

SYNOVIAL TISSUE IN RHEUMATOID ARTHRITIS IS A SOURCE OF OSTEOCLAST DIFFERENTIATION FACTOR

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Objective. Osteoclast differentiation factor (ODF; also known as osteoprotegerin ligand, receptor activator of nuclear factor κ B ligand, and tumor necrosis factor-related activation-induced cytokine) is a recently described cytokine known to be critical in inducing the differentiation of cells of the monocyte/macrophage lineage into osteoclasts. The role of osteoclasts in bone erosion in rheumatoid arthritis (RA) has been demonstrated, but the exact mechanisms involved in the formation and activation of osteoclasts in RA are not known. These studies address the potential role of ODF and the bone and marrow microenvironment in the pathogenesis of osteoclast-mediated bone erosion in RA.

Methods. Tissue sections from the bone-pannus interface at sites of bone erosion were examined for the presence of osteoclast precursors by the colocalization of messenger RNA (mRNA) for tartrate-resistant acid phosphatase (TRAP) and cathepsin K in mononuclear cells. Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to identify mRNA for ODF in synovial tissues, adherent synovial fibroblasts, and activated T lymphocytes derived from patients with RA.

Results. Multinucleated cells expressing both

TRAP and cathepsin K mRNA were identified in bone resorption lacunae in areas of pannus invasion into bone in RA patients. In addition, mononuclear cells expressing both TRAP and cathepsin K mRNA (preosteoclasts) were identified in bone marrow in and adjacent to areas of pannus invasion in RA erosions. ODF mRNA was detected by RT-PCR in whole synovial tissues from patients with RA but not in normal synovial tissues. In addition, ODF mRNA was detected in cultured adherent synovial fibroblasts and in activated T lymphocytes derived from RA synovial tissue, which were expanded by exposure to anti-CD3.

Conclusion. TRAP-positive, cathepsin K-positive osteoclast precursor cells are identified in areas of pannus invasion into bone in RA. ODF is expressed by both synovial fibroblasts and by activated T lymphocytes derived from synovial tissues from patients with RA. These synovial cells may contribute directly to the expansion of osteoclast precursors and to the formation and activation of osteoclasts at sites of bone erosion in RA.

Destruction of marginal and subchondral bone in diarthrodial joints is a common feature of rheumatoid arthritis (RA) and leads to the characteristic marginal erosions seen radiographically. The presence of such erosions is an important factor in predicting a subset of patients likely to progress to further joint damage (1). The pathophysiologic mechanisms of bone loss in RA have not been fully elucidated. Enzymatic breakdown of extracellular matrix components by matrix metalloproteinases (2), cathepsins (3), and mast cell proteinases (4) has been demonstrated to play an important role in cartilage destruction in RA and may contribute to bone loss as well (5). However, resorption of bone requires the removal of mineralized bone matrix, which in physiologic bone remodeling is accomplished by osteoclasts.

Osteoclasts have been implicated in the pathogenesis of bone erosions in RA based on early studies which demonstrated acid phosphatase-positive multinu-

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cleated cells at the pannus–bone interface in areas of subchondral bone loss (6). These analyses were hindered by the lack of phenotypic markers that could definitively identify osteoclasts and their precursor cells. Subsequent *in vitro* studies utilizing marrow coculture systems to generate osteoclasts have delineated the temporal sequence of expression of a number of functional markers of osteoclasts and their precursor cells (7–9). These include the expression of the calcitonin receptor (CTR), which has been shown to be a definitive marker for the identification of the fully functional osteoclast (7,10–12).

We have previously demonstrated that the multinucleated cells present in resorption lacunae in areas of subchondral bone adjacent to calcified cartilage express messenger RNA (mRNA) for CTR, and thus are definitively identified as osteoclasts (13). In addition, some multinucleated and mononuclear cells within invading inflammatory tissues at sites of bone erosion were also noted to express tartrate-resistant acid phosphatase (TRAP) mRNA and enzymatic activity, suggesting that these TRAP-positive cells may represent an expanded pool of osteoclast precursors. In *in vitro* coculture models, the TRAP enzyme is expressed in osteoclast precursors before multinucleation and CTR expression (7–9). However, expression of TRAP activity alone is not restricted to osteoclast precursors, since this enzyme has been noted in other cell types, including macrophage polykaryons (7,14).

Studies of tissues from the cartilage–pannus junction in RA have identified several locally produced cytokines that could provide signals for osteoclast differentiation and bone resorption. These include interleukin-1 α (IL-1 α), IL-1 β , IL-6, IL-11, and tumor necrosis factor α (TNF α) (15–17). However, of the cytokines previously known to affect osteoclastogenesis, only macrophage colony-stimulating factor (M-CSF) is uniquely essential for osteoclast development *in vivo* (18).

In physiologic bone remodeling, it has now been demonstrated that many of the cytokines known to stimulate bone resorption act through the up-regulation of a novel and essential factor for osteoclast development, osteoclast differentiation factor (ODF), also known as osteoprotegerin ligand (OPGL), receptor activator of nuclear factor κ B ligand (RANKL), and tumor necrosis factor–related activation-induced cytokine (TRANCE) (19,20), a membrane-bound member of the TNF family of cytokines. In *in vitro* coculture systems, ODF is sufficient to induce osteoclast differentiation in the presence of M-CSF, and in addition has the capability of directly activating multinucleated osteoclasts (19,20). Further studies have demonstrated that many

bone-resorptive signals including 1,25-dihydroxyvitamin D₃, IL-11, and parathyroid hormone act on the osteoblast/bone stromal cell to up-regulate the expression of membrane-bound and soluble ODF (20,21). The ODF $-/-$ mouse develops severe osteopetrosis and defective tooth eruption and lacks osteoclasts (22), further supporting the critical importance of this factor in osteoclast differentiation.

Because of the presumptive role of osteoclasts in bone resorption in RA, we sought to identify and localize osteoclasts and osteoclast precursor cells at sites of bone erosion and to examine synovial tissues for the expression of ODF. We demonstrate that there is a pool of osteoclast precursors in bone marrow spaces adjacent to invading pannus at sites of erosion in RA. Furthermore, synovial tissue from patients with RA is a source of ODF, implicating this factor in the bone erosions that occur in RA.

PATIENTS AND METHODS

Tissue procurement. Fresh synovial samples were obtained from patients with a diagnosis of RA according to the American College of Rheumatology (formerly, the American Rheumatism Association) criteria (23) or from patients with normal synovium. Normal samples were obtained at the time of carpal tunnel release surgery or were obtained from the National Disease Research Interchange (Philadelphia, PA). The latter included 1 synovial sample from the knee joint of an amputated limb, and 1 from the knee joint of a patient dying of trauma. Tissues from patients with RA were from total joint replacement surgery or synovectomy (knee, 11; elbow, 1; metacarpophalangeal joint and wrist, 4). Also obtained were fibroblasts from human dermal foreskin and 1 tissue sample from giant cell tumor of bone. Tissue procurement was approved by the Institutional Review Board.

Tissue samples were either immediately snap frozen in liquid nitrogen for RNA extraction or cultured for the generation of T cell lines or for adherent synovial fibroblasts. Frozen tissues were stored at -80°C . Samples from the bone–pannus interface were from patients with RA, except for 1 sample, which was from an adult patient with juvenile-onset, polyarticular, rheumatoid factor–positive arthritis. These samples included 3 knees and 2 elbows and were processed under RNase-free conditions. Fixation was with 4% paraformaldehyde or 10% neutral buffered formalin for 2 days, followed by decalcification in 14% EDTA for up to 5 weeks at 4°C .

In situ hybridization. Fixed and decalcified tissues were dehydrated in graded ethanol solutions, transferred to xylenes, and embedded in paraffin. Paraffin sections (4 μm) were placed on 3-aminopropyltriethoxysilane–coated slides, dried overnight, and used immediately or stored at 4°C .

Probe preparation. Probes were prepared according to previously published methods (13). Sense and antisense ^{35}S -labeled RNA probes for the TRAP gene were prepared from a 382-bp fragment of the human TRAP gene complementary DNA (cDNA) generated from a human osteoclast library by

polymerase chain reaction (PCR) and subcloned into pCRII. The probe was prepared by Dr. David Roodman (University of Texas, San Antonio) and corresponds to nucleotides 3–384 of the human TRAP gene cDNA. Sense and antisense probes for the cathepsin K gene were prepared from a 551-bp fragment of the cathepsin K gene generated by PCR from giant cell tumor using the primers 5'-ATAAATCTAGCACCCCTGATG-3' and 5'-TTTGCAGTTTTCTTCTTGA-3', and subcloned into pCRII. Vector DNA was appropriately linearized and in vitro transcribed using either T7 or SP6 polymerase in the presence of ³⁵S-UTP (New England Nuclear, Boston, MA).

Prehybridization/hybridization. Prehybridization and hybridization were performed according to a modification of previously published methods (13). Briefly, slides were deparaffinized in xylenes and rehydrated through graded ethanol solutions. Prehybridization included digestion with proteinase K (20 µg/ml) in 1× phosphate buffered saline (PBS; 8 minutes) followed by fixation in 4% paraformaldehyde (10 minutes) and acetylation in 0.25% acetic anhydride in 100 mmoles/liter of triethanolamine. Hybridization was performed overnight at 52°C. Hybridization solution contained 50% formamide, 0.3M NaCl, 20 mM Tris HCl (pH 7.5), 5 mM EDTA, 10 mM NaH₂PO₄ (pH 8.0), 10% Dextran sulfate, 1× Denhardt's solution, 0.5 mg/ml of total yeast RNA, and 0.1M dithiothreitol.

Radiolabeled probe was added (4 × 10⁴ counts per minute per ml) in a volume of 40 µl/slide. Washes were in graded saline–sodium citrate solutions and included treatment with RNase A (Pharmacia Biotech, Piscataway, NJ) at 37°C for 1.5–2 hours. Air-dried slides were dipped in Kodak NTB-2 emulsion (Eastman Kodak, Rochester, NY), drained, air dried for 1 hour, and placed in a light-proof container with desiccant at 4°C for 6–10 weeks. Slides were developed in Kodak D-19 developer, fixed in Kodak fixer, and counterstained with hematoxylin and eosin.

Reverse transcriptase-PCR (RT-PCR) and Southern blot analysis. First-strand cDNA was generated from synovial tissue-derived total RNA or RNA derived from T lymphocyte lines or from adherent synovial fibroblasts using a First Strand cDNA Synthesis Kit for RT-PCR (Boehringer Mannheim, Indianapolis, IN). Based on the published GenBank sequence for human ODF, the following primers were selected: 5'-CTATTTTCAGAGCGCAGATGGAT-3' and 5'-TATGAGAACTTGGGATTTTGGATGC-3', which generated a 557-bp PCR product. PCR conditions were as follows: denaturation at 95°C for 20 seconds, annealing at 54°C for 45 seconds, extension at 68°C for 45 seconds for 35 cycles, and final extension at 72°C for 15 minutes.

The resulting PCR products were run on 1.5% agarose Tris–borate–EDTA gels and transferred to Magna Lift transfer membrane (MSI, Westborough, MA) for Southern blot analysis. Filters were probed with the ³²P-ATP-labeled oligonucleotide probe 5'-CATCCACCATCGCTTTCTCTGCTC-3'. Sequencing of PCR products was performed on an ABI PRISM 377 DNA Sequencer (Perkin Elmer, Norwalk, CT) using Big Dye Terminator Mix (Perkin Elmer). PCR for GAPDH was performed on each sample as a control using the primers 5'-GCTCTCCAGAACATCATCCCTGCC-3' and 5'-CGTTGTCATACCAGGAAATGAGCTT-3'.

PCR for adherent synovial fibroblast and dermal fibroblast samples was performed as follows. Initial denaturation

was for 5 minutes at 95°C, with a 20-second denaturation step for each subsequent cycle. Annealing was for 45 seconds, beginning at 64°C and decreasing incrementally at the rate of 1° per cycle for 10 cycles, then remaining at 54°C for the remaining 20 cycles. Extension was at 68°C for 45 seconds, and final extension was at 72°C for 15 minutes.

Culture of adherent synovial fibroblasts. Dispersed synovial tissues were prepared by a previously published method (24). Briefly, synovial tissues were minced on tissue culture plates and treated with type I collagenase (4 mg/ml; Worthington, Lakewood, NJ), in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY), incubated for 1 hour at 37°C, treated with 0.25% trypsin for 30 minutes, harvested, and centrifuged at 1,000 revolutions per minute for 10 minutes. Pellets were suspended in 0.05% trypsin–0.02% EDTA for 10 minutes, centrifuged, and resuspended in 50% PBS, 50% DMEM containing 10% fetal calf serum (FCS; Sigma, St. Louis, MO). Cells were then centrifuged and suspended in DMEM, 10% FCS, and plated at a density of 10 × 10⁶ cells/10-cm plate. Cells were initially grown for 7–10 days and subsequently subjected to 2–4 passages.

Culture and expansion of T cells from synovial samples. Synovial tissues were minced into fragments of ~1 mm, placed in 48-well tissue culture plates, and incubated for 11–13 days in complete RPMI (10% FCS, 1 mM L-glutamine, 50 µM β-mercaptoethanol, 100 units/ml of penicillin/streptomycin) with recombinant human interleukin-2 (rHuIL-2; 20–50 units/ml; Pepro Tech, Rocky Hill, NJ).

T cell lines were generated by a modification of previously published methods (25). After culture in rHuIL-2, cells in wells demonstrating lymphocyte growth were pooled, transferred to wells precoated with anti-CD3 (10 µg/ml of UCHT-1; PharMingen, San Diego, CA), and cultured overnight. The T cell lines were then transferred out of anti-CD3-coated wells for subsequent expansion in complete RPMI with rHuIL-2 (8 × 10⁵ cells/ml) given 3 times per week. Further expansions followed a cycle of brief exposure to bound anti-CD3 followed by growth in IL-2-containing medium in 9–13-day cycles.

After several rounds of expansion of sufficient cells for cell surface marker analysis and molecular analysis, T cells were frozen. Cell aliquots were later thawed and placed in TRIzol (Gibco BRL) for extraction of RNA. T cell lines resulting from these outgrowth conditions were >95% CD3+, CD4+, CD45RO+ and oligoclonal, as determined by PCR analysis and subsequent cloning and sequencing of the T cell antigen receptor.

RESULTS

Previous studies in our laboratory have demonstrated that multinucleated cells in bone resorption lacunae at the bone–pannus interface from patients with established RA expressed both TRAP and CTR mRNA, consistent with their definitive identification as osteoclasts (13). In contrast, in several regions, mononuclear and some multinucleated cells remote from the bone surface expressed TRAP mRNA or TRAP enzymatic

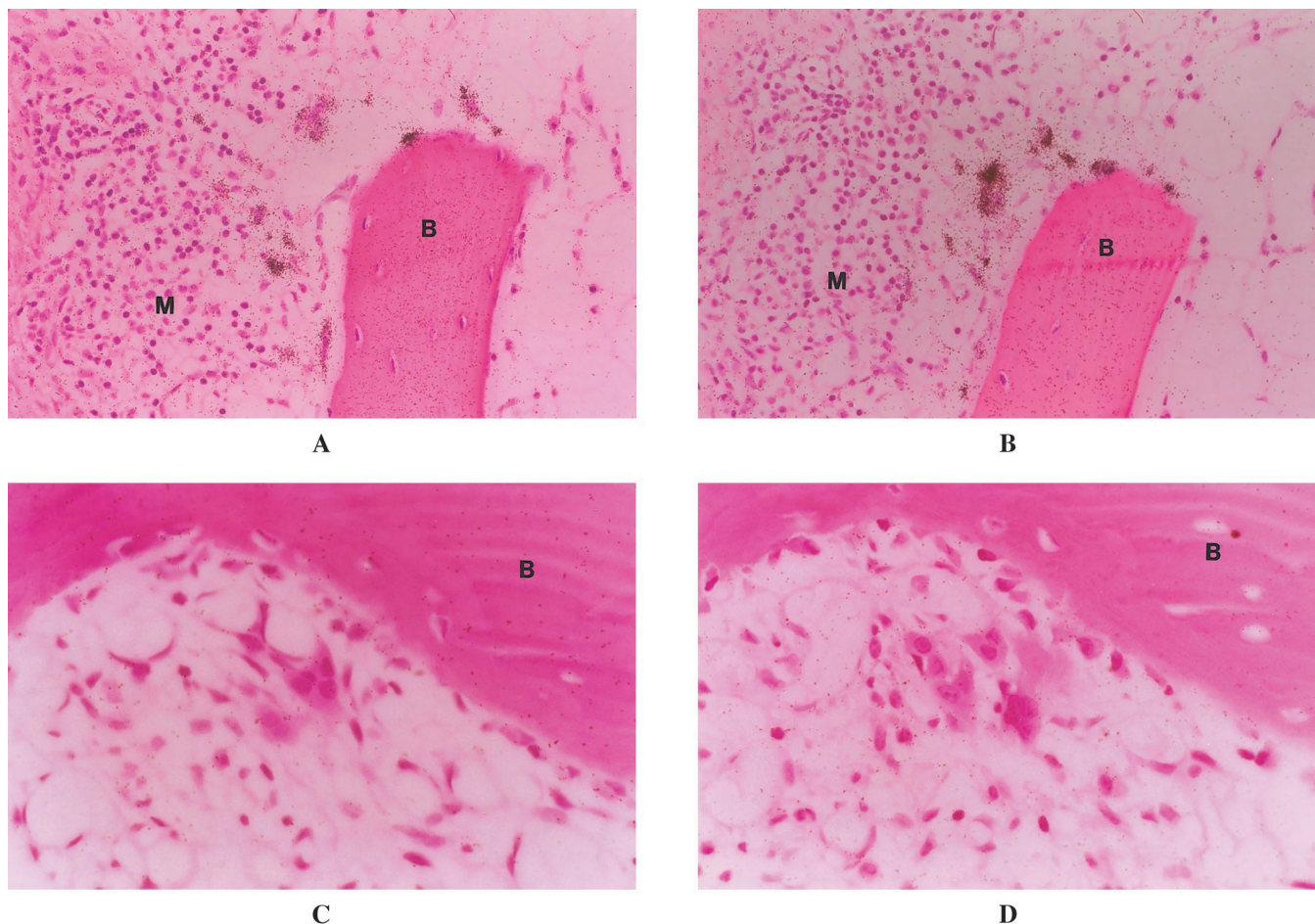


Figure 1. In situ hybridization for the tartrate-resistant acid phosphatase (TRAP) and cathepsin K genes in serial sections of tissue from a rheumatoid arthritis patient undergoing joint replacement surgery. Mononuclear cells are located within the marrow space adjacent to areas of pannus invasion. **A**, Antisense probe for the TRAP gene, demonstrating mononuclear cells expressing mRNA for TRAP. **B**, Antisense probe for the cathepsin K gene, demonstrating mononuclear cells expressing mRNA for cathepsin K. **C**, Sense control probe for the TRAP gene, demonstrating lack of hybridization to mononuclear and multinucleated cells. **D**, Sense control probe for the cathepsin K gene. **M** = marrow; **B** = bone. (Hematoxylin and eosin stained; original magnification $\times 80$ in **A** and **B**; $\times 150$ in **C** and **D**.)

activity, but not CTR. We speculated that these cells represented osteoclast precursors. In the present study, we used the technique of in situ hybridization in serial sections from RA tissues to more rigorously analyze the cells in resorption lacunae and adjacent regions for the expression of functional markers of osteoclasts and their precursors.

Recent analysis of the temporal pattern of gene expression during osteoclast differentiation has demonstrated that transcription of the cathepsin K gene, a cysteine protease implicated in bone resorption (26), and of the TRAP gene is turned on at the stage of the preosteoclast. In addition, extensive surveys of embryonic and adult tissues have demonstrated that colocal-

ization of these 2 markers identifies mononuclear cells as preosteoclasts (27). We therefore used the technique of in situ hybridization in serial tissue sections to determine whether cathepsin K-positive, TRAP-positive cells consistent with the phenotype of preosteoclasts could be identified in and around areas of bone resorption in erosive RA.

Colocalization of cathepsin K and TRAP within mononuclear cells was demonstrated in 4 of 5 cases examined. These cells were primarily located within the marrow space adjacent to areas of pannus invasion into bone marrow (Figure 1). Occasional mononuclear cells expressing both cathepsin K and TRAP were also noted on the articular surface within invading pannus tissues.

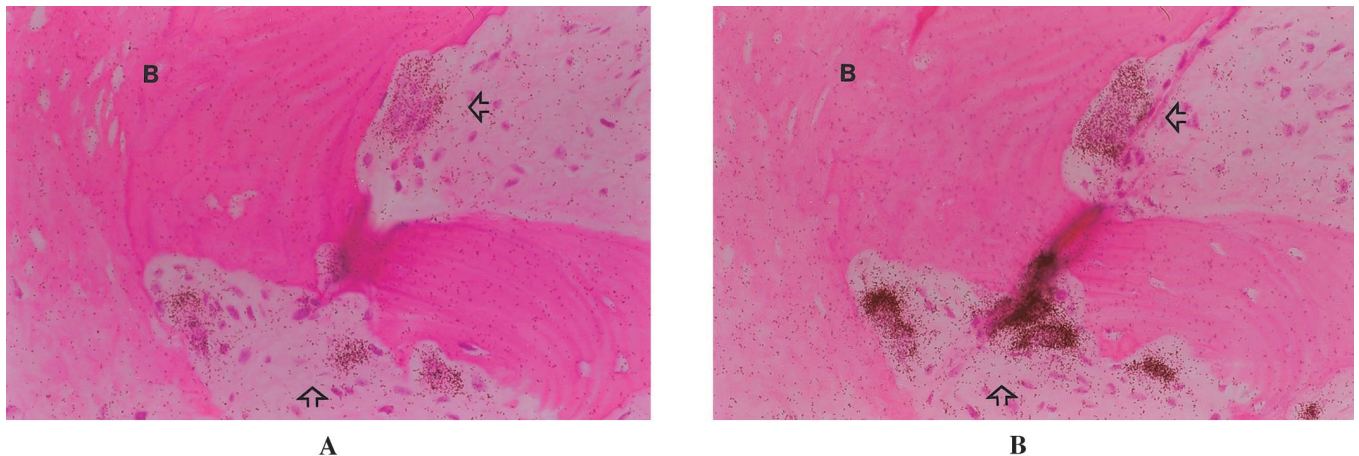


Figure 2. In situ hybridization of tissue from a rheumatoid arthritis patient undergoing joint replacement surgery. **A**, Antisense probe for the tartrate-resistant acid phosphatase (TRAP) gene. Multinucleated cells (arrows) in bone resorption lacunae express mRNA for TRAP. **B**, Antisense probe for the cathepsin K gene, serial section. Multinucleated cells demonstrated in **A** also express mRNA for cathepsin K. **B** = bone. (Hematoxylin and eosin stained; original magnification $\times 100$.)

Previous studies by Hummel et al (28) demonstrated the expression of cathepsin K in multinucleated cells at sites of bone erosion in RA. We confirmed this finding, and demonstrated in serial sections the colocalization within multinucleated cells in bone resorption lacunae of TRAP and cathepsin K in all 5 samples studied (Figure 2).

This histologic picture suggests that differentiation factors may be present within RA synovial tissues that can induce the differentiation of cells of the monocyte/macrophage lineage to become multinucleated osteoclasts with the full potential for bone erosion. The recent identification of ODF, a pivotal cytokine in osteoclast differentiation and activity, prompted us to examine RA synovial tissues for the expression of this cytokine.

We performed RT-PCR on tissue samples from patients undergoing carpal tunnel release surgery (2 samples), on normal synovium (2 samples), and on synovium from patients with RA (10 samples), using primers specific for the ODF gene. Giant cell tumor of bone (1 sample) was also studied, since multinucleated cells with the full phenotype of osteoclasts are present within this lesion, and it has been demonstrated that factors elaborated by this tumor can induce the formation of these osteoclast-like multinucleated cells. Figure 3 demonstrates the Southern blot analysis of products from RT-PCR.

Normal synovial tissues were negative for ODF PCR product. The tissue sample from giant cell tumor of bone was strongly positive for ODF PCR product, and 10 of 10 samples of synovial tissue from patients with RA

showed varying amounts of PCR product for ODF, demonstrating that this osteoclast differentiation factor is being transcribed by cells within RA synovial tissues. Sequence analysis confirmed these RT-PCR products to be the expected 557-bp ODF fragment. PCR product of the expected size for the control gene GAPDH was present in all samples.

To identify the cellular origin of ODF in synovial

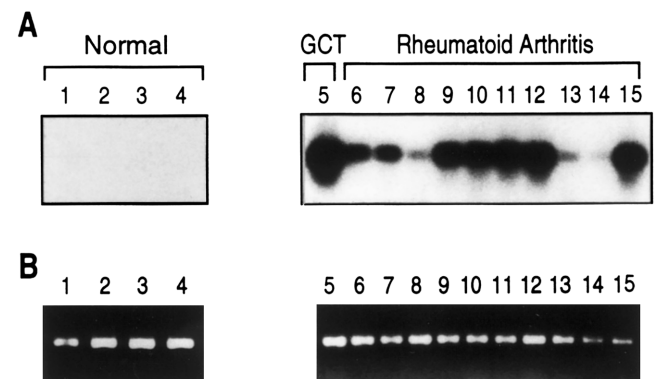


Figure 3. **A**, Southern blot analysis of products from reverse transcriptase–polymerase chain reaction (RT-PCR) from total RNA derived from rheumatoid arthritis (RA) synovial tissue using osteoclast differentiation factor (ODF)–specific primers. Two samples from carpal tunnel release surgery (lanes 1 and 2) and 2 from normal synovial tissues (lanes 3 and 4) are negative for amplification of ODF-specific PCR product. One sample of giant cell tumor of bone (GCT) is positive for the expected 557-bp PCR product (lane 5). Ten RA synovial samples yield ODF-specific PCR product (lanes 6–15). **B**, Ethidium bromide–stained gel demonstrating PCR product of the expected size for GAPDH. Samples correspond to those in **A**.

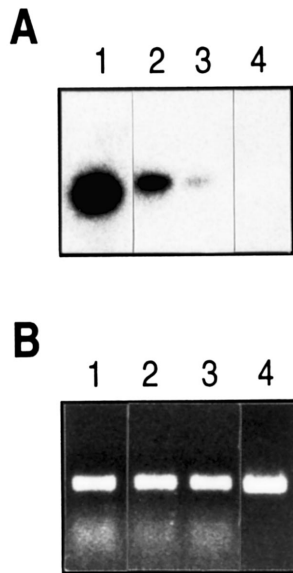


Figure 4. A, Southern blot analysis demonstrating the 557-bp product from reverse transcriptase–polymerase chain reaction (RT-PCR) from adherent synovial fibroblasts using osteoclast differentiation factor–specific primers. Lane 1, Primary culture of dispersed adherent synovial fibroblasts; lane 2, dispersed adherent synovial fibroblasts, passage 2; lane 3, dispersed adherent synovial fibroblasts, passage 4; lane 4, cultured dermal fibroblasts. B, Ethidium bromide–stained gel demonstrating PCR product for GAPDH. Samples correspond to those in A.

tissues derived from patients with RA, we studied 2 cell populations prepared from RA synovial tissues for the expression of ODF mRNA. Previous studies have demonstrated the generation of multinucleated cells with the phenotype of osteoclasts in cocultures of adherent synovial fibroblasts in the presence of peripheral blood cells of the monocyte/macrophage lineage (29,30). We therefore undertook an analysis of cultured adherent fibroblasts from RA synovial samples. Figure 4 is a Southern blot of PCR products amplified with primers specific for the ODF gene from RNA harvested from cultured synovial fibroblasts in primary culture and after increasing numbers of passage. Investigators in our laboratory previously demonstrated that primary cultures of adherent synovial fibroblasts contained small numbers of macrophages, but these cells did not survive after passage (31,32). All 3 samples of cultured synovial fibroblasts demonstrated PCR product for the ODF gene, whereas dermal fibroblasts were negative for this product. PCR product for ODF is increasingly difficult to detect as synovial fibroblasts remain in culture and are passaged.

To explore the possibility that activated T cells

may be a source of ODF in RA, T cell lines were established from 3 RA synovial tissue samples by sequential exposure to anti-CD3 and expansion in IL-2 according to a modification of a previously published method (25). RT-PCR performed on RNA from these T cell lines using ODF-specific primers demonstrated a band of the expected size for the ODF PCR product in all 3 samples. Southern blot analysis confirmed these to be ODF PCR product (Figure 5, lanes 3–5) and demonstrated strong signal in 2 of the 3 samples, and weaker but definite signal in the third sample. Thus, activated T cells from RA synovium expanded in the presence of anti-CD3 are an additional potential source of ODF.

DISCUSSION

The role of osteoclasts as a critical cell type in bone erosion in patients with longstanding RA has been previously reported (6,13,33). We have demonstrated the expression of calcitonin receptor mRNA in multinucleated cells in bone resorption lacunae at the pannus–bone interface in RA erosions, confirming the identification of these cells as osteoclasts (13). We have also previously observed mononuclear cells off the bone surface which are CTR negative and TRAP positive, suggesting that in bone lesions in established RA, oste-

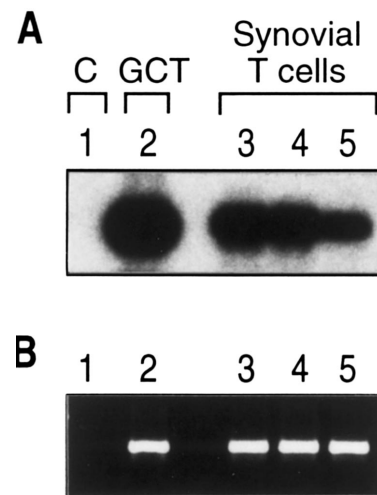


Figure 5. A, Southern blot analysis demonstrating the 557-bp product from reverse transcriptase–polymerase chain reaction (RT-PCR) from activated T cells from rheumatoid arthritis (RA) synovium using osteoclast differentiation factor–specific primers. Lane 1, No template DNA (control [C]); lane 2, giant cell tumor of bone (GCT); lanes 3–5, activated T lymphocytes derived from 3 samples of RA synovial tissues. B, Ethidium bromide–stained gel demonstrating PCR product for GAPDH. Samples correspond to those in A.

oclast precursor cells are generated in and around synovial tissues adjacent to bone surfaces. In the current study, we used serial sections and in situ hybridization techniques to demonstrate that mononuclear cells in these same areas express mRNA for both cathepsin K and TRAP, thus identifying these cells as preosteoclasts (27).

Work in animal models of arthritis has also supported the hypothesis that osteoclasts are generated at sites of bone erosion and that the osteoclasts which form in these areas mediate focal bone erosion. In murine collagen-induced arthritis, TRAP-positive multinucleated cells were observed on day 35 after disease initiation at erosive fronts in the pannus–bone and pannus–subchondral bone junctions of arthritic joints. In addition, multinucleated cells spontaneously generated from these lesions in vitro were calcitonin-receptor positive and produced lacunae on dentin slices, consistent with their identity as fully functional osteoclasts (34). Osteoclast-like multinucleated cells were also observed in areas of extensive bone resorption in the distal tibial diaphysis in rats with adjuvant arthritis (35). These cells expressed the Kat-1 antigen, a marker specifically expressed on the cell surface of rat osteoclasts.

The generation of osteoclast precursor cells, and subsequently, of osteoclasts, in areas of bone erosion in RA and in animal models of arthritis raises the possibility that cells within inflamed synovial tissues may produce a factor or group of factors capable of inducing the differentiation of cells of the monocyte/macrophage lineage into osteoclasts. Several lines of evidence support this possibility.

First, TRAP-positive multinucleated cells with the ability to form resorption pits on dentin slices have been identified in RA synovium remote from bone, suggesting that these cells might participate in bone loss in RA (36). It has also been shown that macrophages isolated from RA synovial tissues can differentiate into cells with the full phenotype of osteoclasts in the presence of UMR 106 rat osteoblast-like cells, 1,25-dihydroxyvitamin D₃, and M-CSF (29). In addition, bone-resorbing multinucleated cells have been formed in primary cultures of RA synovial cells in the presence of 1,25-dihydroxyvitamin D₃ without the addition of osteoblast-like cells or stromal cells; formation of these cells was facilitated by the addition of M-CSF (30). Finally, osteoclast-like multinucleated cells were formed when RA synovial fibroblasts were cocultured with peripheral blood mononuclear cells (30). Taken together, these results provide evidence that synovial macrophages can differentiate into osteoclast-like cells,

and furthermore that synovial fibroblasts may play a role in this process of differentiation.

In physiologic bone remodeling, evidence suggests that several of the cytokines implicated in bone erosion in RA, including IL-1 α , IL-1 β , IL-11, and TNF α (15–17), may act in part by up-regulating the expression of the newly identified ODF. This factor is expressed on the surface of osteoblasts, bone lining, and/or marrow stromal cells. Interaction with its receptor, RANK, which is present on the surface of osteoclast precursor cells, leads to the differentiation of these cells into osteoclasts. Of interest, ODF is identical to TRANCE, which is expressed in antigen-stimulated T cells. In addition to its expression on osteoclast precursors, the ODF receptor, RANK, is expressed on T cells, dendritic cells, and other hematopoietic precursors, and the interaction between ODF and RANK has been implicated in the enhancement of dendritic cell–T cell interactions (37).

In the current study, we demonstrated by RT-PCR that synovial tissues from patients with RA are a source of ODF mRNA. Attempts to identify ODF mRNA by in situ hybridization in RA synovial tissue sections were unsuccessful, suggesting that message levels for this cytokine are not abundant. Messenger RNA for ODF is detected in cultured dispersed synovial fibroblasts, but not in cultured dermal fibroblasts. PCR product for ODF is more difficult to detect in synovial fibroblasts subjected to prolonged culture, suggesting that expression of the ODF gene may be down-regulated under conditions of extended culture. However, this remains to be formally tested with quantitative methods.

An additional potential source of ODF in RA is activated T cells. ODF mRNA has been shown to be expressed in memory T cells, but not in naive T cells. Furthermore, ODF mRNA and cell surface expression are up-regulated in CD4⁺ T cells upon TCR/CD3 stimulation, and expression on CD4⁺ T cells is enhanced by costimulation through CD28 (37). We demonstrate in this study the expected finding that CD4⁺ T cell lines derived from RA synovial tissues and expanded by anti-CD3 express mRNA for ODF, suggesting that activated T cells may contribute to bone loss in RA via cell–cell contact with cells of the monocyte/macrophage lineage, leading to differentiation of these cells into osteoclasts. However, if activated T cells do contribute to osteoclast differentiation in RA, other regulatory factors must be present, since we have observed lymphocyte-rich areas in and around bone erosions in established RA where we do not find cathepsin K–positive, TRAP-positive osteoclast precursor cells (personal observations). In addition, activated T cells

are present in many disease states in which erosion of bone is not a prominent feature.

These data demonstrate that synovial tissues in RA are a source of the potent osteoclast differentiation factor and that synovial fibroblasts and activated T lymphocytes are both potential sources of ODF. The regulation of ODF activity in RA is undoubtedly complex. Although RA synovial tissues remote from bone are a source of ODF, osteoclasts and their precursors are identified primarily in sites of pannus invasion into bone and bone marrow, suggesting that the bone environment is playing an important role in this process. Osteoprotegerin (OPG) is a decoy receptor for ODF and has been demonstrated to block osteoclast differentiation in in-vitro culture systems. In the absence of OPG, mice develop severe osteoporosis (38). Interactions between ODF and OPG may be important in regulating the activity of ODF in synovial tissues, and these interactions may be modulated at the interface between invading synovial tissues and bone. Further studies will be needed to directly address the role of OPG in pathologic bone loss. Nonetheless, ODF may be an important cytokine in bone erosions in RA, and as such, it represents a possible therapeutic target for the prevention of bone loss in this disease.

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