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BONE RESORPTION BY TARTRATE-RESISTANT ACID PHOSPHATASE-POSITIVE MULTINUCLEAR CELLS ISOLATED FROM RHEUMATOID SYNOVIUM

Y. FUJIKAWA, M. SHINGU,* T. TORISU, I. ITONAGA and S. MASUMI

*Department of Orthopaedic Surgery, Oita Medical University, Oita and *Department of Clinical Immunology, Medical Institute of Bioregulation, Kyushu University 69, Beppu, Japan 874*

SUMMARY

Inflammatory reactions in rheumatoid arthritis (RA) often cause severe joint destruction. However, the mechanism of bone destruction is still a matter of controversy. To determine whether multinuclear cells found in the rheumatoid synovium can resorb bone, isolated synovial cells were assessed for tartrate-resistant acid phosphatase (TRAP) staining and the ability to resorb bone in a dentine resorption assay. TRAP-positive multinuclear cells were found in six out of 10 samples. These six samples showed resorption pit formation on dentine slices. The other four samples did not form resorption pits. The results of this study demonstrate that TRAP-positive multinuclear cells isolated from the rheumatoid synovium form resorption pits on dentine slices. Our results suggest that inflamed synovial cells in rheumatoid joints might participate in bone destruction.

KEY WORDS: Bone resorption, Rheumatoid arthritis, Pit formation, Synovial cells, Osteoclast-like cells, Tartrate-resistant acid phosphatase.

RHEUMATOID arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the synovium, which often leads to joint destruction [1, 2]. One of the major pathological changes in RA is the formation of pannus, leading to consequent joint destruction with bone and cartilage erosion [3]. There is also hyperplasia of the cells of the synovial lining which is believed to be a consequence of the influx of type A synoviocytes derived from bone marrow [4, 5].

Osteoclasts are the cells predominantly responsible for bone resorption, and they have been found at the pannus/bone interface in rheumatoid joints [6]. These cells increase in number and activity very early in the disease process. Giant cells have also been noted in RA synovial tissue. These cells may be of different type and derived from a range of mononuclear precursors. Macrophage-derived giant cells are commonly described [7] and are considered to be distinct from osteoclasts [8], despite a number of common features, including their probable origin from bone marrow precursors [9]. Previous studies have shown that some of these giant cells have immunohistochemical and cytochemical characteristics of osteoclasts [10, 11], and that some are capable of bone resorption [12].

We have noted the presence of tartrate-resistant acid phosphatase (TRAP)-positive multinuclear cells in dissociated synovial cells isolated from rheumatoid synovium. This study investigated the bone-resorbing ability of TRAP-positive multinuclear cells in rheumatoid synovium using the dentine pit formation assay. The data presented show that TRAP-positive multinuclear cells isolated from rheumatoid synovium form resorption pits on dentine slices.

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Correspondence to: Y. Fujikawa, Department of Orthopaedic Surgery, Oita Medical University, Idaigaoka 1-1, Hasama-machi, Oita-gun, Oita, Japan 879-55.

PATIENTS AND METHODS

Patient population

Ten synovial tissue specimens were obtained from patients with RA, as defined by the 1987 revised criteria of the American College of Rheumatology [13], at the time of knee joint replacement. Specimen radiographs of the synovium were taken and patients showing radiographically detected calcification of the synovial membrane were excluded from the study. The synovial membranes of all patients were taken for haematoxylin and eosin histological study, and those samples showing bone fragments in the synovial membrane were also excluded from this study.

Preparation of synovial cells

The synovium was cut into small pieces (~1–2 mm) and washed thoroughly in phosphate-buffered saline (PBS) (0.01 M phosphate, 0.138 M NaCl, pH 7.4). The synovial tissue specimens were enzymatically dissociated using 0.2% collagenase (Sigma Chemical Co., St Louis, MO) at 37°C for 30 min, and then using 0.25% trypsin (Chiba Laboratories, Chiba, Japan) and 0.08% deoxyribonuclease I (Sigma Chemical Co., St Louis, MO) at 37°C for 60 min, filtered through a nylon mesh and extensively washed. The dispersed synovial cells were suspended in an RPMI 1640 medium (RPMI; Gibco Laboratories, Grand Island, NY) with 10% fetal calf serum (FCS; Gibco Laboratories, Grand Island, NY), 100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 ng/ml fungizone and 2 mmol/ml L-glutamine.

TRAP histochemical staining

The isolated synovial cells were cultured for 48 h and adherent cultured synovial cells were fixed with 30 mM

citrate/acetone for 15 min, air dried and stained for TRAP using a kit from Sigma.

Dentine resorption assay

The assay system for dentine resorption by isolated synovial cells *in vitro* slightly modified from the original methods of Boyde *et al.* [14] was used [15]. Transverse slices of dentine (diameter 4 mm, $\sim 200\ \mu$m thick) were cleaned by ultrasonication in multiple changes of distilled water, sterilized using 70% ethanol, and left overnight under UV light. Twenty slices were placed in a 10 mm plate (#3001; Becton Dickinson Labware Co., Becton, NJ). The synovial cell preparation was suspended at 1×10^6 cells/ml in the culture medium and a 2.0 ml aliquot was transferred onto the slices. After 2 h of being cultured at 37°C in a 5% carbon dioxide incubator, the slices were removed and gently washed in fresh medium. The slices were then placed in a new 10 mm plate and incubated for 48 h with 2.0 ml medium.

At the end of the culture period, the slices were placed in 1 M NH_4OH for 30 min and cleaned by ultrasonication to remove adherent cells. The slices were then stained with Mayer's haematoxylin solution (haematoxylin, 1 g/l; $NaIO_3$, 0.2 g/l; $AlNH_4(SO_4)_2 \cdot 12H_2O$, 50 g/l; CH_3COOH, 7.5 g/l; pH 2.8) for 40–60 s, washed with distilled water, cleaned by ultrasonication and finally air dried.

Resorption pits visualized by Mayer's haematoxylin staining were identified by light microscopy, and then slices were coated with gold and examined in a Hitachi S-800 scanning electron microscope.

RESULTS

The replicated wells which were stained for TRAP had 1×10^{-4} isolated adherent synovial cells in each well. TRAP-positive multinuclear cells (Fig. 1) were found in six of 10 samples. In these samples, the percentage of TRAP-positive multinuclear cells varied from 0.1 to 0.8%. TRAP-negative multinuclear cells (Fig. 2) were also noted amongst them in isolated synovial cells and their number was greater than those of TRAP-positive multinuclear cells. Many TRAP-positive mononuclear cells were found in those samples which contained TRAP-positive multinuclear cells; however, four samples that did not contain TRAP-positive multinuclear cells also showed a small number of TRAP-positive mononuclear cells.

The isolated cells of all 10 samples were examined by the dentine pit formation assay. Resorption pits formed by isolated synovial cells on a dentine slice were visualized with Mayer's haematoxylin under transmitted light. Isolated cells from those six samples that contained TRAP-positive multinuclear cells produce resorption pits when cultured on dentine slices. The number of slices showing resorption pit formation in

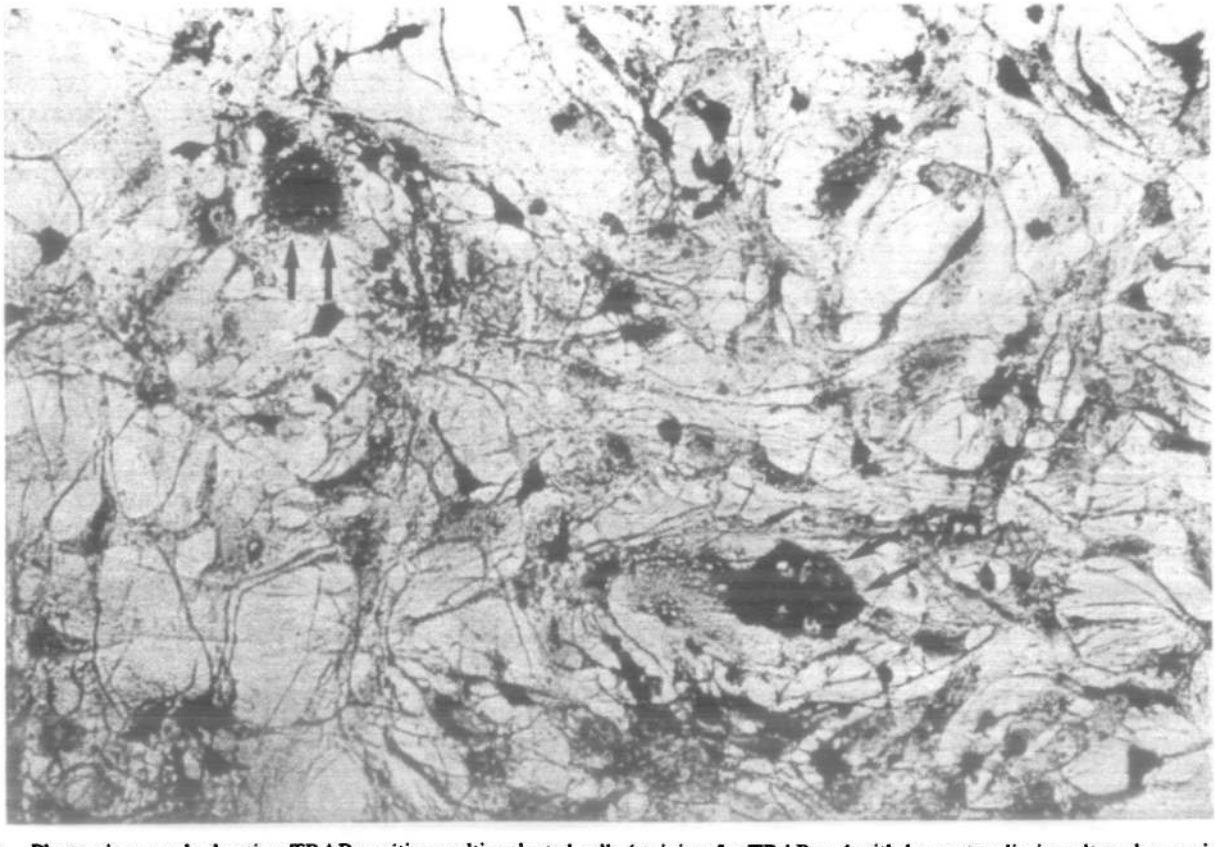

FIG. 1.—Photomicrograph showing TRAP-positive multinucleated cells (staining for TRAP and with haematoxylin in cultured synovial cells). This shows TRAP-positive multinuclear cells (arrows) isolated from rheumatoid synovium (×200).

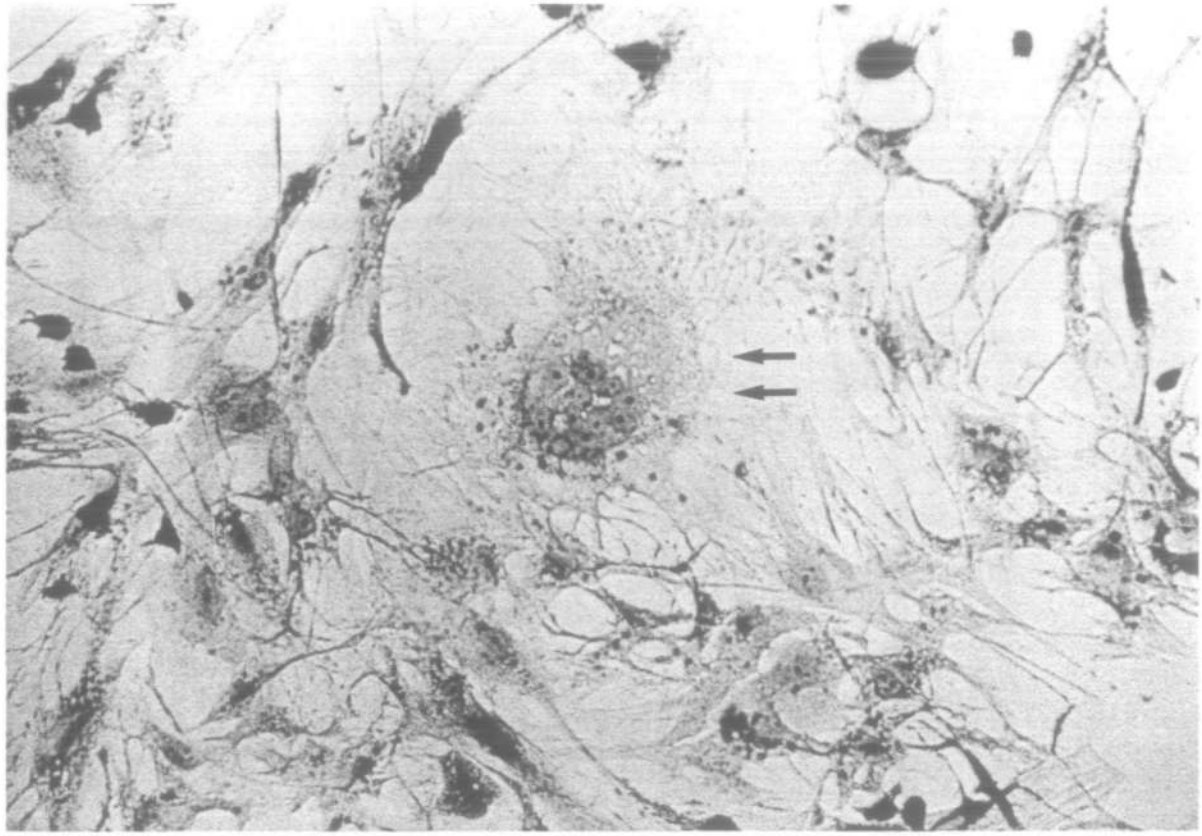

FIG. 2.—Photomicrograph showing TRAP-negative multinucleated cell (staining for TRAP and with haematoxylin in cultured synovial cells). This shows TRAP-negative multinuclear cell (arrows) isolated from rheumatoid synovium (×400).

each sample is shown in Table I. The number of resorption pits in one slice varied from one to five. Isolated cells from the four samples that did not contain TRAP-positive multinuclear cells showed no resorption pit formation.

The resorbed areas stained with Mayer's haematoxylin were also examined by scanning electron microscopy. Typical lacunar resorption pits were seen in all slices at the same areas stained with Mayer's haematoxylin (Fig. 3).

DISCUSSION

We show that TRAP-positive multinuclear cells were found amongst dissociated cells isolated from rheumatoid synovium where neither calcification nor bone fragments were demonstrated on radiographical and histological examination. In this report, we also describe the bone-resorbing ability of cells isolated from rheumatoid synovium using the dentine pit formation assay.

TABLE I
The results of TRAP staining and dentine resorption assay

Patient no.	TRAP-positive multinuclear cells	Number of slices that formed resorption pits
1	−	0/20
2	+	2/20
3	+	4/20
4	−	0/20
5	+	1/20
6	−	0/20
7	+	2/20
8	+	3/20
9	−	0/20
10	+	2/20

As far as we know, only one group has been successful in demonstrating bone resorption by cells isolated from human soft tissue, including inflammatory cells derived from the joint capsule of hip arthroplasties, giant cell tumour of tendon sheath and cells isolated from the rheumatoid synovium [12, 16, 17]. One other study that examined whether cells isolated from rheumatoid synovium were capable of bone resorption failed to demonstrate these [11]. A possible explanation for this is that there are a very small number of TRAP-positive multinuclear cells that have bone-resorbing ability in rheumatoid synovium. For this reason, in this study we examined many dentine slices for evidence of bone resorption by cells from each sample and identified resorption pit formation in a few slices.

Another possible explanation of our observation is that synovial cells cultured *in vitro* are overexposed to a factor that may activate bone resorption in the RA joint. Agents such as parathyroid hormone, 1,25-dihydroxyvitamin D_3 and interleukin (IL)-3 are known to increase the number of TRAP-positive cells per

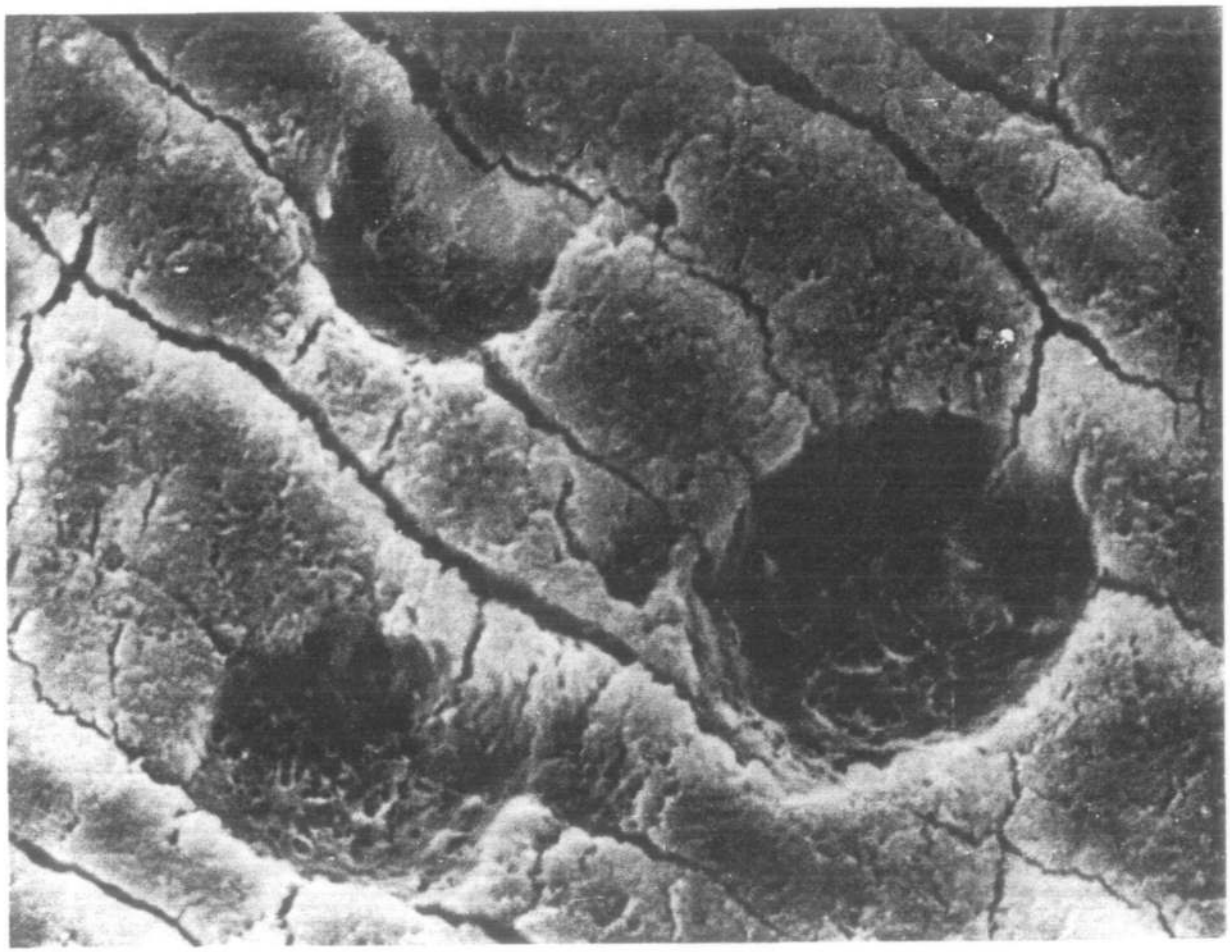

FIG. 3.—Photoelectromicrograph showing resorption pits on the dentine slice surface. This shows an area of dentine surface on which resorption pits were confirmed by light microscope. Lacunar resorption pits were seen on the dentine slice surface (×2500).

culture, and to cause fusion of TRAP-positive precursors into multinucleated osteoblasts [18–20]. IL-1 and IL-6 are now recognized as potent stimulators of osteoclastic bone resorption *in vivo* and *in vitro*, and also as potent stimulators of osteoclast-like cell formation in murine bone marrow cultures [21–24]. We also examined the pit formation assay in some cultured RA synovial cells in the presence of parathyroid hormone, 1,25-dihydroxyvitamin D_3, IL-1, IL-3 or IL-6. There is, however, no difference between samples with and without these factors in our examination of both TRAP staining and the dentine pit formation assay (data not shown).

Collins [25] and Grimley and Sokoloff [26] were the first to note giant cells in the rheumatoid synovium. Recently, Wilkinson *et al.* [10] have reported that there are two major groups of giant cells in the rheumatoid synovium, one of which shows TRAP activity and prominent expression of CD51(23C6). We also found that there are two major groups of multinuclear cells within isolated adherent synovial cells derived from the rheumatoid synovium, one which was characterized by strongly TRAP positive staining. This indicates that there are multinuclear cells in the rheumatoid synovium which satisfy several criteria for the identification of osteoclasts: namely, high TRAP activity, presence of the α v β 3 heterodimer of the vitronectin receptor and multinuclearity [14–16].

In the present study, we used a simple pit formation assay system established by Tamura *et al.* [15] to show that these isolated cells were also capable of bone resorption. This fulfilled the absolute functional criterion of lacunar bone resorption which indicates that these cells are osteoclasts. We used dentine slices instead of bone slices for the pit formation assay, since dentine has a homogeneous structure and is free of vascular canals and osteocyte lacunae, both of which are present in bone slices. We also used Mayer's haematoxylin to visualize resorption pits. Scanning electron microscopy has been used as one of the most reliable tools to identify and quantify resorption pits. However, we found a very small number of resorption pits formed by cells isolated from rheumatoid synovium and, therefore, many dentine slices had to be scanned to identify resorption pits in each RA synovial cell. To identify and quantify the resorption assay for many dentine slices, it is reasonable to use light microscopy before using scanning electron microscopy.

In conclusion, we have found that [1] there are strongly TRAP-positive multinuclear cells amongst

isolated rheumatoid synovial cells and that [2] these osteoclast-like cells can form resorption pits on dentine slices. A critical problem which needs to be resolved in further studying TRAP-positive multinuclear cells derived from rheumatoid synovium is the preparation of a large viable number of these TRAP-positive cells. The data obtained suggest that TRAP-positive multinuclear cells which infiltrate the rheumatoid synovium may be partly responsible for the bone destruction seen in RA patients, although the origin of these cells remains unclear.

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