Mod Rheumatol (2004) 14:123–129
DOI 10.1007/s10165-004-0278-5

ORIGINAL ARTICLE

Hiroyuki Mizumura · Shinji Nishihara · Yuji Kishimoto
Yasuo Morio · Ryota Teshima

Tartrate-resistant acid phosphatase-positive cells in the synovial–cartilage junction and bone marrow during the progression of collagen-induced arthritis in adult rats

Received: May 19, 2003 / Accepted: October 9, 2003

Abstract We investigated the time-course changes in bone destruction in rats with collagen-induced arthritis (CIA). The synovial–cartilage junction (SCJ) and epiphyseal bone marrow of the femoral posteromedial condyle in CIA rats were evaluated histologically and immunohistologically at 2, 3, 4, 6, and 8 weeks after sensitization. Two weeks after sensitization, tartrate-resistant acid phosphatase (TRAP)-positive multinuclear cells formed resorption lacunae on the lateral side of the cortical bone under the SCJ. No resorption lacunae were observed in bone marrow. Three weeks after sensitization, resorption lacunae on the lateral side of the cortical bone expanded, but no bone marrow invasion by pannus was observed. In bone marrow, many TRAP-positive multinuclear cells appeared and formed resorption lacunae in subchondral bone. Four weeks after sensitization, cortical bone was destroyed, and pannus had invaded the bone marrow. After six weeks, trabecular bone and subchondral bone plate were extensively resorbed by TRAP-positive cells. Bone destruction in CIA began with the appearance of TRAP-positive cells on the lateral side of the cortical bone under the SCJ, followed by the TRAP-positive multinuclear cells in bone marrow, which were morphologically unconnected to the SCJ lesions. These histological findings suggested that bone destruction in the early stage of arthritis occurred in two anatomically different regions.

Key words Bone destruction · Collagen-induced arthritis (CIA) · Osteoclast · Tartrate-resistant acid phosphatase (TRAP)

H. Mizumura (✉) · S. Nishihara · Y. Kishimoto · Y. Morio · R. Teshima
Department of Orthopedic Surgery, Faculty of Medicine, Tottori University, 36-1 Nishi-cho, Yonago 683-8504, Japan
Tel. +81-859-34-8115; Fax +81-859-34-8903
e-mail: masamizu@go2.enjoy.ne.jp

Introduction

Many histopathological studies of rheumatoid arthritis (RA) have been performed using human samples and experimental models.[1–4] The regions responsible for bone destruction have been localized to the synovial membrane or bone marrow (or both), but the detailed progression processes of bone destruction have not been clarified. Moreover, although bone destruction is thought to start in a bare area, no definitive proof has been obtained.

Collagen-induced arthritis (CIA) is used as an experimental animal model of RA because it induces lesions histopathologically similar to those seen with RA: chronic synovitis, pannus formation, and bone and cartilage destruction.[5,6] To clarify the initiation of bone destruction in RA, we investigated the time course of histological changes in CIA rats, focusing on tartrate-resistant acid phosphatase (TRAP) and proliferating cell nuclear antigen (PCNA) staining in the synovial–cartilage junction (SCJ) and epiphyseal bone marrow.

Materials and methods

Animals

Female Sprague-Dawley (SD) rats aged 24 weeks were used (Shimizu Laboratory Supply, Kyoto, Japan). Rats were given drinking water and food ad libitum during the study period. Tap water and CE-2 (CREA Japan, Tokyo, Japan) (calcium content 1.18 g/100 g, phosphorus 1.09 g/100 g, vitamin D_3 25 010 U/100 g) were given for drinking water and food, respectively. The experiment was carried out under the control of the committee in accordance with the Guidelines for Animal Experimentation, Faculty of Medicine, Tottori University and performed in Tottori University Animal Center controlled at 24°C (room temperature) with 12-h lighting between 7:00 a.m. and 7:00 p.m.

Induction of arthritis

According to the method reported by Trentham et al.,[6] an emulsion of 0.5 mg of bovine type II collagen (Cosmo Bio, Tokyo, Japan) and 0.5 ml of incomplete Freund's adjuvant (Difco, Detroit, MI, USA) was injected intracutaneously at the base of the rats' tails in the CIA group ($n = 35$). Half of this volume of the same emulsion was additionally injected 1 week later. The control group ($n = 10$) received the same volume of physiological saline.

Evaluation of arthritis

After the first sensitization, every week during the entire follow-up period the rats were assessed by one of us (H.M.) for signs of arthritis. The severity of the arthritis was graded on a 0–4 scale as follows: 0, normal; 1, swelling, redness, or both in one joint; 2, swelling, redness, or both in more than one joint; 3, swelling, redness, or both of the entire paw; 4, deformity, ankylosis, or both. Each paw was graded, and the scores for all four limbs were summed so the maximum score per rat was 16. Paw thickness was assessed using calipers to measure the thickness of the hind paw.

Tissue preparation

In the CIA group, groups of six rats with arthritis were randomly selected and killed 2, 3, 4, 6, and 8 weeks after the initial sensitization. In the control group, two animals were killed at each time point. After the rats were killed, bilateral knee joints were excised and fixed in 4% buffered paraformaldehyde/phosphate-buffered saline (PBS) (pH 7.4) at 4°C for 16 h, then decalcified in 10% ethylenediaminetetraacetic acid (EDTA) in PBS (pH 7.4) for 2 weeks. After decalcification, the specimens were embedded in paraffin. Serial sagittal sections (6 μm) of the knee joint were prepared using a microtome. The tissue sections were dried on silane-coated superfrost slide glasses (Matsunami Glass Industries, Osaka, Japan) at 37°C for 16 h and stained with hematoxylin and eosin (H&E), safranin O, TRAP, and immunostaining (labeled streptavidin-biotinylated antibody method). The femoral posteromedial condyle SCJ (area S) and epiphyseal bone marrow (area M) were observed (Fig. 1).

TRAP staining

According to the method reported by Suzuki et al.[7] deparaffinized tissue sections washed with PBS for 5 min were kept in PBS containing 1 mM magnesium and 1 mM calcium for 16 h to activate EDTA-inactivated TRAP, and the sections were washed with PBS for 10 min. Then, according to the method reported by Fujikawa et al.[8] the sections were stained with a solution (Sigma, St. Louis, MO, USA) containing acetate buffer (pH 5.0), naphthol AS-BI phosphate, Fast Garnet GBC, and 50 mM sodium tartrate at 37°C for 1 h. Harris hematoxylin solution (Sigma) was used for counterstaining.

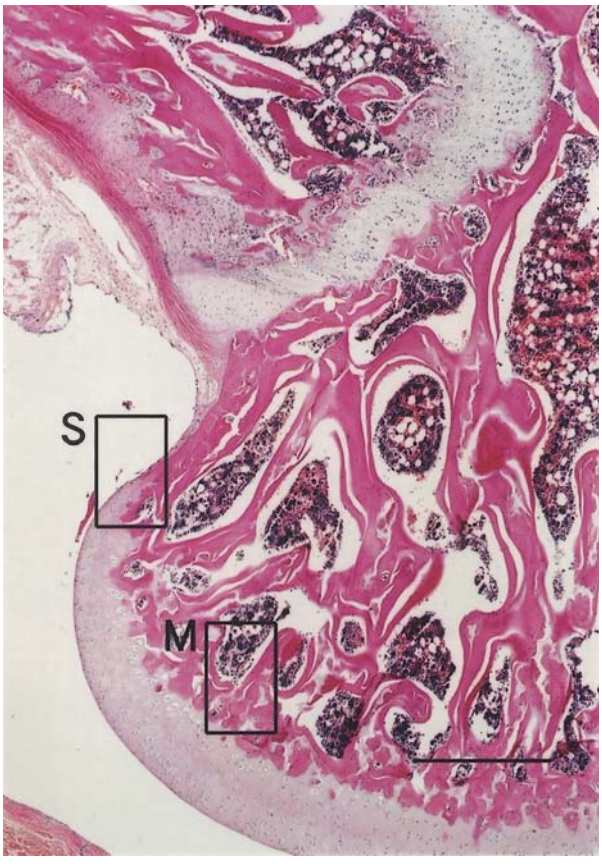

Fig. 1. Regions observed in the femoral posteromedial condyle. Areas *S* and *M* indicate synovial–cartilage junction and epiphyseal bone marrow, respectively. H&E, ×40. *Bar* 500 μm

Immunohistological staining

Staining with PCNA was performed according to the method reported by Suzuki et al.[9] Deparaffinized tissue sections were washed with PBS for 5 min and kept in 3% hydrogen peroxide solution for 15 min to block endogenous peroxidase. After washing with PBS for 5 min, the sections were kept in 10% normal rabbit serum at 37°C for 10 min, then reacted with the primary monoclonal anti-PCNA antibody (PC10, undiluted mouse anti-human antibody; Nichirei, Tokyo, Japan) at 4°C overnight. For the negative control, normal nonimmune mouse serum was used as the primary antibody. After reaction with antibody, staining was completed using a HISTOFINE SAB-PO kit and DAB (3.3-diaminobenzidine tetrahydrochloride) color development kit (Nichirei).

Evaluation of TRAP-positive cell counts

The TRAP-positive cells in the SCJ and bone marrow of the femoral posteromedial condyle were investigated to compare the number of TRAP-positive cells. Three tissue sec-

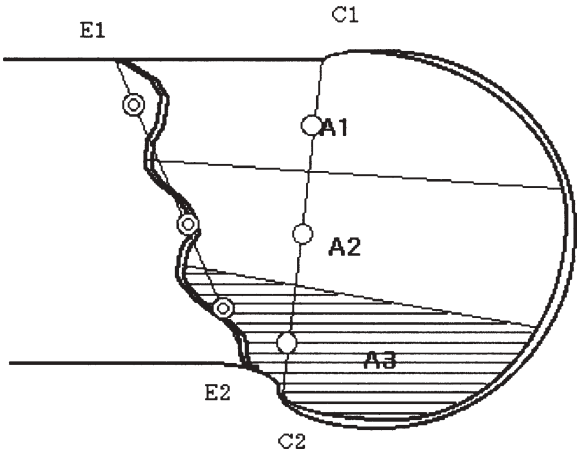

Fig. 2. Region of tartrate-resistant acid phosphatase (TRAP)-positive cell counts in epiphyseal bone marrow. Epiphyseal bone marrow is divided into three regions (*A1*, *A2*, *A3*) by the two lines connecting two points each equally divided lines *E1–E2* and *C1–C2* into three portions

tions were randomly selected from each knee joint containing the posteromedial condyle and anterior articular cartilage in the control group and the experimental groups at weeks 2, 3, and 4 after sensitization, and TRAP-positive cells in the SCJ were counted.

For counting TRAP-positive cells in bone marrow, the line connecting the intersection of the epiphyseal line and the anterior cortex (E1) to the intersection of the epiphyseal line and the posterior cortex (E2) was equally divided into three portions, and the two division points were connected to two points equally dividing into three the line connecting the anterior end (C1) with the posterior end (C2) of the articular cartilage; the bone marrow was thus divided into three regions: A1–A3. TRAP-positive cells in the A3 region were counted as the number of bone marrow TRAP-positive cells (Fig. 2).

The mean TRAP-positive cell counts in the SCJ and A3 region for three tissue sections were regarded as the TRAP-positive cell counts of each knee joint. Differences between the TRAP-positive cell counts in the SCJ and A3 region were compared in the CIA group for each week. The *t*-test was used for statistical analysis, and a *P* value of 0.05 or less was regarded as significant.

Results

Incidence of arthritis, arthritis score, hind paw thickness

Altogether, 33 (93%) of the 35 rats in the CIA group developed arthritis. Arthritis onset was 10 days after the initial sensitization. The arthritis score gradually increased and reached a plateau during the fourth week. The hind paw thickness reached its maximum during the fourth week and then decreased. This course of arthritis was constant, and no difference was observed among the animals (Fig. 3).

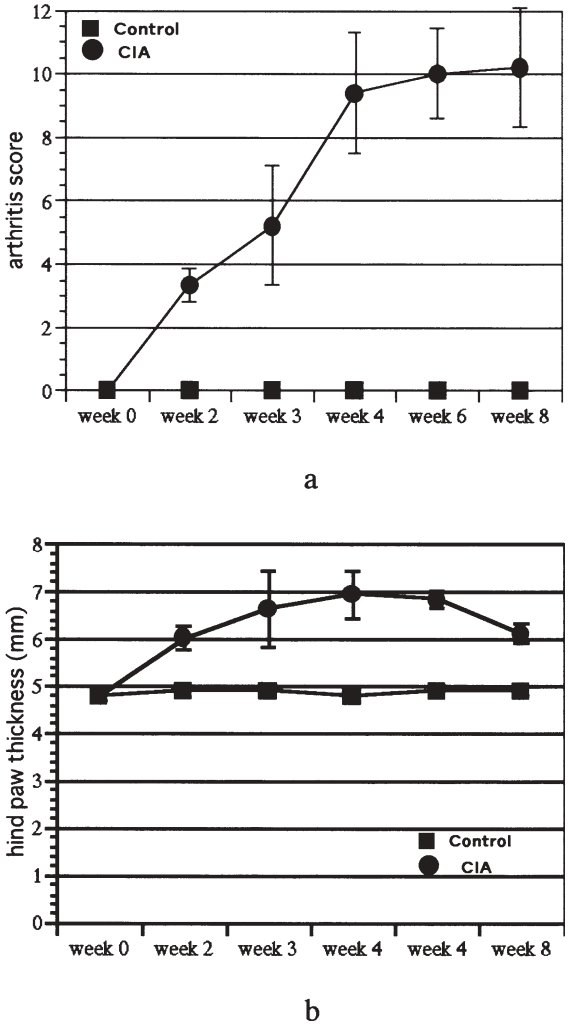

Fig. 3. Arthritis score and hind paw thickness in each group (*weeks 0–8*). The arthritis score gradually increased and reached the plateau during the fourth week (**a**). The hind paw thickness reached a maximum during the fourth week and then decreased (**b**)

Histological and immunohistological findings

Control group

In all 10 control animals, a monolayer of fibroblast-like (Fb) cells lining the articular cavity side and fibrous tissue comprised the SCJ. A few Fb cells were PCNA-positive (Fig. 4a). No TRAP-positive cells were noted in the synovial membrane, cartilage, bone, or epiphyseal bone marrow. The articular cartilage structure was normal, and the middle to deep layers were homogenously stained with safranin O.

CIA group

Week 2 group. By week 2, the Fb cells in the CIA group increased on the articular cavity side of the SCJ and formed four- or five-cell layers. Intercellular fibrous tissues hypertrophied, forming pannus, and began to cover the articular cartilage surface. Most of the cells forming the pannus were Fb cells, with a few lymphocytes and plasma cells. Most Fb

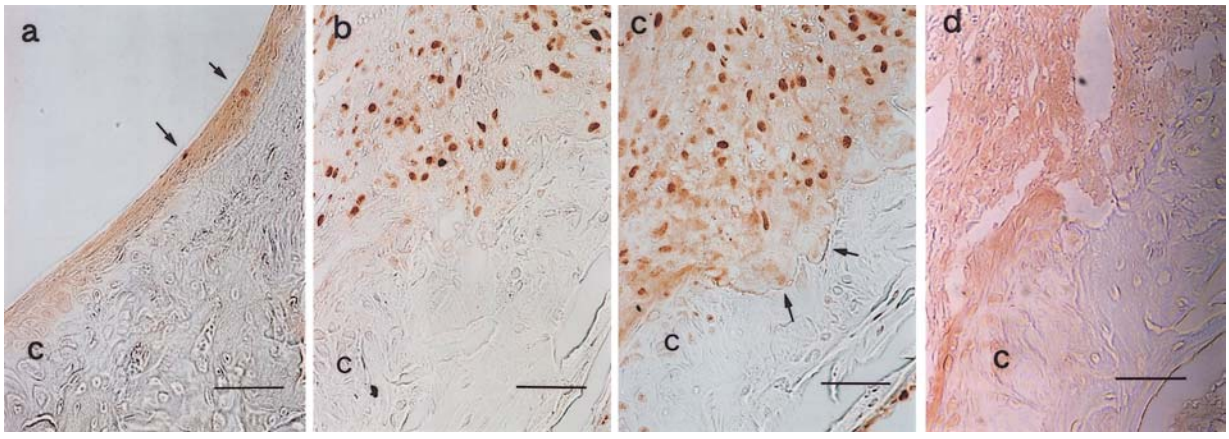

Fig. 4. Sections of synovial–cartilage junctions from control rats (**a**) and CIA rats 2 weeks (**b**) and 3 weeks (**c**) after sensitization. The negative control (**d**) reacted with the normal nonimmune mouse serum. C, cartilage. Proliferating cell nuclear antigen (PCNA) stain, ×400, *bar* 50 μm. **a** There are few PCNA-positive cells (*arrows*) among Fb cells from a control rat. **b** Note the many PCNA-positive cells in the pannus in CIA rats 2 weeks after sensitization. **c** There are many PCNA-positive cells and PCNA-negative giant cells (*arrows*) on the lateral side of cortical bone from CIA rats 3 weeks after sensitization. **d** There are no PCNA-positive cells in the pannus from CIA rats 2 weeks after sensitization in the negative control

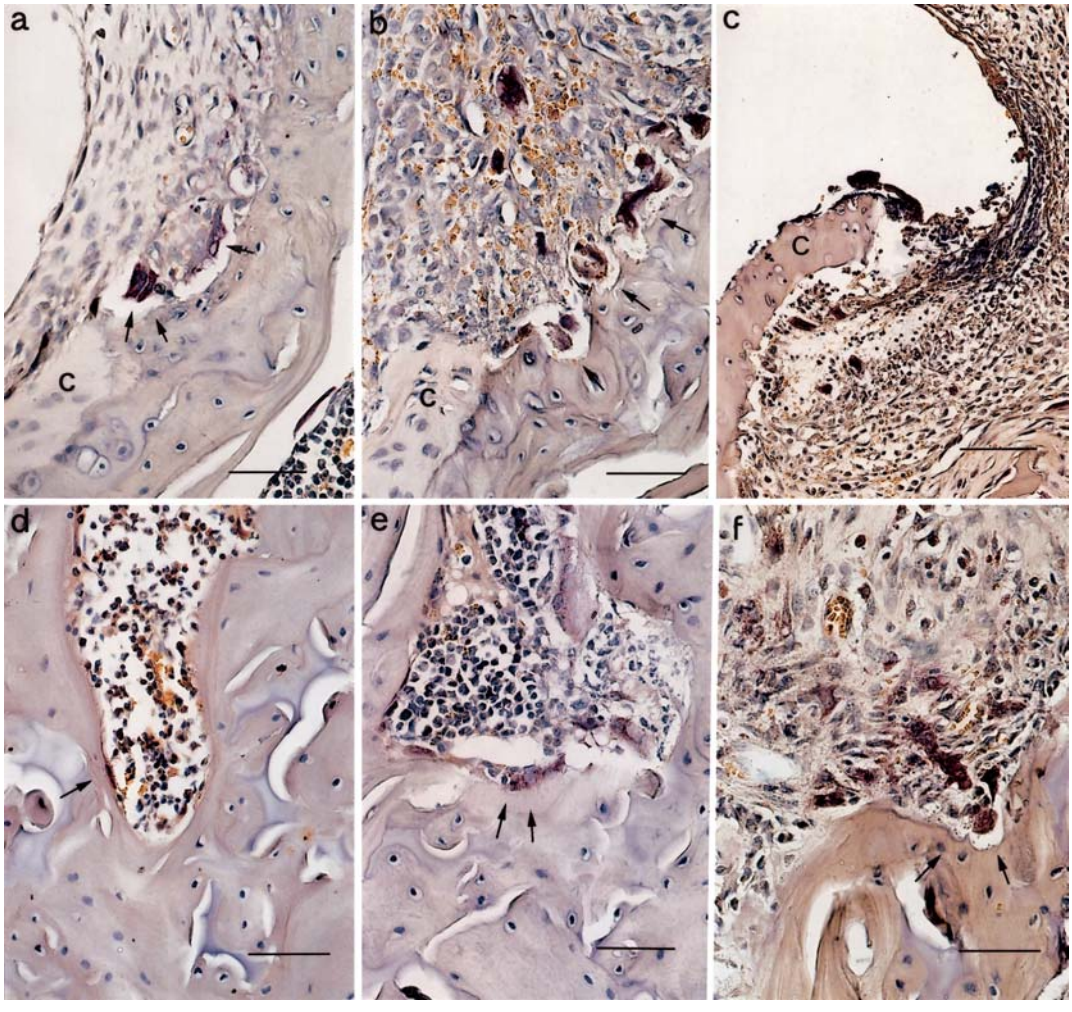

Fig. 5. Synovial–cartilage junctions (**a**, **b**, **c**) and epiphyseal bone marrow (**d**, **e**, **f**) from CIA rats 2 weeks (**a**, **d**), 3 weeks (**b**, **e**), and 4 weeks (**c**, **f**) after sensitization. **a** TRAP-positive multinuclear cells and resorption lacunae (*arrows*) on the lateral side of cortical bone. *C*, cartilage. **b** TRAP-positive cells form resorption lacunae on the lateral side of cortical bone (*arrows*). **c** Pannus destroys cortical bone and invades the bone marrow. **d** TRAP-positive cells in bone marrow are mononuclear and spindle-shaped (*arrow*). No resorption lacunae are seen. **e** TRAP-positive multinuclear cells form shallow resorption lacunae in the bone marrow (*arrows*). **f** Many TRAP-positive cells morphologically unconnected to the pannus form deep resorption lacunae in the bone marrow (*arrows*). **a**, **b**, **d**, **e**, **f**: TRAP stain, ×400, *bar* 50 μm. **c**: TRAP stain, ×200, *bar* 100 μm

cells were PCNA-positive. Many capillary vessels formed in the pannus. TRAP-positive cells appeared in the region adjacent to the lateral surface of the SCJ cortical bone, forming shallow resorption lacunae. Morphologically, the TRAP-positive cells were large multinuclear cells with an irregular contour (Fig. 5a), and they were PCNA-negative (Fig. 4b). No PCNA-positive cells were observed in negative control tissue (Fig. 4d), nor were they seen in capillary vessels or regions adjacent to cartilage in the pannus.

In the epiphyseal bone marrow cavity, TRAP-positive cells were observed in the region close to the subchondral bone plate; however, there were fewer TRAP-positive cells than in the SCJ, and no resorption lacunae were formed. Most of these cells were spindle-shaped mononuclear cells with a morphology different from that of TRAP-positive cells on the lateral surface of cortical bone (Fig. 5d). No TRAP-positive cells were observed in the central bone marrow cavity or on the epiphyseal growth cartilage side.

Slight irregularity and shallow ulceration were observed on the articular cartilage surface covered by pannus. Proliferating Fb cells covered the surface of the ulcer. The safranin O staining intensity in the articular cartilage was similar to that in the controls.

Week 3 group. At week 3, PCNA-positive Fb cells had grown further in the pannus and were uniformly distributed (Fig. 4c). There were many plasma cells in the pannus. TRAP-positive multinuclear cells increased on the lateral surface of cortical bone. These cells widely destroyed the lateral surface of cortical bone, but no cells invaded the bone marrow cavity (Fig. 5b).

Many TRAP-positive cells appeared over the epiphyseal bone marrow cavity. Subchondral TRAP-positive cells were large multinuclear cells and formed resorption lacunae in the subchondral bone plate and trabecular bone (Fig. 5e). TRAP-positive cells were also observed in the central bone marrow cavity and trabecular bone near the epiphyseal growth cartilage, but many of these cells were spindle-shaped and mononuclear, forming no resorption lacuna.

The area of destruction of the noncalcified zone in the margin of the articular cartilage was wider and deeper than at week 2. The safranin O staining intensity in the noncalcified layer of articular cartilage decreased. The pannus that destroyed the noncalcified cartilage were adjacent to the exposed calcified zone with many TRAP-positive cells on the surface, showing resorption of the calcified zone.

Week 4 group. Pannus had destroyed the SCJ cortical bone and invaded the bone marrow at week 4. The density of PCNA-positive Fb cells in the pannus was similar to that at week 3, and the cells were distributed uniformly. There were many TRAP-positive multinuclear cells at the tips of the pannus, forming resorption lacunae in trabecular bone. The subchondral bone plate in the marginal region of the articular cartilage was also destroyed by TRAP-positive cells on the pannus surface (Fig. 5c).

There were more TRAP-positive cells resorbing the subchondral bone plate and trabecular bone in bone marrow than in the samples prepared 3 weeks after sensitization (Fig. 5f). These TRAP-positive cells were unconnected to pannus. Resorption lacunae were also formed in trabecular bone near the epiphyseal growth cartilage. Most TRAP-positive cells were multinuclear, and there were few mononuclear cells.

Pannus covered the entire surface of the articular cartilage. Most of the noncalcified zone was lost, and the calcified zone remained. However, the calcified zone was being resorbed from the marginal region by TRAP-positive multinuclear cells.

Week 6 and week 8 groups. At weeks 6 and 8, pannus had widely invaded the subchondral bone marrow cavity. PCNA-positive cells in the pannus had decreased compared to those in the week 4 samples. Resorption lacunae were observed in trabecular bone near the epiphyseal growth cartilage, but the trabecular structure remained. The calcified zone of articular cartilage slightly remained in the central region. Many TRAP-positive multinuclear cells were seen on the bone marrow side of the residual calcified zone, forming resorption lacunae. No TRAP-positive cells were observed on the articular cavity side.

Histological findings at each week after sensitization remained constant among the arthritis groups. There were no differences among the animals.

TRAP-positive cell counts in the SCJ and bone marrow

No TRAP-positive cells were observed in the synovial membrane, articular cartilage, or epiphyseal bone marrow cavity of the control group. There were significantly more TRAP-positive cells on the surface of hypertrophic

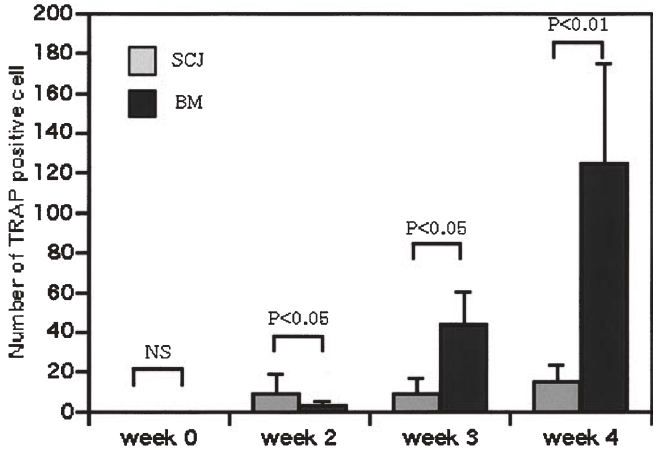

Fig. 6. TRAP-positive cell counts in synovial–cartilage junction (*SCJ*) and epiphyseal bone marrow (*BM*) each week after sensitization. There were significantly more TRAP-positive cells on the surface of the pannus in the synovial–cartilage junction than in the A3 bone marrow region 2 weeks after sensitization ($P < 0.05$). After week 3, there were significantly more TRAP-positive cells in the bone marrow cavity than on the surface of the SCJ pannus, and the difference was more marked during week 4 than week 3

pannus in the SCJ than in the A3 bone marrow region 2 weeks after sensitization ($P < 0.05$). After week 3, there were significantly more TRAP-positive cells in the bone marrow cavity than on the surface of the SCJ pannus, and the difference was more marked at week 4 than at week 3 (Fig. 6).

Discussion

Time-course changes in the SCJ and epiphyseal bone marrow of the femoral posteromedial condyle were observed in this study with regard to bone destruction in adult rats with CIA. Few studies[10,11] have investigated bone destruction over time in CIA, and most such studies have been performed in young animals.[10] The epiphysis grows in young animals, and so osteoclasts appear during the bone modeling process. Adult rats were used in this study to avoid changes due to bone modeling during the growth period. No TRAP-positive cells were observed in the epiphyseal bone marrow cavity in the control group during the observation period. The femoral posteromedial condyle was selected for observation because SCJ is histologically easy to identify, and bone erosion frequently develops in this region in patients with RA.[12,13]

The bone destruction process in CIA observed in this study may be summarized as follows. During the early stage of CIA, pannus formation occurred in the SCJ. The pannus consisted of many PCNA-positive fibroblast-like cells and a few TRAP-positive multinuclear cells, the latter of which were negative for PCNA staining.

Bone destruction began with the appearance of TRAP-positive multinuclear cells, forming resorption lacunae on the lateral surface of the cortical bone under the SCJ 2 weeks after sensitization. TRAP-positive cells were also observed around the subchondral trabecular bone in the epiphyseal bone marrow at the same time point, but there were fewer of these cells than in the SCJ and no resorption lacunae were formed. At week 3 after sensitization, destruction of the lateral surface of the cortical bone had expanded, but there was no bone marrow invasion by pannus. In contrast, more TRAP-positive multinuclear cells appeared in the epiphyseal bone marrow than in the SCJ, and the cells formed resorption lacunae in the subchondral bone plate and trabecular bone.

At week 4 after sensitization, the cortical bone was destroyed by pannus containing TRAP-positive cells at the tip, and the pannus had invaded the bone marrow. Later, the trabecular bone and subchondral bone plate in the epiphyseal bone marrow were destroyed by TRAP-positive cells at the tip of the pannus and TRAP-positive cells unconnected with the pannus. Only calcified cartilage remained by weeks 6 and 8.

Chew et al.[14] reported that histological investigation of adjuvant arthritis in rabbits showed that edema developed in the synovial membrane, multinuclear leukocytes appeared, synovial cells grew, and osteoclasts and osteoblasts were observed in the granulation tissue adjacent to cortical bone in the SCJ during the early stage. Kuratani et al.[15] found TRAP-positive cells of the outer bone surface and in the bone marrow 4 weeks after sensitization in adjuvant arthritic rats. The findings in the above two reports were consistent with those observed in CIA rats, and the findings in the report of Chew et al. absolutely corresponded to those observed 2 weeks after sensitization in our study: synovial cell growth and appearance of TRAP-positive cells on the lateral side of cortical bone.

Toritsuka et al.[16] reported that differentiation of undifferentiated cells to osteoclasts was promoted in RA bone marrow in humans, and Nakagawa et al.[4] reported that bone resorption in the SCJ was related to abnormal stromal cells that migrated from epiphyseal bone marrow into the joint through the canal in cortical bone in a bare area in CIA rats.

If preceding lesions are present in bone marrow, as in these reports, TRAP-positive cells may appear first in bone marrow, with bone destruction possibly beginning on the bone marrow side. However, at 2 weeks after sensitization, when TRAP-positive cells on the lateral side of the cortical bone were still multinuclear and formed resorption lacunae, TRAP-positive cells in the epiphyseal bone marrow were mononuclear and formed no resorption lacuna, suggesting that bone destruction in the SCJ preceded bone destruction in the bone marrow.

Bromley and Woolley[17] reported that osteoclasts without continuity to pannus destroy subchondral bone. This finding was consistent with our finding that TRAP-positive cells appeared in bone marrow and began resorbing the subchondral bone plate and trabecular bone before pannus destroyed the cortical bone and entered the bone marrow. This suggested that bone destruction at an early stage of CIA occurred in two anatomically different regions: the lateral side of the cortical bone and the bone marrow.

This study was unable to determine the origin of osteoclasts that appeared on the lateral side of the cortical bone. Fassbender et al.[18,19] searched for the origin in undifferentiated mesenchymal cells of synovial membrane tissue, and Fujikawa et al.[8] and Takayanagi et al.[20] assumed that the origin was synovial membrane A cells, which they based on their findings after culturing the cells in the presence of osteoclast differentiation factor. Furthermore, Fujikawa et al.[21] reported that peripheral blood monocytes differentiate to osteoclasts in culture.

There are various hypotheses regarding the destruction of articular cartilage, including involvement of cytokines and proteases produced in hypertrophied synovial membrane and apoptosis of chondrocytes. There have been few reports, however, on the destruction of calcified cartilage. Bromley and Woolley[17] reported that calcified cartilage was destroyed by chondroclasts with the same morphological and histochemical properties as osteoclasts. Resorption of calcified cartilage by TRAP-positive multinuclear cells was observed in this study, which was consistent with Bromley and Woolley's finding. Calcified cartilage remained until the terminal stage of joint destruction, but it is not clear whether this finding indicates high resistance of calcified cartilage to resorption or if it is derived from the anatomical

property that calcified cartilage is located between non-calcified cartilage and the subchondral bone plate.

Conclusions

Bone destruction in CIA began with the appearance of TRAP-positive cells on the lateral side of the cortical bone, followed by the appearance of TRAP-positive multinuclear cells in the bone marrow, which were morphologically unconnected to the SCJ lesions. These histological findings suggested that bone destruction during the early stage of CIA occurred in two anatomically different regions.

Acknowledgments Part of this study was supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (no. 13671509) and a grant from the Japan Orthopaedics and Traumatology Foundation, Inc. (no. 0125). We thank Drs. D. Yamasaki, M. Enokida, and K. Nawata for their technical help.

References

1. Allard SA, Muirden KD, Maini RN. Correlation of histopathological features of pannus with patterns of damage in different joints in rheumatoid arthritis. Ann Rheum Dis 1991;50:278–83.
2. Bromley M, Woolley DE. Histopathology of the rheumatoid lesion. Arthritis Rheum 1984;27:8:857–63.
3. Shiozawa S, Shiozawa K, Fujita T. Morphologic observations in the early phase of cartilage-pannus junction. Arthritis Rheum 1983;26:4:472–8.
4. Nakagawa S, Toritsuka Y, Wakitani S, Denno K, Tomita T, Owaki H, et al. Bone marrow stromal cells contribute to synovial cell proliferation in rats with collagen induced arthritis. J Rheumatol 1996;23:2098–103.
5. Durie FH, Fava RA, Noelle RJ. Collagen-induced arthritis as a model of rheumatoid arthritis. Clin Immunol Immunopathol 1994;73:11–8.
6. Trentham DE, Townes AS, Kang AH. Autoimmunity to type II collagens: an experimental model of arthritis. J Exp Med 1977;146:857–68.
7. Suzuki Y, Nishikaku F, Nakatsuka M, Koga Y. Osteoclast-like cells in murine collagen induced arthritis. J Rheumatol 1998;25:6:1154–60.
8. Fujikawa Y, Shingu M, Torisu T, Itonaga I, Masumi M. Bone resorption by tartrate-resistant phosphate-positive multinuclear cells isolated from rheumatoid synovium. Br J Rheumatol 1996; 35:213–7.
9. Suzuki K, Katoh R, Kawaoi A. Immunohistochemical demonstration of proliferating cell nuclear antigen (PCNA) in formalin-fixed, paraffin-embedded sections from rat and human tissues. Acta Histochem Cytochem 1992;25:13–21.
10. Hayashida K, Ochi T, Fujimoto M, Owaki H, Shimaoka Y, Ono K, et al. Bone marrow changes in adjuvant-induced and collagen induced arthritis. Arthritis Rheum 1992;35:2:241–5.
11. Smith RJ, Sly LM. Type II collagen-induced arthritis in the diabetic-resistant biobreeding rat: inflammatory and histopathological features of joint pathology and effects of anti-inflammatory and antirheumatic drugs on this chronic arthritis process. J Pharmacol Exp Ther 1996;277:3:1801–13.
12. Resnick D, Niwayama G. Rheumatoid arthritis. In: Manke D, editor. Diagnosis of bone and joint disorders. 2nd ed. Philadelphia: Saunders; 1988. p. 954–1067.
13. Murray RO, Jacobson HG. Collagen diseases, arthritides and soft tissue abnormalities. In: The radiology of skeletal disorders. 2nd ed. New York: Churchill Livingstone; 1977. p. 810–23.
14. Chew MWK, Henderson B, Edwards JCW. Antigen-induced arthritis in the rabbit: ultrastructural changes at the chondrosynovial junction. Int J Exp Pathol 1990;71:879–94.
15. Kuratani T, Nagata K, Kurita T, Hotokebuchi T, Nakashima A, Iijima T. Induction of abundant osteoclast-like multinucleated giant cells in adjuvant arthritic rats with accompanying disordered high bone turnover. Histol Histopathol 1998;13:751–9.
16. Toritsuka Y, Nakamura N, Lee SB, Hashimoto J, Yasui N, Shino K, et al. Osteoclastogenesis in iliac bone marrow of patients with rheumatoid arthritis. J Rheumatol 1997;5:1690–6.
17. Bromley M, Woolley DE. Chondroclasts and osteoclasts at subchondral sites of erosion in the rheumatoid joint. Arthritis Rheum 1984;27:9:968–75.
18. Fassbender HG. The potential aggressiveness of synovial tissue in rheumatoid arthritis. J Pathol 1983;139:399–406.
19. Fassbender HG. Histomorphological basis of articular cartilage destruction in rheumatoid arthritis. Collagen Relat Res 1983;3: 141–55.
20. Takayanagi H, Oda H, Yamamoto S, Kawaguchi H, Tanaka S, Nishikawa T, et al. A new mechanism of bone destruction in rheumatoid arthritis: synovial fibroblasts induce osteoclastogenesis. Biochem Biophys Res Commun 1997;240:279–86.
21. Fujikawa Y, Quinn J, Sabokbar A, McGee JO, Athanasou NA. The human osteoclast precursor circulates in the monocyte fraction. Endocrinology 1996;137:4058–60.