINT-INFILTRATING CELLS IN THE SF

The joint-infiltrating cells in the SF were collected by lavage and counted. There was a significant (P < 0.01)
increase in the average number of cells in the SF of the patient group (14.6 ± 10.8 × 10^6 cells), as compared to the SF of the control group (0.8 ± 0.1 × 10^6 cells). Furthermore there was no significant differences in the average percentage of viable cells in the SF of the patient group (96.3 ± 5.7%) as compared with the SF of control group (98.6 ± 2.4%).

Differential of cultured joint-infiltrating cells into multinucleated bone-resorbing giant cells

The joint-infiltrating cells from the SF of TMD patients were cultured in the presence of 10⁻⁷ M 1,25(OH)₂D₃ or 10⁻⁷ M PGE₂ and 20 ng/ml rhM-CSF for 4 weeks. After 2 weeks of incubation, the cells had increased in number. Under light microscopy, large, spindle-shaped, adherent cells that resembled fibroblastic cells were observed [Fig. 1(a)]. After 4 weeks of incubation, the cell cultures had reached confluence, and fibroblastic cells predominated [Fig. 1(B)]. Multinucleated giant cells were seen after removal of the other cells by brief treatment with trypsin/EDTA. These large monocyte- or macrophage-like cells were multinucleated and positive for TRAP [Fig. 2(A and B)]. Although multinucleated giant cells could be derived from the SF cells from most of the joints, they were not inducible from the cells collected from three TMJs of the TMD patients or from any of the TMJs of the control group. The numbers of joint-infiltrating cells in these joints were lower than that in other joints affected by TMD (data not shown).

Formation of resorption pits on dentin slices and calcium phosphate-coated discs

Calcium resorption, which is a distinctive function of osteoclasts, was observed after incubation for 14 days of calcium phosphate-coated discs (Osteologic) with cells from the SF of the patients. Numerous resorption pits were observed by phase-contrast microscopy. Giant cells were observed near the resorption pits [Fig. 3(A)]. The resorption pits were clearly visible after removal of the cells with bleach [Fig. 3(B)]. Moreover, resorption pits were formed on dentin slices that were incubated with cells from the SF of TMD patients [Fig. 4(A and B)], and were clearly visible in scanning electron micrographs (SEMs) [Fig. 4(B)].

Expression of RANKL mRNA after treatment with 1,25(OH)₂D₃ or PGE₂

After sub-culturing, the fibroblast-like cells from the SF of the TMD patients were treated with 10⁻⁷ M 1,25(OH)₂D₃ or 10⁻⁷ M PGE₂, to clarify the effects of stimulation on RANKL expression. RANKL mRNA was up-regulated after 24 h of stimulation with either agent (Fig. 5).

Cell surface markers of the fibroblast-like cells

All of the fibroblast-like cells were positive for prolyl 4-hydroxylase [Fig. 6(A)] and vimentin [Fig. 6(B)], which are fibroblast markers. In contrast, the cells stained negative
for the macrophage marker and dendritic cell marker [Fig. 6(C and D)]. No immunostaining was observed for control specimens that were incubated with the secondary antibody alone (data not shown).

FORMATION OF MULTINUCLEATED BONE-RESORBING GIANT CELLS BY CO-CULTURE OF FIBROBLAST-LIKE CELLS AND PERIPHERAL BLOOD MONOCYTES

After the fibroblast-like cells from TMD SF were co-cultured with peripheral blood monocytes in the presence of 20 ng/ml rhM-CSF and either $10^{-7}$ M 1,25(OH)$_2$D$_3$ or $10^{-7}$ M PGE$_2$ for 4 weeks, numerous TRAP-positive multinucleated cells were observed [Fig. 7(A)]. However, in cultures of peripheral blood monocytes alone, no multinucleated cells [Fig. 7(B)] were detected following stimulation with either $10^{-7}$ M 1,25(OH)$_2$D$_3$ or $10^{-7}$ M PGE$_2$. The multinucleated cells formed in the co-cultures displayed osteoclastic characteristics.

EFFECT OF OPG ON OSTEOCLASTOGENESIS IN CO-CULTURE OF FIBROBLAST-LIKE CELLS AND PERIPHERAL BLOOD MONOCYTES

We investigated the effect of OPG on co-culture of fibroblast-like cells and peripheral blood monocytes in the presence of 20 ng/ml rhM-CSF and $10^{-7}$ M 1,25(OH)$_2$D$_3$ for 3 weeks. TRAP-positive multinucleated giant cell formation was significantly inhibited by OPG (10 ng/ml, 100 ng/ml) in this culture system [Fig. 8].

DETECTION OF THE sRANKL CONTENT IN SF

Using an ELISA, we detected sRANKL in both groups of SF. Each detectable sRANKL concentration (pg/ml) shown was calculated per 1 mg of SF total protein. The levels of sRANKL in the SF of the patient group were significantly higher than the control group [Fig. 9].

Discussion

In this study, we collected joint-infiltrating cells from the SF of the TMJs of patients with TMD and demonstrated that these cells could be induced to differentiate into TRAP-positive multinucleated bone-resorbing giant cells. To the best of our knowledge, this is the first study to investigate the molecular and cellular characteristics of joint-infiltrating cells from the SF of TMJs. Although the development of multinucleated giant cells was induced from SF cells isolated from most of the joints, we could not induce multinucleated giant cells from cells collected from three TMJs of patients with TMD or from any of the TMJs of the control group. The numbers of joint-infiltrating cells were lower in these joints than in other joints affected by TMD, and these cells did not proliferate in culture.
In TMD, capillary hyperemia and synovial hyperplasia lead to an increase in vascular permeability, resulting in the exudation of white blood cells, as well as inflammatory mediators and various proteins into the joint spaces of the TMJs. Clinically, these inflammatory changes result in pain, soft tissue swelling, crepitus, and disability. The effective vascular permeability across the blood–synovial barrier is a key factor in controlling SF volume and joint effusions. The hydraulic permeability of the synovium increases at pathological intra-articular pressures, which is a phenomenon of potential importance for effusion kinetics. In addition, the average number of joint-infiltrating cells in the SF of the TMD patient group was significantly higher than that in the SF of the control group in this study. These results suggest that the vascular permeability across the blood–synovial barrier of the TMJ is increased by inflammation in TMD patients, with consequent increased extravasation of white blood cells into the joint space. Therefore, the SF of the TMJ of TMD patients may contain more cells that have the capacity to differentiate into preosteoclasts.

Recently, we reported that multinucleated bone-resorbing osteoclast-like cells could be generated from peripheral monocytes that differentiated into TRAP-positive mononuclear cells when induced by RA nurse-like cells. In addition, it appears that certain cytokines in the SF of RA patients are responsible for osteoclast-like cell formation. Furthermore, we detected TRAP-positive mononuclear cells, which differentiated into multinucleated bone-resorbing giant cells, in the SF of patients with RA and OA.

Quinn and Bazan were the first to identify the presence of PGE2 in the SF of inflamed, dysfunctional TMJs and to show a significant correlation with the level of acute synovitis, which was assessed arthroscopically. Numerous subsequent studies have identified additional pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α. The pro-inflammatory cytokines (PGs, ILs, and TNF-α) are potential regulators of osteoclastogenesis. Kotake et al. have demonstrated that high levels of IL-6, soluble IL-6 receptor, and IL-17 in the SF of patients with RA appear to enhance osteoclastogenesis and promote joint destruction. Kobayashi et al. have demonstrated that TNF-α stimulates osteoclast differentiation in the presence of M-CSF in mice and humans. These combined findings suggest...
that the microenvironment of the joint space offers favorable conditions for the differentiation of precursors to osteoclasts when the production of inflammatory cytokines is increased during TMD.

More recently, the molecular determinants of osteoclastogenesis have been identified; RANKL and M-CSF are indispensable factors for osteoclastogenesis and are produced by osteoblasts and bone marrow stromal cells. RANKL has been reported as a potent inducer of osteoclast development from monocytes and is a key molecule in osteoclastogenesis. Shigeyama et al. have recently reported that synovial fibroblasts in RA patients may promote osteoclastogenesis by expressing RANKL. In the present study, RANKL mRNA expression was increased by treatment of adherent fibroblast-like cells with 1,25(OH)2D3 or PGE2. In addition, when OPG was added to co-culture of fibroblast-like cells and peripheral blood monocytes, osteoclastogenesis was inhibited in a dose dependent manner. The effect of OPG on osteoclastogenesis in this co-culture system was similar to that observed in co-culture of RA synovial fibroblasts with peripheral blood mononuclear cells. These results reveal that these populations contain stromal cells, and RANKL expressed on these fibroblast-like cells may support osteoclast differentiation of peripheral blood mononuclear cells. Furthermore, sRANKL was detected in both groups of SF. The levels of sRANKL in SF of the patient group were significantly higher than control group. It is also suggested that sRANKL in the SF of TMD patients may have activated osteoclastogenesis in TMJs.

In order to identify the influence of synovial fibroblastic cells on osteoclast formation in our culture system, fibroblast-like cells were co-cultured with peripheral monocytes in the presence of 1,25(OH)2D3 or PGE2. The results indicate that fibroblast-like cells from the SF of patients with TMD have the property of stromal cells, i.e., that of supporting differentiation of SF monocytes/macrophages into osteoclasts. The fibroblast-like cells in the SF of TMD patients were positive for fibroblast cell markers, such as prolyl 4-hydroxylase and vimentin, in immunocytostaining experiments. In contrast, these cells were negative for macrophage and dendritic cell markers. These results suggest that fibroblast-like cells obtained from the SF of TMD patients by culturing are predominantly type B fibroblast-like cells, which are thought to originate from the synovial membrane.

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Fig. 7. Examination of capability of supporting osteoclast formation on fibroblast-like cells from the SF of patients with TMD in co-cultured with peripheral blood monocyte. Numerous TRAP-positive multinucleated cells were appeared in co-culture of fibroblast-like cells and peripheral blood monocyte (A). Culture of only peripheral blood monocytes under the same condition, there were only a few two nuclei-cells (B).

Fig. 8. Effect of OPG on osteoclastogenesis in co-culture of fibroblast-like cells and peripheral blood monocytes. Inhibitory effects of TRAP-positive multinucleated giant cells formation in response to 10 ng/ml and 100 ng/ml of OPG in this culture system. Each value represents mean ± SD. *P < 0.05 vs absence of OPG.

Fig. 9. ELISA for sRANKL in SF. Each detectable sRANKL concentration (pg/ml) shown was calculated per 1 mg of SF total protein. Each value represents mean ± SD. n = 5; *P < 0.05 vs control.
Although the development of multinucleated giant cells could be induced from the SF cells of most of the TMD patients, we were unable to derive giant cells from three TMJs of the TMD patients or from any of the TMJs of the control group. The numbers of joint-infiltrating cells were lower in these joints than in other joints affected by TMD (data not shown), which suggests that osteoclast induction may be dependent upon the number of joint-infiltrating cells and/or the grade of inflammation.

In conclusion, joint-infiltrating cells from the SF of TMD patients may play important roles in the pathogenesis of these disorders, which are characterized by progressive bone destruction or remodeling, as well as in the enhanced function of hematopoietic cells, such as preosteoclast-like cells. These findings may shed light on the molecular mechanisms of bone resorption or remodeling in the pathophysiology of TMJ. Assuming that the formation of these cells could be controlled, treatment modalities for TMJ diseases could be modified drastically. However, further studies concerning the characterization of these osteoclastic cells are required to clarify the roles of these cells in TMD pathogenesis.

Acknowledgment
This work was supported by the Ministry of Education, Science, Sports, and Culture of Japan (Grant-in-Aid for Science Research no. 15791181 to Dr H. Takano).

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