Osteoclast-like cells in an *in vitro* model of bone destruction by rheumatoid synovium

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**Abstract**

**Objective.** Osteoclasts may be involved in the process of rheumatoid bone destruction. To test this hypothesis, we developed an *in vitro* model of bone destruction by osteoclast-like cells derived from cultured rheumatoid synovial tissue without using any inducers.

**Methods.** Synovial tissues were obtained from rheumatoid arthritis and osteoarthritis patients and tissue pieces of about 2 mm\(^3\) that contained synovial lining were cultured. Multinucleated cells derived from cultured synovial tissues were studied cytochemically and morphologically for osteoclast-specific markers.

**Results.** Fibroblast-like and macrophage-like cells from the tissue pieces proliferated in the coexistence of lymphocytes. After 14 days of culture, multinucleated cells with tartrate-resistant acid phosphatase activity appeared. These cells expressed vacuolar H\(^+\)-ATPase, the vitronectin receptor and cathepsin K. Although binding of \(^{125}\)I-labelled salmon calcitonin was very low, the cells contained ringed structures of F-actin and showed strong bone-resorbing activity on ivory slices. Proliferation of macrophage-like cells and formation of multinucleated cells continued during 6 months of culture in the presence of fibroblast-like cells. The bone-resorbing activity of multinucleated cells derived from rheumatoid synovial tissue was much higher than that of cells from osteoarthritic synovial tissue, and was related to the disease activity of rheumatoid arthritis.

**Conclusion.** Our culture system reproduced *in vitro* the process of bone destruction by rheumatoid synovium, including the proliferation and fusion of precursor cells, polarization, activation and bone tissue resorption. This system may provide a tool for understanding the mechanisms of bone destruction in rheumatoid arthritis and for the development of new therapies to prevent bone destruction.

**Key words:** Rheumatoid arthritis, Bone destruction, Osteoclasts, Synovial tissue macrophages, *In vitro* model.

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by bone and cartilage destruction in the joints. Characteristic histopathological features of RA are hyperplasia of synovial lining cells with a dense microvasculature and infiltration of inflammatory mononuclear cells with lymphoid follicle formation [1].

Multinucleated giant cells are uncommon in the rheumatoid synovium, but some of the synovial lining cells appear to be multinucleated [2]. Human macrophages obtained at arthroplasty have been shown to differentiate into osteoclastic bone-resorbing cells [3]. Although it remains unclear whether these osteoclast-like cells originate from bone tissue or synovial tissue, recent studies have demonstrated that synovial cells differentiate into osteoclasts in the presence of osteoblast-like stromal cells and osteoclast-inducing factors, such as 1\(\alpha\)25-dihydroxy vitamin D\(_3\) (1\(\alpha\),25-(OH)\(_2\)D\(_3\)) and macrophage colony stimulating factor (M-CSF) [4]. Osteoclasts are considered to arise from haematopoietic stem cells of the monocyte-macrophage lineage and osteoclast progenitors are thought to be recruited to the bone from the haematopoietic tissues (bone marrow and spleen) [5-7].

Various studies have been performed using enzymatically dispersed non-lymphoid synovial lining layer cells to clarify the mechanisms of bone destruction in
RA. Cells isolated from the synovium by collagenase digestion contain lymphocytes, macrophages, and type A and type B synoviocytes. Because the monocytes, macrophages and lymphocytes usually die and only the fibroblast-like cells are perpetuated in culture, these experiments have been performed using fibroblast-like type B synoviocytes [8, 9].

In the present study, we developed a novel in vitro model of bone destruction that employed osteoclast-like cells differentiated from rheumatoid synovial macrophages without using any inducers. The formation of osteoclast-like cells persisted for more than 6 months in our culture system and was correlated with the clinical activity of RA. This culture system may be useful for understanding the mechanism of bone degradation by rheumatoid synovium and for screening new drugs that can prevent bone destruction in RA and other types of inflammatory arthritis.

Materials and methods

Preparation and culture of synovium

The study protocol was approved by the Review Board of St Marianna University School of Medicine. Synovial samples were obtained from 16 RA patients at the time of joint surgery, which consisted of synovectomy in five patients, total knee replacement in 10 patients, and total hip replacement in one patient (Table 1). The RA patients were divided into two groups: active and inactive RA. Active RA was defined as three or more swollen joints and C-reactive protein concentration > 1.0 mg/dl. Eight samples of osteoarthritis (OA) synovium were used as controls (total knee replacement in four patients and total hip replacement in four patients). The samples were immediately soaked in 0.01 M phosphate-buffered saline (PBS), pH 7.4, containing antibiotics and were washed several times in this buffer. Part of each sample was frozen in dry ice:acetone for haematoxylin-eosin staining and immunocytochemistry, while the remaining tissue was cut under a stereoscopic microscope into small pieces of about 2 mm³ that contained synovial lining and excluded fatty tissue. The tissue pieces were then placed in an eight-well chamber slide (one piece per well) and cultured in Dulbecco’s Minimum Eagle Medium supplemented with 10% fetal calf serum. Half of the medium was replaced every 3 days.

Enzyme histochemistry

Cells were fixed with acetone:glutaraldehyde for tartrate-resistant acid phosphatase (TRAP) and non-specific esterase (NSE) staining. TRAP and NSE were visualized using commercial kits (Sigma, St Louis, MO, USA). Cathepsins K and L were stained histochemically using a synthetic substrate, Z-Phe-Arg-4-methoxy-β-naphthylamide (Z-Phe-Arg-4MβNA) (Bachem, Bubendorf, Switzerland) by the method of Dodds et al. [10] with slight modification. The peptide used in the assay could also be processed by cathepsin B, but was more sensitive to cathepsins K and L, and the reaction was performed in the presence of 4 M urea to inhibit cathepsin B activity [11]. After the cells had been fixed with acetone: glutaraldehyde, the samples were preincubated and activated

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*CRP: C-reactive protein; normally < 0.3 mg/dl.  
*bCS, corticosteroids; MTX, methotrexate; SASP, sulphasalazine; NSAIDs, non-steroidal anti-inflammatory drugs.
in 10 mM sodium acetate buffer (pH 5.4) with 5 mM EDTA (ethylenediamine tetraacetate) and 20 mM cysteine. Then the cells were reacted with Z-Phe-Arg-4MNA at 37°C in the presence of 4 mM urea, after which the 4-methoxy-2-naphthylamine released in the reaction was post-coupled with Fast Garnet GBC (Sigma) to yield a red colour.

**Immunocytochemistry**

For immunocytochemistry, cultured cells were fixed with cold ethanol and stained by an indirect immunoperoxidase technique. The primary antibodies used included monoclonal antibodies directed against CD3 (Leu 4) (Becton Dickinson, San Jose, CA, USA); CD14 (TÜK 4), CD20 (L20), CD68 (KP1) and prolyl-4-hydroxylase (5B5) (Dako, Glostrup, Denmark); CD9 (NALM-6) and CD51 (WNK147), vitronectin receptor α-chain (Chemicon International, CA, USA); and cathepsin K (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The polyclonal antibody against vacuole-type proton ATPase was a kind gift from Dr Takashi Sawai (Department of Pathology, Iwate Medical University, Morioka, Japan). The primary antibodies were diluted 1:100–1000 (IgG concentration 0.5–2.5 μg/ml). A horseradish peroxidase-labeled secondary antibody to mouse or rabbit immunoglobulin (Amersham International, Poole, UK) was diluted 1:20. Incubation with the antibody was for 30 min, and was followed by peroxidase staining and methyl green counterstaining.

**Electron microscopy**

Cultured cells were washed twice with PBS and were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 2% sodium chloride for 1 h at room temperature and for an additional hour at 4°C. The specimens were washed with the buffer solution containing sodium chloride, post-fixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer (pH 7.4) for 2 h at 4°C, and then stained with 1% tannic acid in 50 mM maleic acid buffer solution (pH 6.0) for 2 h at 4°C in a dark box. The washed cells were dehydrated in a graded ethanol series, detached from the chamber slide and embedded in Spurr’s resin. Ultrathin sections were cut on an ultramicrotome (Ultracut E, Reichert, Depew, NY, USA) and stained with both uranyl acetate and lead citrate solution before observation with a transmission microscope (Jeol 1200EX-II).

**Calcitonin receptor assay**

[125I]Salmon calcitonin (sCT) (New England Nuclear/ Dupont, Boston, MA, USA) was incubated with or without an excess of cold sCT (10 million-fold), washed, and developed by microautoradiography to demonstrate the calcitonin receptor (CTR) [12]. Briefly, the cells were plated on chamber slides and were cultured for 4 weeks. At the end of culture, the cells were incubated with [125I]sCT (50,000 c.p.m./ml) in the presence or absence of cold sCT (10−12 M; Sigma) at 37°C for 2 h. Then the cells were washed twice with PBS to remove non-specific radioactivity and were fixed with 2.5% glutaraldehyde in PBS. Slides were dipped in LM-1 photographic emulsion (1 : 1 dilution with 1.7% glycerol; Amersham, Arlington Heights, IL, USA) for autoradiography.

**Actin ring formation assay**

The distribution of microfilaments was evaluated by the detection of F-actin with fluorescein isothiocyanate (FITC)-conjugated phalloidin, as described previously [13]. Cells were fixed in 3.7% formaldehyde in PBS for 10 min at room temperature. After washing with PBS, the cells were permeabilized by incubation with 0.1% Triton X-100 in PBS for 3 min at 0°C. Chamber slides were washed with PBS and stained for TRAP to identify osteoclast-like cells, and then incubated for 3 h with FITC-conjugated phalloidin solution. Next, the slides were washed with PBS, mounted with Fluorsave (CalbioChem-Novabiochem, San Diego, CA, USA), and examined under a confocal fluorescence microscope (MRC 600, Bio-Rad, Tokyo, Japan).

**TRAP and cathepsin K/L activities in cell lysates**

Cells were lysed in distilled water with 0.1% Triton X-100. The TRAP activity of the cell lysate was measured by a colorimetric assay using p-nitrophenyl phosphate as the substrate. The reaction was performed at 37°C for 60 min in the presence of 10 mM tartrate and 500 μM ascorbate, and the release of p-nitrophenol was detected at 410 nm. The cathepsin K/L activity was determined by the method of Barrett and Kirschke [14] with slight modification [15]. The reaction mixture contained the cell lysate, 5 mM EDTA, 100 mM Z-Phe-Arg-4MNA, 20 mM cysteine and 4 mM urea in sodium acetate buffer (pH 5.4). Incubation was at 37°C for 60 min and the 4-methoxy-β-naphthylamine released was coupled with Fast Garnet GBC (Sigma). After extraction of the coupled product in n-butanol, the colour was measured at 520 nm.

**Bone resorptive activity**

Circular slices of ivory were placed in the wells of a 96-well culture plate and synovial tissues were plated on to the slices. The cells derived from the synovial tissues were cultured for 30 days as described above and were then scraped off with 2 N NaOH with a rubber policeman. Then the slices were stained with Coomassie brilliant blue to detect lacunar resorption pits; the pits were also evaluated by scanning electron microscopy. After sputter-coating with gold in an ion sputter-coater (Joel JFC-1100), the slices were examined under a scanning electron microscope (Joel JSM-840A).

To investigate the difference between RA and OA synovium, the pits stained by Coomassie brilliant blue were counted under a microscope by two investigators. Although some pits were fused, the numbers from the two investigators were similar and the mean value was taken for statistical analysis.
Statistics
Differences between the mean values for the two groups were assessed with the Mann–Whitney U-test, and $P < 0.05$ was considered to indicate significance.

Results
Development of multinucleated cells
When pieces of RA synovial tissue were cultured in medium containing 10% fetal calf serum, three types of cells were obtained, including spindle-shaped fibroblast-like cells, macrophage-like cells and lymphocytes. The number of lymphocytes depended on the disease activity of RA, and active RA synovium yielded a larger number of lymphocytes. Adherent cells, such as fibroblast-like cells and macrophages, proliferated and then spread over the surface of the culture well. After 10–14 days the layer of fibroblast-like cells showed partial sloughing and multinucleated giant cells with an eosinophilic cytoplasm appeared in the sloughed areas (Fig. 1A). At first, the multinucleated cells were rather small and most of them contained fewer than 10 nuclei. As culture continued, the multinucleated cells became larger and cells containing more than 50 nuclei appeared. Although lymphocytes disappeared within 3 months of the start of culture, the proliferation of macrophage-like cells and the formation of multinucleated cells continued for over 6 months in coexistence with the fibroblast-like cells.

Histochemical analysis
Both multinucleated cells and mononucleate macrophage-like cells were positive by TRAP staining (Fig. 1B). NSE was detected in the mononuclear cells and small multinucleated cells, but the large multinucleated cells with many nuclei displayed equivocal NSE activity (data not shown). Cathepsin K/L activity was observed in both multinucleated cells and mononuclear macrophage-like cells by enzyme histochemistry and was confirmed by immunocytochemistry using a monoclonal antibody for cathepsin K (Fig. 2A, B). Cathepsin K expression was undetectable in fibroblast-like cells both by enzyme histochemistry and immunocytochemistry.

To further characterize the multinucleated cells, various phenotypic features of osteoclasts and macrophages were investigated. Multinucleated cells expressed vacuolar proton ATPase and the vitronectin receptor (Fig. 2C, D). The cells also expressed CD68 and CD9, but not CD14 (Fig. 3A–C). Mononuclear macrophage-like cells showed strong expression of CD68 and CD9 and weak expression of CD14. In frozen sections of synovial tissue, a large number of cells in the synovial lining layer were TRAP-positive, and a few TRAP-positive cells were also dispersed in the sublining (data not shown). Most of the synovial lining cells expressed CD68 and CD9, but CD14-positive cells were less common (data not shown). Expression of CD14 and CD68 by the sublining cells was similar. Small lymphocytes in the culture of rheumatoid synovium were mostly CD3-positive and CD20-negative (data not shown).

Ultrastructural findings
On electron microscopy, the multinucleated cells displayed a ruffled border-like structure, consisting of complicated finger-like projections of the cytoplasm. The cytoplasm contained numerous vacuoles, endoplasmic reticulum, mitochondria and electron-dense lysozyme-type granules (Fig. 4A). Filamentous elements were prominent in the peripheral cytoplasm. The mononuclear cells in these cultures exhibited a similar appearance (ruffled border-like structure and vacuole- and lysosome-rich cytoplasm), suggesting that they were the precursors of the osteoclast-like multinucleated cells (Fig. 4B).

CTR expression
Figure 5 shows microautoradiographs of $[^{125}I]$sCT binding to multinucleated cells formed during the culture of rat bone marrow cells in the presence of 1α,25-(OH)2D3, and binding to multinucleated cells derived from human rheumatoid synovial tissue. As reported previously [13], TRAP-positive osteoclasts derived from rat bone marrow cells demonstrated a high level of $[^{125}I]$sCT binding (Fig. 5A). In contrast, the multinucleated cells from synovial tissue cultures showed very weak $[^{125}I]$sCT binding (Fig. 5B).

Actin ring and pit formation
The multinucleated cells derived from synovial tissue clearly demonstrated ringed structures consisting of F-actin bands after incubation with FITC-conjugated phalloidin (Fig. 6A). When the synovial tissues were cultured on ivory slices, numerous resorption pits were formed after 4–8 weeks of culture. The resorption pits tended to occur in clusters and some of them were deep enough to penetrate the slices (Fig. 6B, C).

Comparison between RA and OA synovium
The development of osteoclast-like cells and bone resorptive activity were compared between synovial tissue cultures from RA and OA patients. The clinical features of the patients are shown in Table 1. All RA patients were being treated with non-steroidal anti-inflammatory drugs and disease-modifying anti-rheumatic drugs or corticosteroids. TRAP-positive multinucleated cells were numerous in cultures from RA synovium, especially from the synovium of patients with active RA, compared with the cultures from OA synovium (Table 2). The formation of pits was prominent in the cultures from RA synovium and was related to the disease activity of RA. Cultures of synovial tissue from RA patients showed higher TRAP activity and higher cathepsin K/L activity than cultures from OA synovium (Table 2). The differences in cathepsin K activity and pit formation were greater between RA and OA osteoclast-like cells than the differences in cell number and TRAP activity.
Discussion

RA is an inflammatory joint disease in which chronic synovitis leads to the destruction of bone and cartilage [1]. However, the mechanisms of bone destruction by rheumatoid synovium have not yet been established. Osteoclastic bone resorption involves several cellular and molecular processes [7]. When osteoclasts change from the resting (non-polarized) state to the polarized (resorbing) state, striking alterations occur in the organization of their cytoskeleton. The osteoclasts adhere to the surface of bone via integrin molecules, mostly via the vitronectin receptor, and develop specialized structures called podosomes in the clear zone, which consist mainly of F-actin [16-18]. After these cytoskeletal changes, osteoclasts come to possess bone-resorbing activity that

![Image A](image1.png)

![Image B](image2.png)

**Fig. 1.** (A) Multinucleated cells after culture of RA synovial tissues for 2 weeks. Multinucleated cells (arrows) are seen intermingled with fibroblastic cells and lymphocytes. Haematoxylin–eosin staining, original magnification ×20. (B) TRAP-positive giant cells with numerous unstained nuclei were observed in the 8-week culture of rheumatoid synovial tissue. Original magnification ×40.

![Image C](image3.png)

![Image D](image4.png)

**Fig. 2.** Expression of cathepsin K by multinucleated cells derived from synovial tissue (A, B). Cathepsin K/L activity (A) was localized in the cytoplasm by enzyme histochemistry. Immunolocalization of cathepsin K was confirmed using a monoclonal antibody (B). Vacuolar proton ATPase (C) and CD51 (vitronectin receptor z-chain) (D) were detected by immunocytochemistry in multinucleated cells derived from synovial tissues. Original magnification ×100.
involves demineralization by acid and degradation of the bone matrix by lysosomal proteinases. Transport of protons into resorption lacunae is mediated by vacuolar proton ATPase (V-ATPase) [19]. While a number of proteases are known to exist in osteoclasts, recent studies have demonstrated that acidic cysteine proteinase, cathepsin K and a neutral proteinase (matrix metalloproteinase 9), play a crucial role in osteoclast-mediated matrix degradation [20]. Both protons and these lysosomal enzymes are secreted into the sealed space beneath the ruffled border to degrade bone matrix.

In the present study, the synovium-derived osteoclast-like cells showed typical ring-like configurations of polymerized actin, which play a critical role in cell attachment and polarization. The ultrastructural appearance of these cells (numerous vacules, endoplasmic reticulum, mitochondria in the cytoplasm with a ruffled border-like structure, and peripherally oriented filaments) was similar to that of osteoclasts, as reported previously [21]. In addition, V-ATPase and cathepsin K were demonstrated in the synovium-derived osteoclast-like cells by immunocytochemical localization or histochemical staining. A recent study demonstrated that cathepsin K expression is confined to osteoclasts in bone tissue and is not detectable in other tissues [22]. Therefore, it is reasonable to consider that polarized cells secreting protons and proteases would have the potential to degrade the matrix and to invade bone tissue adjacent to rheumatoid synovium.

The major criteria for identification of osteoclasts include TRAP activity, expression of the CTR and the vitronectin receptor (zvβ3) and pit-forming activity [7]. In the present study, synovium-derived multinucleated cells exhibited weak binding of $^{125}$I-calcitonin compared with osteoclasts generated by culture of bone marrow cells with 1x25-(OH)$_2$D$_3$. As we did not investigate CTR mRNA expression, it remains unclear whether these cells completely lacked the CTR or showed very low CTR expression. In the present study, binding of

![Fig. 3](image1.png)

**Fig. 3.** Multinucleated cells derived from synovial tissue showed immunocytochemical expression of (A) CD68, (B) CD9 and (C) CD14. The cells strongly expressed CD68 and CD9 but not CD14. Original magnification $\times$40 (A, B), $\times$80 (C).

![Fig. 4](image2.png)

**Fig. 4.** Electron micrographs of (A) a multinucleated giant cell and (B) a macrophage-like mononuclear cell derived from synovial tissue. Both cells exhibit a ruffled border-like structure with numerous vacules, endoplasmic reticulum, mitochondria, and electron-dense lysosomal granules. Filaments are abundant in the peripheral cytoplasm. Compare the size of the multinucleated cell with that of a lymphocyte (arrow). Original magnification $\times$1000 (A), $\times$1500 (B). Scale bar= 5 μm.
radioactive calcitonin was determined using osteoclast-like cells cultured for 4 weeks, so it is possible that CTR expression was down-regulated during long-term culture. Further studies of CTR and calcitonin responsiveness after various culture periods would be necessary to solve this issue.

Several animal studies have demonstrated that monocytes and macrophages isolated from inflammatory lesions or tumours are capable of differentiating into osteoclastic bone-resorbing cells when co-cultured with bone-derived stromal cells in the presence of 1α,25-(OH)2D3 [23, 24]. A recent study showed that mononuclear phagocytes from rheumatoid synovial tissue could differentiate into multinucleated cells, which fulfilled the criteria for osteoclasts both cytochemically and functionally when co-cultured with osteoblast-like UMR 106 cells in the presence of 1α,25-(OH)2D3 and M-CSF [4]. These findings suggest that synovial macrophages may be precursors of bone-resorbing cells at sites of rheumatoid joint destruction.

Multinucleated osteoclasts originate from haematopoietic progenitor cells of the monocyte–macrophage lineage [5, 6] and their progenitor cells are recruited from haematopoietic tissues such as bone marrow and spleen. In the present study, synovium-derived osteoclast-like cells expressed CD9 and CD68, suggesting that these cells originated from the monocyte–macrophage family. However, the osteoclast-like cells did not express CD14. Electron microscopy showed that the mononuclear cells possessed a ruffled border membrane and vacuole- and mitochondria-rich cytoplasm,
like osteoclasts. The macrophage-like mononuclear cells in synovial cultures expressed both CD9 and CD68, while showing low expression of CD14. Our histological study of synovial tissue specimens demonstrated that the synovial lining cells expressed CD68, CD9 and TRAP with weak expression of CD14. These findings are consistent with the report by Mulherin et al. [25]. CD9 is one of the haemopoietic antigens and belongs to the transmembrane 4 superfamily. It is usually expressed by pre-B cells, platelets, megakaryocytes and monocytes, and has also been detected on osteoclasts [26]. Because it forms a complex with the β1 and β3 integrins [27], CD9 expressed on synoviocytes may be involved in cell-to-cell or cell-to-extracellular matrix adhesion. Our findings suggested strongly that the precursor of the osteoclast-like multinucleated cells was the CD9+, CD68+ macrophage-like cells in the synovial lining.

Osteoclasts are terminally differentiated cells with a limited lifespan. Isolated murine osteoclasts survive for no longer than 1 week, while time-course studies of osteoclast formation in murine bone marrow cultures or co-cultures have shown that most osteoclasts disappear within 5 days [7]. Two cytokines, interleukin (IL)-1 and M-CSF, act directly on mature osteoclasts and prolong their lifespan in culture [28, 29]. In our culture system, multinucleated osteoclast-like cells were generated consistently in the absence of additional humoral factors such as vitamin D3 and M-CSF, and co-culture with osteoblast-like stromal cells was unnecessary. Furthermore, the generation of osteoclast-like cells persisted for as long as 6 months. Although we did not examine the lifespan of the osteoclast-like multinucleated cells, it seems likely that cytokines produced by the cultured synovial tissue prolonged their survival. The persistent and extensive proliferation of macrophage-like synoviocytes meant that the supply of precursor cells and their fusion into maintained multinucleated cells continued for a long period. In this regard, our culture system was able to reproduce in vitro the process of bone destruction by rheumatoid synovium, including proliferation and fusion of precursor cells, polarization, activation, and resorption of bone tissue. Our system may thus provide a tool for research into the mechanism of bone destruction in RA and the prevention of such destruction as well as for the study of osteoclastic bone resorption.

Recent studies have demonstrated that receptor activator of nuclear factor κB ligand (RANKL), expressed on osteoblasts and stromal cells, plays an important role during the commitment and differentiation of progenitors to osteoclasts [30, 31]. Our culture system contained fibroblast-like cells that expressed prolyl-4-hydroxylase in their cytoplasm. This enzyme is essential for the formation of collagen and has been demonstrated in type B fibroblast-like synovial lining cells [32]. It seems likely that these cells act as supporting cells during osteoclast-like cell formation.

Multinucleated osteoclast-like cells were formed in cultures of synovial tissues from both RA and OA patients, indicating that there was no essential difference between the RA and OA macrophage-like synoviocytes. However, more osteoclast-like cells were generated more rapidly in cultures from RA synovium compared with OA synovium. Also, the bone-resorbing activity of osteoclast-like cells from OA synovial tissue was much weaker than that of RA synovium-derived osteoclast-like cells. It has been reported that RA synovium produces various cytokines, such as IL-1, IL-6 and tumour necrosis factor α, and prostaglandins [33–35]. Differences in the production of cytokines and chemical mediators might explain these differences in the formation and activity of osteoclast-like cells between RA and OA. In addition, numerous lymphocytes were observed in the cultures from RA synovium. Fibroblast-like synoviocytes isolated from RA patients show morphological and functional activation [8], and it has been reported that activated T cells [36] and synovial fibroblasts [37] express RANKL. Further investigations will be necessary to elucidate the role of lymphocytes and fibroblast-like cells during the formation of osteoclast-like cells in terms of RANK-RANKL regulation.

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