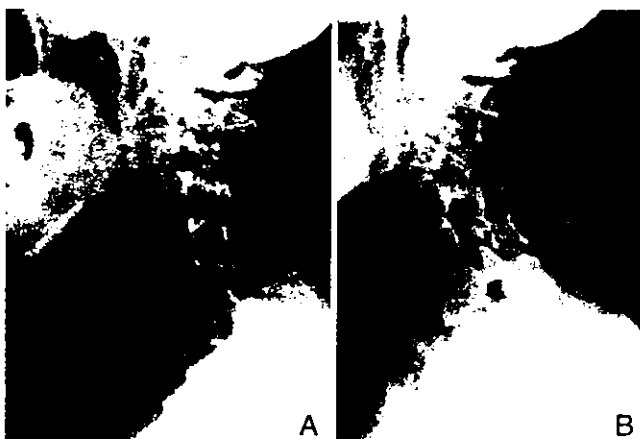FIG. 2. Lateral radiographs obtained in a 55-year-old woman with nonmutilating-type RA. A: Preoperative radiograph revealing subaxial lesions, such as vertebral slippage at C3–4, facet joint erosion, endplate erosion, and spinous process erosion. Vertical subluxation was stabilized. B: Five years after laminoplasty, deterioration of vertebral slippage was not found. Neurological impairment had improved from Ranawat Class IIIA to Class II.

seems unrelated to the number of fusion levels. In most patients, other than those with mutilating-type RA, not only was radiological stability achieved after laminoplasty but sufficient mobility in the cervical spine remained for daily life (Fig. 2).

Even if subaxial subluxation is not so severe at surgery, it is often progressive irrespective of treatment in patients with mutilating-type RA. Oda, et al.,[19] stated that the RA subset reported by Ochi, et al.,[18] represents a good indicator of progression of subluxation at both upper and subaxial cervical regions, and that the mutilating-type RA group is at high risk for development of subaxial subluxation. In our study, deterioration of slippage and postoperative changes to cervical alignment were found significantly more often in the group with mutilating-type RA than in the other. In the group with nonmutilating-type RA, 17 (94%) of 18 patients experienced improved myelopathy and no recurrence of neurological deterioration, whereas five (42%) of 12 patients with mutilating-type RA experienced poor results. Intergroup clinical outcome thus differed significantly. Significant deterioration of cervical alignment due to increased slippage or vertebral collapse requiring revision surgery was noted in three patients (27%) in the mutilating-type RA group. This deterioration of alignment was the main reason for poor results in these patients. Although preoperative radiologically documented disorders in patients with both mutilating- and nonmutilating-type RA were relatively mild, mutilating-type RA was associated with a significantly higher rate of deterioration of subaxial subluxation after laminoplasty.

The study population did not represent all patients who had undergone surgery for subaxial lesions related to RA. Many patients with severe subaxial lesions underwent posterior spinal fusion and placement of instrumentation across the entire cervical spine and were not enrolled in the present study. Retrospectively, we found vertebral slippage of less than or equal to 5 mm at only one or two levels in most patients. In these patients, nonmutilating-

type RA was associated with good results, whereas mutilating-type RA was associated with poor results. We therefore believe that patients with nonmutilating-type RA can benefit from laminoplasty for myelopathy due to mild subaxial lesions. Conversely, good laminoplasty-related results in patients with mutilating-type RA should not be expected, even if radiological changes appear trivial before surgery.

Conclusions

Based on our results, we found that most patients with RA, except those with mutilating-type disease, can benefit from laminoplasty for subaxial lesions in which the degree of slippage is less than or equal to 5 mm in only one or two levels. Neurological improvements and preserved neck ROM ensure high quality of life in patients with RA for a long period. Radiological changes were also trivial. Laminoplasty can be undertaken more widely for compression-related myelopathy associated with subaxial lesions in patients with nonmutilating-type RA.

Disclaimer

References

1. Agarwal AK, Peppelman WC, Kraus DR, et al: Recurrence of cervical spine instability in rheumatoid arthritis following previous fusion: can disease progression be prevented by early surgery? J Rheumatol 19:1364–1370, 1992

2. Baggenstoss AH, Bickel WH, Ward LE: Rheumatoid granulomatous nodules as destructive lesions of vertebra. J Bone Joint Surg Am 34:601–609, 1952

3. Bland JH: Rheumatoid arthritis of the cervical spine. J Rheumatol 1:319–342, 1974

4. Boden SD, Dodge LD, Bohlman HH, et al: Rheumatoid arthritis of the cervical spine. A long-term analysis with predictors of paralysis and recovery. J Bone Joint Surg Am 75:1282–1297, 1993

5. Cabot A, Becker A: The cervical spine in rheumatoid arthritis. Clin Orthop 131:130–140, 1978

6. Clark CR, Goetz DD, Menezes AH: Arthrodesis of the cervical spine in rheumatoid arthritis. J Bone Joint Surg Am 71: 381–392, 1989

7. Conaty JP, Mongan ES: Cervical fusion in rheumatoid arthritis. J Bone Joint Surg Am 63:1218–1227, 1981

8. Eulderink F, Meijers K: Pathology of the cervical spine in rheumatoid arthritis: a controlled study of 44 spines. J Pathol 120:91–108, 1976

9. Fujiwara K, Yonenobu K, Ochi T: Natural history of upper cervical lesions in rheumatoid arthritis. J Spinal Disord 10: 275–281, 1997

10. Heywood AW, Learmonth ID, Thomas M: Cervical spine instability in rheumatoid arthritis. J Bone Joint Surg Br 70: 702–707, 1988

11. Itoh T, Tsuji H: Technical improvements and results of laminoplasty for compressive myelopathy in the cervical spine. Spine 10:729–736, 1985

12. Iwasaki M, Kawaguchi Y, Kimura T, et al: Long-term results of expansive laminoplasty for ossification of the posterior longitudinal ligament of the cervical spine: more than 10 years follow up. J Neurosurg 96:180–189, 2002

13. Kubo S, Goel VK, Yang SJ, et al: Biomechanical evaluation of

cervical double-door laminoplasty using hydroxyapatite spacer. **Spine 28**:227–234, 2003

14. Kudo H, Iwano K: Surgical treatment of subaxial cervical myelopathy in rheumatoid arthritis. **J Bone Joint Surg Br 73:** 474–480, 1991
15. Kurokawa T, Tsuyama N, Tanaka H: [Enlargement of spinal canal by the sagittal splitting of the spinal process.] **Bessatsu Seikeigeka 2:**234–240, 1984 (Jpn)
16. Lipson SJ: Rheumatoid arthritis in the cervical spine. **Clin Orthop 239:**121–127, 1989
17. Matsunaga S, Sakou T, Nakanisi K: Analysis of the cervical spine alignment following laminoplasty and laminectomy. **Spinal Cord 37:**20–24, 1999
18. Ochi T, Iwase R, Yonemasu K, et al: Natural course of joint destruction and fluctuation of serum C1q levels in patients with rheumatoid arthritis. **Arthritis Rheum 31:**37–43, 1988
19. Oda T, Fujiwara K, Yonenobu K, et al: Natural course of cervical spine lesions in rheumatoid arthritis. **Spine 20:**1128–1135, 1995
20. Olerud C, Larsson BE, Rodriguez M: Subaxial cervical spine subluxation in rheumatoid arthritis. A retrospective analysis of 16 operated patients after 15 years. **Acta Orthop Scand 68:** 109–115, 1997
21. Ranawat CS, O'Leary P, Pellici P, et al: Cervical spine fusion in rheumatoid arthritis. **J Bone Joint Surg Am 61:**1003–1010, 1979
22. Redlund-Johnell I, Pettersson H: Subaxial antero-posterior dislocation of the cervical spine in rheumatoid arthritis. **Scand J Rheumatol 14:**355–363, 1985
23. Santavirta S, Konttinen YT, Sandelin J, et al: Operations for the unstable cervical spine in rheumatoid arthritis. Sixteen cases of subaxial subluxation. **Acta Orthop Scand 61:**106–110, 1990
24. Satomi K, Nishu Y, Kohno T, et al: Long-term follow-up studies of open-door expansive laminoplasty for cervical stenotic myelopathy. **Spine 19:**507–510, 1994
25. Winfield J, Cooke D, Brook AS, et al: A prospective study of the radiological changes in the cervical spine in early rheumatoid disease. **Ann Rheum Dis 40:**109–114, 1981
26. Yonezawa T, Tsuji H, Matsui H, et al: Subaxial lesions in rheumatoid arthritis. Radiographic factors suggestive of lower cervical myelopathy. **Spine 20:**208–215, 1995

ORIGINAL ARTICLE

Preferential reductions of paraarticular trabecular bone component in ultradistal radius and of calcaneus ultrasonography in early-stage rheumatoid arthritis

Masaaki Inaba · Mayumi Nagata · Hitoshi Goto
Yasuro Kumeda · Keisuke Kobayashi
Kiyoshi Nakatsuka · Takami Miki · Shinsuke Yamada
Eiji Ishimura · Yoshiki Nishizawa

Abstract Rheumatoid arthritis (RA) is a major cause of secondary osteoporosis and is frequently associated with both paraarticular and generalized osteoporosis. The present study was designed to investigate the preferential sites of reduction of bone mineral density (BMD), in the early stage of RA, with special emphasis on the differential effect of RA on BMD in trabecular and cortical components. The participants (30 RA patients and 26 healthy participants) were all female with disease duration of less than 1 year. BMD in the radius was measured at 4% (ultradistal site) and 20% (midshaft) to the ulnar length proximal to the end of radius by peripheral quantitative computed tomography. BMD in lumbar spine was measured by dual X-ray absorptiometry and the osteo-sono assessment index (OSI) of the calcaneus by ultrasound. RA patients showed lower BMD preferentially in the trabecular component, but not in cortical bone component of the ultradistal radius than age-matched normal controls. Calcaneus OSI was also significantly reduced. The radial midshaft and lumbar spine did not differ significantly between RA patients and normal controls. Trabecular BMD in the ultradistal radius exhibited negative correlations with serum CRP, ESR, and RF, and calcaneus OSI with M-HAQ score. In conclusion, it was suggested that disease activity of RA and impairment of daily physical activity might be a significant determinant of deterioration of bone structure in paraartciular distal radius and calcaneus, respectively, in early-stage RA patients.

M. Inaba (✉) · M. Nagata · H. Goto · Y. Kumeda
K. Kobayashi · K. Nakatsuka · T. Miki · S. Yamada
E. Ishimura · Y. Nishizawa
Department of Metabolism, Endocrinology
and Molecular Medicine,
Osaka City University Graduate School of Medicine,
1-4-3, Asahi-machi, Abeno-ku, 545-8585 Osaka, Japan
E-mail: inaba-m@med.osaka-cu.ac.jp
Tel.: +81-6-66453806
Fax: +81-6-66453808

Introduction

Rheumatoid arthritis (RA) is frequently associated with paraartciular and generalized osteoporosis, although the exact mechanism is as yet unknown [1, 2]. We have reported that several mechanisms are involved in the development of osteoporosis in RA patients [3, 4, 5]. One of the major mechanisms of paraarticular osteoporosis involves increased release into affected joints of inflammatory cytokines with bone-resorptive activity, such as interleukin 1 (IL-1), interleukin 6 (IL-6), and tumor necrosis factor (TNF) [4, 5, 6, 7, 8]. Malnutrition [9], impairment of physical activity [10], and drugs for RA such as steroids [11] and methotrexate [12] may play an important role in the development of generalized osteoporosis.

Since several mechanisms contribute to the development of osteoporosis in RA and the importance of each mechanism in the development of osteoporosis at each site may differ, it is possible that reduction of bone mass in RA patients may develop at different sites with different kinetics. Previous study reported that loss of trabecular bone in the distal radius was more rapid than those of the radial midshaft or lumbar spine in earlier stages of RA, although its mechanism and the data on changes in cortical bone component were not known exactly [13]. The clinical application of peripheral quantitative computed tomography (pQCT) made it possible to measure volumetric bone densities of total, trabecular, and cortical bone components separately in the radius [14]. We measured bone mineral density (BMD) in the radius at 4% (ultradistal site) and 20% (midshaft) to the ulnar length proximal to the end of radius by pQCT, as indexes of paraarticular and gen-

eralized osteoporosis [13, 14]. Furthermore, lumbar spine (L2-L4) BMD and calcaneus osteo-sono-assessment index (OSI) were measured as indexes of generalized osteoporosis.

This background prompted us to determine the preferential site of reduced BMD in early RA patients among ultradistal and midshaft of radius, lumbar spine, and calcaneus, and its mechanism, particularly focusing on the different effect of RA on the trabecular and cortical components.

Patients and methods

Participants

The participants were all female and consisted of 30 RA patients and 26 healthy controls, from whom written informed consent was obtained. The study was approved by Ethics Committee of Osaka City University Hospital. The RA patients were recruited consecutively from the patients of the Outpatient Clinic of Rheumatology at Osaka City University Hospital and were diagnosed according to the 1987 revised American Rheumatism Association criteria [15]. As controls, we selected 26 healthy participants from people who participated in a local health check program at the Osaka City University Hospital. The mean ages of the RA patients and healthy controls were 52.1 ± 11.9 years and 54.4 ± 11.3 years, respectively. The duration of RA was less than 1 year.

To avoid confounding by other known risk factors of osteoporosis, we selected participants using the following exclusion criteria: (1) smoking, (2) renal disease possibly causing secondary hyperparathyroidism, (3) endocrine disorders, (4) liver disease, (5) malnutrition (serum albumin < 3.0 g/dl), (6) the administration of any medication including steroid and methotrexate that might affect bone and mineral metabolism, and (7) severely disabled patients unable to walk unassisted.

Serum samples were collected from the 30 RA patients and relevant laboratory variables measured (blood sedimentation rate, blood concentration of hemoglobin [Hb], platelets, serum concentrations of C-reactive protein [CRP], rheumatoid factor [RF], immunoglobulin G [IgG], IgA and IgM, and albumin). All of the 30 RA patients were receiving multiple medication, with 21 patients taking nonsteroidal anti-inflammatory drugs (NSAIDs), 9 gold, 11 salazosulfapyridine, and 15 bucillamine.

Assessment of activity of daily life (ADL) score

Physical function and health-related quality of life were assessed using a self-administered questionnaire modified from the Stanford Health Assessment Questionnaire, named the modified Health Assessment Questionnaire (M-HAQ) [16]. M-HAQ score is relevant to assess ADL in Japanese RA patients [17, 18].

Peripheral quantitative computed tomography (pQCT)

pQCT measurements were performed at 4% (ultradistal site) and 20% (midshaft site) to the ulnar length proximal to the end of radius with a single 2.5-mm thick CT slice on the nondominant side using an XCT-960 scanner (Stratec Inc, Pforzheim, Germany), as described previously [19, 20, 21]. Briefly, the bone mineral content (g/mm), the cross-sectional bone area, and the bone mineral density (g/cm³) were determined at the ultradistal site for the entire cross section as well as for the trabecular compartment. After the determination of the entire bone contour, the outer 55% of voxels were concentrically peeled off. The remaining 45% of voxels were defined as the trabecular region [19, 20]. The peeled-off area was defined as the cortical plus subcortical area [19, 20]. At the midshaft

site, BMD was measured only for the entire cross section as the cortical component because of the paucity of trabecular bone at this site [19, 21]. The former measurement site was chosen to analyze the effect of RA on the trabecular volumetric component of paraarticular bone separately from the cortical component [13, 20], and the latter to measure the effect of RA on the cortical volumetric component of generalized bone [21]. Image processing and calculation of numerical values were performed using the manufacturer's software package. The precision of the pQCT procedure ranged from 1% to 2%, depending upon the parameter assessed [22].

BMD measurement at lumbar spine

BMD was measured in the lumbar spine (L2-L4) by dual-energy X-ray absorptiometry (DXA; QDR-4500A, Hologic, Waltham, MA), essentially as previously described [23]. The precision of the measurement of lumbar spine BMD using DXA was less than 1.8%.

Quantitative ultrasound assessment of calcaneus

Quantitative ultrasound assessment of calcaneus was performed using an ultrasound system (Acoustic Osteo-Screener, AOS-100; Aloka, Tokyo, Japan), as previously described [24]. Briefly, the AOS-100 measures both speed of sound (SOS) and an attenuation-related parameter called the transmission index (TI). These measurements yield a derived parameter, the osteo sono-assessment index (OSI), which has been proposed to be an estimate of the elastic modulus of the calcaneus. Precision of the OSI parameter was 2.2% [25].

Statistical analysis

Values are expressed as mean $\pm$ SD unless otherwise indicated. Statistical analysis was performed with the Stat View V system (Abacus Concepts, Berkeley CA) for the Apple computer. Comparison of mean values between the two groups was performed by Student's t-test. The correlation coefficients were calculated by simple regression analysis. P values of less than 0.05 were considered significant.

Results

Clinical profiles of RA patients and healthy controls

The RA patients and healthy controls chosen were all female to exclude the effect of gender on bone mass. There were no significant between-group differences in the proportion of premenopausal and postmenopausal women, or in age, height, body weight, or body mass index (Table 1).

BMD at various sites in RA patients and controls

Table 2 shows that BMD measured by pQCT in trabecular bone at the ultradistal radius was significantly lower in RA patients than in normal controls ($p < 0.0001$). Interestingly, however, total bone BMD at the ultradistal radius in RA patients did not differ significantly from that in controls ($p > 0.10$). Cortical and plus subcortical bone BMD at ultradistal radius did not

Table 1 Clinical profiles of RA patients and healthy controls. Values are mean ± SD; n.s. not significant ($p > 0.10$), as determined by χ-square test. Comparison of mean values between two groups were performed by Student's t-test

	RA patients ($n = 30$)	Healthy controls ($n = 26$)	p value
Female/male	30/0	26/0	n.s.
Premenopausal/Postmenopausal	16/14	14/12	n.s.
Age (years)	52.1 ± 11.9	50.4 ± 13.8	n.s.
Height (cm)	155.8 ± 5.5	153.6 ± 4.1	n.s.
Body weight (kg)	52.8 ± 7.6	52.7 ± 7.8	n.s.
Body mass index (kg/m^2)	21.8 ± 2.8	22.4 ± 3.6	n.s.

Table 2 Comparison of bone mass at various sites, between RA patients and healthy controls. Data are shown as mean ± SD; n.s. not significant ($p > 0.10$) by Student's t-test

Site of bone mass measurement	RA patients ($n = 30$)	Healthy controls ($n = 26$)	p value
BMD in distal radius 4% (mg/cm^3)			
Trabecular bone component	147.3 ± 39.0	199.7 ± 51.6	< 0.0001
Total bone component	371.9 ± 66.9	392.3 ± 88.4	n.s.
Cortical plus subcortical bone component	874.2 ± 8.6	875.1 ± 12.7	n.s.
BMD in distal radius 20% (mg/cm^3)	1128 ± 68.6	1133 ± 67.0	n.s.
BMD in lumbar spine L2-L4 (g/cm^2)	0.952 ± 0.183	0.962 ± 0.173	n.s.
Calcaneus OSI (x10^6)	2.349 ± 0.247	2.498 ± 0.362	< 0.05

differ significantly between RA patients and normal controls ($p > 0.10$). BMD at midshaft radius did not differ significantly between RA patients and normal controls. Nor did BMD at the lumbar spine measured by DXA differ significantly, although calcaneus OSI measured ultrasonographically was significantly lower in RA patients ($p < 0.05$).

Correlation of RA activity with trabecular BMD in the ultradistal radius and with calcaneus OSI in RA patients

We next determined the significance of the effect of RA on trabecular bone component in the radius and calcaneus. Table 3 shows the summary of simple correlations of trabecular BMD in ultradistal radius and calcaneus

Table 3 Summary of simple correlation of clinical variables indicating RA activity with trabecular BMD in radius 4% and calcaneus OSI. Correlation of clinical variables indicating RA activity was examined with Z-score, the number of standard deviations by which a given measurement differs from the mean for a gender- and age-matched healthy Japanese population, of ultradistal trabecular BMD and calcaneus OSI

Clinical variables	Trabecular BMD in radius 4% (Z-score)		Calcaneus OSI (Z-score)	
	r	p	r	p
C-reactive protein	−0.348	0.076[†]	−0.119	0.571
Blood sedimentation rate	−0.487	0.010*	0.001	0.996
Rheumatoid factor	−0.670	0.001*	−0.323	0.115
Immunoglobulin G	−0.146	0.485	−0.193	0.367
Immunoglobulin M	−0.097	0.643	−0.082	0.702
Immunoglobulin A	−0.126	0.550	0.259	0.222
Platelet	−0.030	0.882	−0.126	0.549
Albumin	0.217	0.278	−0.089	0.673

*$p < 0.05$, †$p < 0.10$

OSI with various variables indicating RA disease activity. Of the clinical variables, blood sedimentation rate and rheumatoid factor showed a significant and negative correlation, and C-reactive protein a tendency to negative correlation with trabecular BMD in the ultradistal radius. In contrast, none of RA disease marker correlated significantly with calcaneus OSI.

Correlation between calcaneus OSI Z-score and M-HAQ score

We next examined the effect of daily physical activity on calcaneus OSI, and on BMDs in radius and lumbar spine. A negative correlation, although not significant probably due to the small number of RA patients, was found between the two parameters ($r = -0.313$, $p = 0.087$) (Fig. 1). However, M-HAQ score did not correlate either with BMDs in trabecular bone at the ultradistal radius ($r = -0.194$, $p = 0.323$), in midshaft radius ($r = -0.222$, $p = 0.237$), or lumbar spine ($r = -0.281$, $p = 0.125$).

Discussion

The present study demonstrated that early-stage RA patients showed significant reductions in the trabecular BMD of the paraarticular ultradistal radius and in the trabecular bone-rich calcaneus OSI compared with healthy controls. However, the mechanisms of lower bone mass in these sites may differ. Negative correlations of trabecular BMD in the paraarticular ultradistal radius with CRP, BSG, and RF in RA patients suggests that local inflammation in the affected joints may be an important factor for BMD reduction in paraarticular trabecular bone. In contrast, a negative correlation of calcaneus OSI with M-HAQ score, but not with any
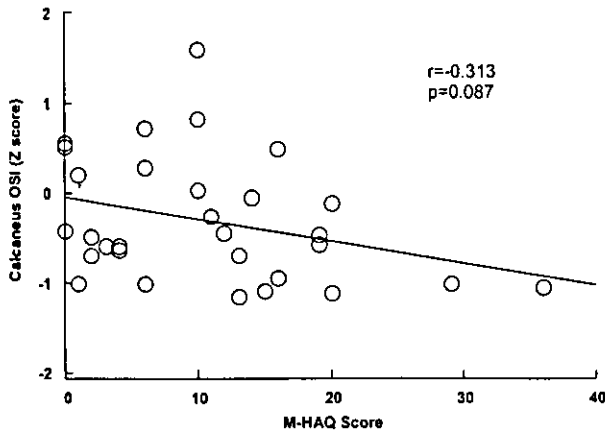
Fig. 1 Negative correlation between calcaneus OSI and M-HAQ score in 30 female RA patients. A negative correlation, although not significant due probably to small number of patients, was found between calcaneus OSI and M-HAQ score ($r = -0.313$, $p = 0.087$)

inflammation marker, suggests the impairment of physical activity as a major factor for reduction of calcaneus OSI.

pQCT is able to measure the BMD of the trabecular component of the distal radius separately from the cortical component [19, 20, 21]. However, the measured cortical bone component does not exactly comprise cortical bone because of the inclusion of subcortical bone, the endosteal portion of the cortex, and also trabecular bone component, since the Stratec pQCT system defines outer 55% of voxels as cortical bone area [19, 26]. Since the present study showed that trabecular BMD, but not total or cortical plus subcortical BMD, in the ultradistal radius was reduced in early RA patients (Table 2), it was concluded that the BMD of the trabecular component of the ultradistal radius was preferentially reduced in early RA patients compared with age- and gender-matched controls, in contrast to the maintenance of BMD observed in the cortical component. A previous report demonstrated that loss of trabecular bone component in the ultradistal radius was more rapid in RA than those of the radial midshaft or lumbar spine, although they did not measure the rate of cortical bone loss in the ultradistal radius [13]. Furthermore, we elucidated the importance of RA-induced inflammation on the loss of paraarticular trabecular bone as evidenced by a negative correlation between inflammation marker and trabecular BMD in the ultradistal radius.

Previous reports of ours have indicated that humoral factors with bone resorptive activity, such as IL-1, IL-6, TNF [5], and hepatocyte growth factor [3], and extrarenal activation of vitamin D [4], may contribute to BMD reduction in paraarticular trabecular bone. Since these are humoral factors that activate bone resorption, it is reasonable to suppose that metabolically active trabecular bone is more susceptible than cortical bone to the enhancement of bone resorption by humoral bone

resorptive factors. Previous reports have shown that DXA-determined total BMD in the forearm, which consists of 85% cortical bone and 15% trabecular bone, is significantly reduced in RA patients compared with control participants and that number of years since menopause, M-HAQ score for the upper extremities, and BSG appear to be significant determinants of forearm BMD [26]. Since DXA cannot discriminate trabecular BMD from cortical BMD and since the RA patients analyzed were at a more advanced stage, the conclusion drawn from the previous study may contrast with that of the present study. Furthermore, a longitudinal study has shown that RA patients lose bone mass in spine, femoral neck, and Ward's triangle more rapidly than healthy controls, resulting in generalized bone loss at a more advanced stages of RA [27]. Recent reports have shown that the risk factors for generalized osteoporosis in RA patients increase with longer duration and greater severity of the disease [20], suggesting that the sites of osteoporosis may differ between RA patients in an early stage and those in more advanced stages.

Although the measurement of calcaneus ultrasound includes not only bone density but also bone elasticity and architecture, parameters of quantitative ultrasound assessment of the calcaneus are mostly related to BMD [28, 29]. In fact, daily physical activity has a beneficial effect on bone mass in weight-bearing bone such as the lumbar spine and calcaneus [30]. The validity of quantitative ultrasound measurement of calcaneus was demonstrated in advanced stage RA patients with disease duration of more than 10 years, as evidenced d by a significant reduction of ultrasound measures in RA patients and a significant association of radiological modified Larsen score with ultrasound measures [31]. We have shown that the calcaneus OSI was significantly reduced probably due to reduced physical activity in RA patients, as reflected by a negative correlation between two parameters (Fig. 1). However, although bone mass in lumbar spine is also heavily influenced by physical activity [32], a significant reduction was not observed (Table 2). Aerobics and weight training increase both the calcaneus OSI and the lumbar spine BMD significantly, but to a lesser extent at the latter site [30, 32]. Since the calcaneus is thus more susceptible than the lumbar spine to physical activity and since the RA patients analyzed in the present study were restricted to those with disease duration of less than 1 year, it is possible that calcaneus OSI, but not lumbar spine BMD, decreased significantly.

To precisely know the effect of RA on bone mass in early RA patients, patients who are now, or have a history of, taking drugs that might affect bone metabolism, such as steroid and methotrexate, were excluded, although the latter drug might not have any adverse effect on bone metabolism [33, 34].

In summary, it was demonstrated that RA patients in the early stage of the disease showed significant reductions in the trabecular BMD of the ultradistal radius and OSI of the calcaneus. BMD at the former site exhibited

significant and positive correlations with biochemical indicators for RA disease activity, and the calcaneus OSI negative correlation with daily physical activity score. The findings therefore suggest that joint inflammation is a significant determinant of paraarticular osteoporosis, and impairment of daily activity of calcaneus osteoporosis in early stage RA patients.

References

1. Inaba M, Ishimura E (2002) Secondary osteoporosis. In: Morii H, Nishizawa Y, Massry SG (eds) Calcium in internal medicine. Springer, Berlin Heidelberg New York, pp 347–360
2. Deodhar AA, Woolf AD (1996) Bone mass measurement and bone metabolism in rheumatoid arthritis: a review. Br J Rheumatol 35:309–322
3. Yukioka K, Inaba M, Furumitsu Y et al (1994) Levels of hepatocyte growth factor in synovial fluid and serum of patients with rheumatoid arthritis and release of hepatocyte growth factor by rheumatoid synovial fluid cells. J Rheumatol 21:2184–2189
4. Inaba M, Yukioka K, Furumitsu Y et al (1997) Positive correlation between levels of IL-1 or IL-2 and 1,25(OH)2D/25-OH-D ratio in synovial fluid of patients with rheumatoid arthritis. Life Sciences 61:977–985
5. Furumitsu Y, Inaba M, Yukioka K et al (2000) Levels of serum and synovial fluid pyridinium crosslinks in patients with rheumatoid arthritis. J Rheumatol 27:64–70
6. Manolagas SC (1995) Role of cytokines in bone resorption. Bone 17 [Suppl]:63s–67s
7. Miyasaka N, Sato K, Goto M et al (1988) Augmented interleukin-1 production and HLA-DR expression in the synovium of rheumatoid arthritis patients. Arthritis Rheum 31:480–486
8. Gowen M, Wood DD, Ihrie EJ et al (1983) An interleukin 1 like factor stimulates bone resorption in vitro. Nature 306:378–380
9. Inaba M, Morii H, Katsumata T et al (2000) Hyperparathyroidism is augmented by ovariectomy in Nagase analbuminemic rats. J Nutr 130:1543–1547
10. Neville CE, Murray LJ, Boreham CA et al (2002) Relationship between physical activity and bone mineral status in young adults: the Northern Ireland young hearts project. Bone 30:792–798
11. Canalis E, Delany AM (2002) Mechanisms of glucocorticoid action in bone. Ann N Y Acad Sci 966:73–81
12. Minaur NJ, Kounali D, Vedi S et al (2002) Methotrexate in the treatment of rheumatoid arthritis. II. In vivo effects on bone mineral density. Rheumatology 41:741–749
13. Sambrook PN, Ansell BM, Foster S, Gumpel JM, Hesp R, Reeve J (1985) Bone turnover in early rheumatoid arthritis. 2. Longitudinal bone densities. Ann Rheum Dis 44:58–584
14. Boonen S, Cheng XG, Nijs J et al (1997) Factors associated with cortical and trabecular bone loss as quantified by peripheral computed tomography (pQCT) at the ultradistal radius in aging women. Calcif Tissue Int 60:164–170
15. Arnett FC, Edworthy SM, Bloch DA et al (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31:315–324
16. Pincus T, Summey JA, Soraci SA Jr et al (1983) Assessment of patient satisfaction in activities of daily living using a modified Stanford Health Assessment Questionnaire. Arthritis Rheum 26:1346–1353
17. Maeda T, Yamada T, Nagamine R et al (2002) Involvement of CD4+, CD57+ T cells in the disease activity of rheumatoid arthritis. Arthritis Rheum 46:379–384
18. Nakamura H, Ueki Y, Sakito S et al (2000) Clinical effects of actarit in rheumatoid arthritis: improvement of early disease activity mediated by reduction of serum concentrations of nitric oxide. Clin Exp Rheumatol 18:445–450
19. Gorai I, Nonaka K, Kishimoto H et al (2001) Cut-off values determined for vertebral fracture by peripheral quantitative computed tomography in Japanese women. Osteoporos Int 12:741–748
20. Shibuya K, Hagino H, Morio Y et al (2002) Cross-sectional and longitudinal study of osteoporosis in patients with rheumatoid arthritis. Clin Rheumatol 21:150–158
21. Lochmuller, E-ML, Lill CA, Kuhn V et al (2002) Radius bone strength in bending, compression, and falling and its correlation with clinical densitometry at multiple sites. J Bone Miner Res 17:1629–1638
22. Ashizawa N, Nonaka K, Michikami S et al (1999) Tomographical description of tennis-loaded radius: reciprocal relation between bone size and volumetric BMD. J Appl Physiol 86:1347–1351
23. Inaba M, Nishizawa Y, Mita K et al (1999) Poor glycemic control impairs the response of biochemical parameters of bone formation and resorption to exogenous 1,25-dihydroxyvitamin D3 in patients with type 2 diabetes. Osteoporos Int 9:525–531
24. Kumeda Y, Inaba M, Goto H et al (2002) Increased thickness of the arterial intima-media detected by ultrasonography in patients with rheumatoid arthritis. Arthritis Rheum 46:1489–1497
25. Tsuda-Futami E, Hans D, Njeh CF, Fuerst T, Fan B, Li J et al (1999) An evaluation of a new gel-coupled ultrasound device for the quantitative assessment of bone. Br J Radiol 72:691–700
26. Iwamoto J, Takeda T, Ichimura S (2002) Forearm bone mineral density in postmenopausal women with rheumatoid arthritis. Calcif Tissue Int 70:1–8
27. Gough AKS, Lilley J, Eyre S et al (1994) Generalised bone loss in patients with early rheumatoid arthritis. Lancet 344:23–27
28. Hans D, Wu C, Njeh CF et al (1999) Ultrasound velocity of trabecular cubes reflects mainly bone density and elasticity. Calcif Tissue Int 64:18–23
29. Njeh CF, Fuerst T, Diessel E, Genant HK (2001) Is quantitative ultrasound dependent on bone structure? A reflection. Osteoporos Int 12:1–15
30. Friedlander AL, Genant HK, Sadowsky S, Byl NN, Gluer CC (1995) A two-year program of aerobics and weight training enhances bone mineral density of young women. J Bone Miner Res 10:574–585
31. Sambrook P, Raj A, Hunter D et al (2001) Osteoporosis with low dose corticosteorids: Contribution of underlying disease effects and discriminatory ability of ultrasound versus bone densitometry. J Rheumatol 28:1063–1067
32. Van Marken Lichtenbelt WD, Fogelholm M, Ottenheijim R, Westerterp KR (1995) Physical activity, body composition and bone density in ballet dancers. Br J Nutr 74:439–451
33. Minaur NJ, Kounali D, Vedi S, Compston JE, Beresford JN, Bhalla AK (2002) Methotrexate in the treatment of rheumatoid arthritis. II. In vivo effects on bone mineral density. Rheumatology (Oxford) 41:741–749
34. Cranney AB, McKendry RJ, Wells GA, Ooi DS, Kanigsberg ND, Kraag GR, Smith CD (2001) The effect of low dose methotrexate on bone density. J Rheumatol 28:2395–2399

図説：ビスホスホネートの作用機序

宇田川信之　　髙 橋 直 之

図説

ビスホスホネートの作用機序

宇田川信之[1]　　高橋 直之[2]

ビスホスホネートは強力な骨吸収抑制作用を示し，腰椎骨密度を増加させ，骨粗鬆症による椎体骨折発生を低下させることが報告されている．最近本邦においても，ビスホスホネートの経口剤が骨粗鬆症の治療薬として認可され，その効果について注目されている．ビスホスホネートが有する骨吸収抑制のメカニズムについては，現在まで様々な実験結果が報告されてきている．

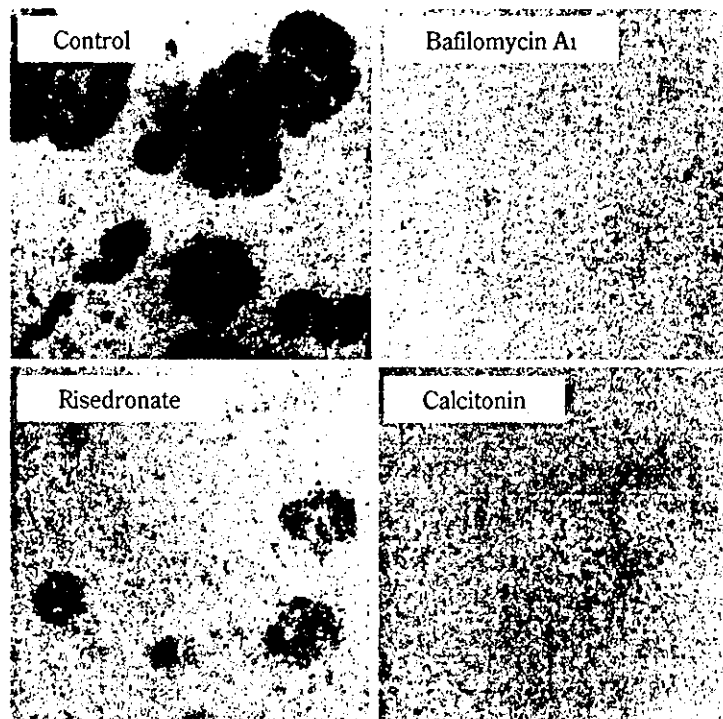マウスの共存培養系で形成された破骨細胞を象牙質切片上で培養すると，多数の吸収窩を形成する．ビスホスホネート(リセドロネート)はカルシトニンや液胞型プロトンポンプ阻害剤(バフィロマイシン)と同様に，吸収窩形成活性を強力に抑制した(図1).

図　1

マウスの骨芽細胞と骨髄細胞を活性型ビタミンDの存在下で共存培養して形成された破骨細胞を回収後，象牙質切片上で24時間培養する．培養後細胞を除去し，ヘマトキシリン染色を施すと，多数の吸収窩の形成が観察できる．ビスホスホネートの添加により，強力な骨吸収抑制作用が認められる．

[1]Nobuyuki Udagawa：松本歯科大学 生化学　Department of Biochemistry, Matsumoto Dental University

[2]Naoyuki Takahashi：松本歯科大学総合歯科医学研究所 硬組織疾患制御再建学　Division of Hard Tissue Research, Institute for Oral Science, Matsumoto Dental University

　著者らは，ビスホスホネート（チルドロネート）は極性化した波状縁を有する破骨細胞に直接作用することを示す実験結果を，波状縁形成を欠如している多核破骨細胞（遺伝性大理石骨病を呈する oc/oc マウス由来の破骨細胞）を用いて報告した[1]．この結果は，骨表面に付着したビスホスホネートが波状縁を有する破骨細胞によって取り込まれ，破骨細胞を不活性化させることにより骨吸収を阻害することを示している（図2）．
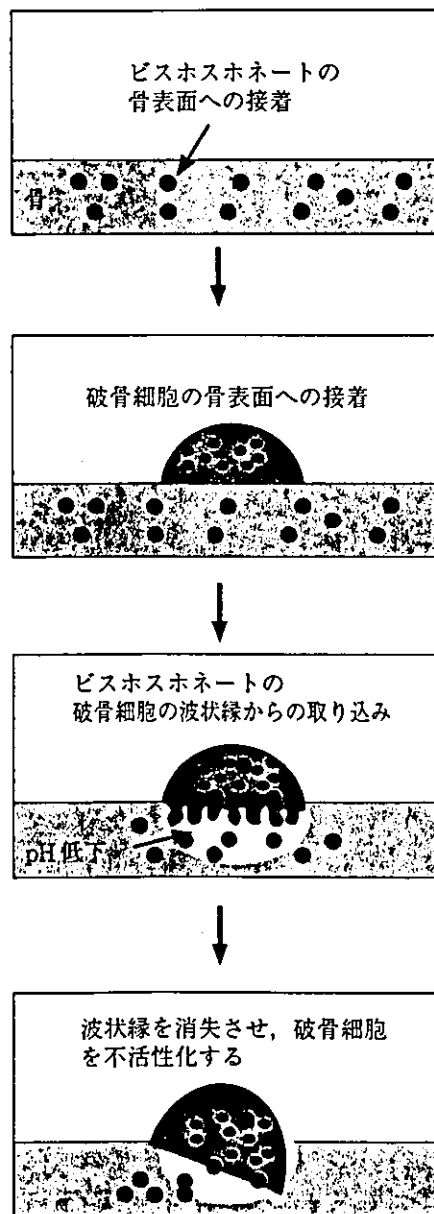

図　2
ビスホスホネートは直接破骨細胞に取り込まれ，波状縁を消失させ，骨吸収機能を不活性化させる．

　英国の Mike J. Rogers は，窒素を含むビスホスホネートの骨吸収阻害メカニズムとして，コレステロール生合成経路であるメバロン酸代謝酵素の阻害を介するという説を提唱した[2]．彼らの実験結果は，ビスホスホネートはメバロン酸からファルネシルピロリン酸の合成を抑制し，Small GTP 結合蛋白質の細胞膜への結合を阻害し，破骨細胞の細胞骨格の破壊やアポトーシスを誘導することを示している(図 3)．
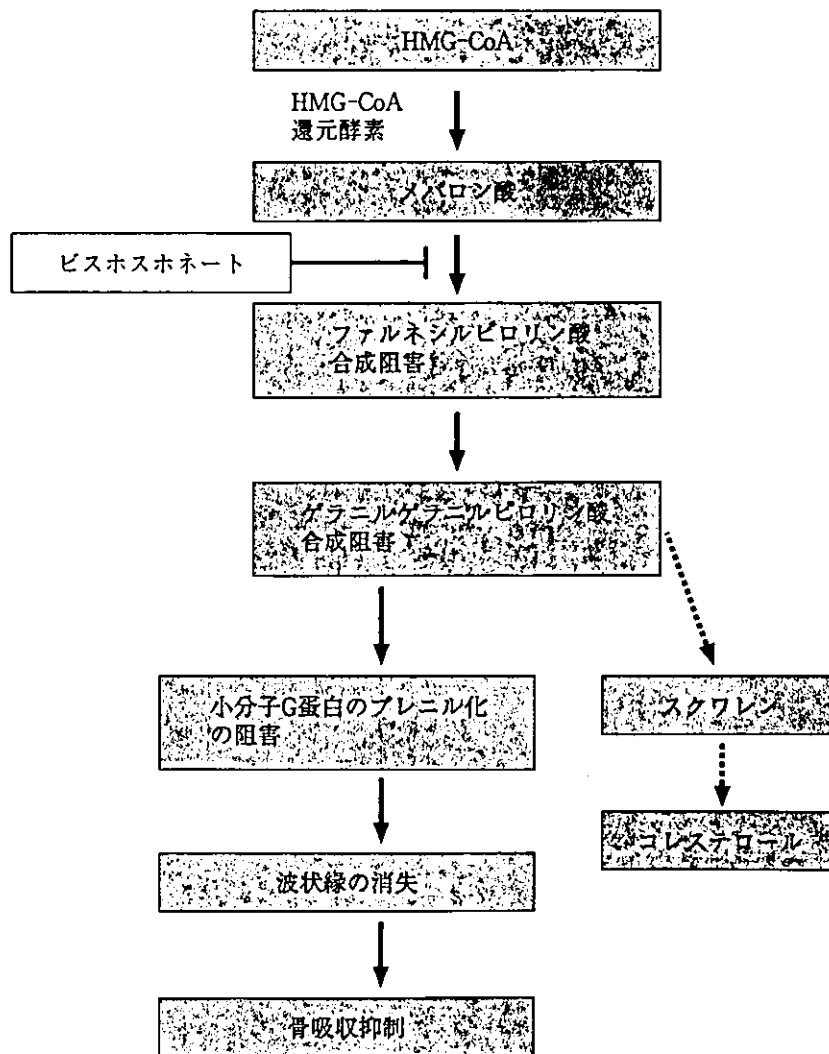

図 3　破骨細胞のコレステロール合成経路(メバロン酸経路)に対するビスホスホネートの作用

　著者らは最近，破骨細胞分化因子である RANKL のデコイ受容体である OPG の遺伝子欠損マウスにビスホスホネート（リセドロネート）を投与する実験を行った[3]．OPG 欠損マウスは破骨細胞による骨吸収の亢進によって重篤な骨粗鬆症を呈するが，ビスホスホネートの皮下投与（30 日間）により，破骨細胞性の骨吸収が抑制され，腰椎骨量の回復が認められた．図 4 は，OPG 欠損マウスと正常マウスにビスホスホネートを投与した後の大腿骨の所見であり，ビスホスホネートによる骨粗鬆症の治療効果が認められた．
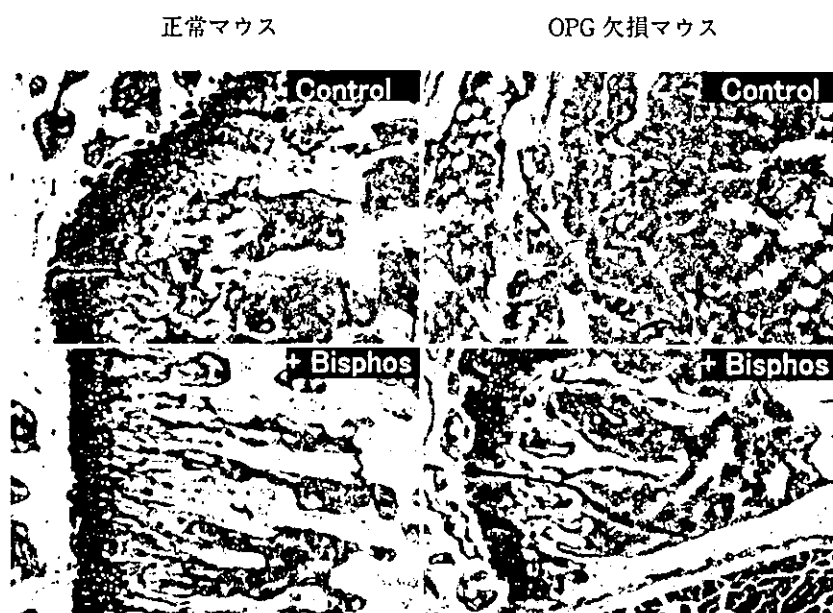
正常マウス　　　　　　　　　OPG 欠損マウス

図　4

　14 週齢雄の OPG 欠損マウスおよび正常マウス（C57BL/6J）にビスホスホネートを毎日皮下投与（30 日間）した．OPG 欠損マウスにおいては，大腿骨成長板の破壊がみられ，著明な骨粗鬆化が認められる．ビスホスホネートの投与によって，OPG 欠損マウスおよび正常マウスともに，骨量の増加が認められる．（トルイジンブルー染色および TRAP 染色の 2 重染色）

文　　献

1) Murakami H, et al: A possible mechanism of the specific action of bisphosphonates on osteoclasts: tiludronate preferentially affects polarized osteoclasts having ruffled borders. Bone　17: 137-144, 1995.

2) Fisher JE, et al: Alendronate mechanism of action: geranylgeraniol, an intermediate in the mevalonate pathway, prevents inhibition of osteoclast formation, bone resorption, and kinase activation in vitro. Proc Natl Acad Sci USA　96: 133-138, 1999.

3) 中村美どりほか：OPG 遺伝子欠損マウスに対するビスホスホネート（BP）の投与実験－骨代謝共役因子（カップリングファクター）の存在様式に関する研究．歯科基礎医学会雑誌　44（抄録号），2002．（印刷中）

4. 骨吸収を促進する炎症性サイトカインと細菌菌体成分の作用機構

高橋　直之[1]，小林　泰浩[1]，宇田川信之[2]

[1]松本歯科大学総合歯科医学研究所硬組織疾患制御再建学部門，[2]同生化学講座

はじめに

　骨組織は一生涯を通じて骨吸収と骨形成を繰り返し，この現象は骨リモデリングと呼ばれる。骨形成は未分化間葉系細胞から分化した骨芽細胞が，一方，骨吸収は造血系細胞から分化した破骨細胞が担っている。近年の急激な高齢化社会への移行に伴い社会問題化している骨粗鬆症は，破骨細胞による過剰な骨吸収あるいは骨芽細胞による骨形成の低下などにより，骨リモデリングのバランスが崩れ，骨量が減少することにより引き起こされる。また，慢性関節リウマチや歯周炎においても骨吸収が骨形成を凌駕し，骨量が減少する。1998 年，骨芽細胞が発現する破骨細胞の分化と機能を調節する破骨細胞分化因子（osteoclast differentiation factor：ODF）がクローニングされ，骨吸収調節メカニズムの一端が分子レベルで明らかにされた。このODFは TNF（tumor necrosis factor）ファミリーの属する新規のサイトカインで，Choi らのグループによって報告された TRANCE，あるいは Immunex のグループによって報告された RANKL と同一分子であった。以来，免疫学の主役と思われていたサイトカインのファミリーが，破骨細胞の形成や機能を制御する知見も集積されてきた。本稿では，破骨細胞の分化と活性制御の分子メカニズムとともに，炎症性サイトカインと細菌菌体成分の骨吸収調節機構について概説したい。

1. 骨芽細胞による破骨細胞分化の調節

　破骨細胞は，骨組織に存在し骨吸収を司る多核細胞である。破骨細胞は単球・マクロファージ系の前駆細胞より分化する。この破骨細胞の分化と機能発現は，骨形成を司る骨芽細胞・骨髄細胞由来間質細胞（本稿では両細胞群を骨芽細胞とする）により厳密に調節されている。1998 年，骨芽細胞が発現する膜結合因子である破骨細胞分化因子（ODF）がクローニングされ，骨吸収の調節メカニズムの一端が分子レベルで明らかにされた[1]。このODFは，すでに報告されていた TRANCE（TNF-related activation-induced cytokine）[2] あるいは RANKL（receptor activator of NF-κB）[3] と同一分子であった（なお本稿では，破骨細胞分化因子の名称として，RANKL を用いる）。すなわち，骨芽細胞は破骨細胞の分化に必須な因子である M-CSF（macrophage colony-stimulating factor）と RANKL を産生し，破骨細胞の形成を誘導する[4]。また骨芽細胞は，RANKL のデコイレセプターである分泌型タンパクの OPG（osteoprotegerin）も産生し，破骨細胞の形成と機能を抑制する作用も有する[5,6]。骨芽細胞による M-CSF の発現は恒常的であるのに対し，RANKL の発現は活性型ビタミン D_3[1,25(OH)$_2$D$_3$]，PTH（parathyroid hormone），PGE$_2$（prostaglandin E$_2$），IL-11（interleukin 11）などの骨吸収因子により誘導される[4]（図1）。1,25(OH)$_2$D$_3$ はビタミンDレセ
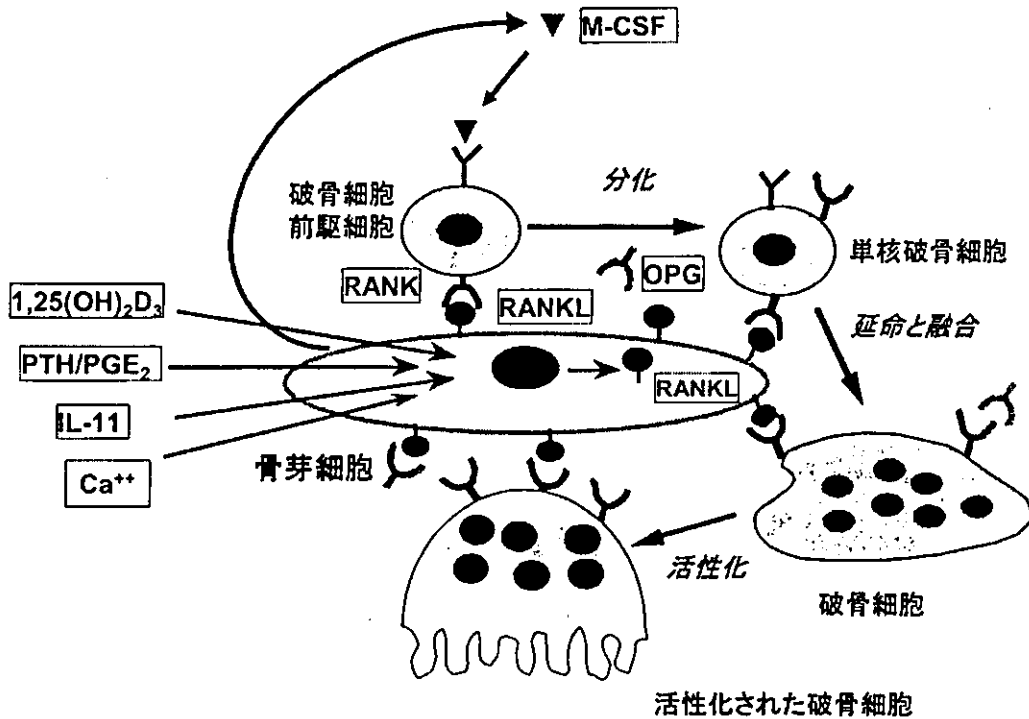
図 1　破骨細胞の分化と機能を調節する骨芽細胞の作用

プター（VDR）を介し，IL-11やIL-6は共通の
シグナル伝達因子であるgp130を介して
RANKLの発現を上昇させる。また，PTHや
PGE$_2$のレセプターからのシグナルはcAMPを
介してRANKL発現を上昇させると考えられる。
骨吸収を促進する1,25(OH)$_2$D$_3$，PTH，PGE$_2$
は，骨芽細胞によるOPGの産生も抑制し，破骨
細胞形成を促進する。さらに，骨芽細胞の細胞内
Caレベルが上昇するような薬物処理や培養液の
Ca^{++}濃度を増加させるとRANKLの発現が誘導
される[7]。このRAKLの発現誘導は，細胞内Ca
濃度の上昇により活性化されたPKC（protein
kinase C）が仲介すると考えられている。一方，
RANKLのレセプターであるRANKを発現する
破骨細胞前駆細胞は，骨芽細胞との細胞間接触を
介してRANKLを認識し，M-CSFの存在下で
破骨細胞に分化する。また，成熟破骨細胞も
RANKを発現しており，RANKLからの刺激に
より骨吸収活性が誘導される[8]（図1）。

2.　TNFα/IL-1による破骨細胞の分化と活性化の制御

　TNFαは破骨細胞の前駆細胞である骨髄由来
マクロファージに直接作用し破骨細胞への分化を
誘導する[9,10]。このTNFαによる破骨細胞形成
促進作用はRANKLのデコイ受容体であるOPG
によって抑制されず，TNF I型ならびにII型受
容体に対する中和抗体によって強力に抑制され
た。これらの知見は，TNFαはRANKL-
RANKシステムを介さずに破骨細胞分化を誘導
することを示唆する。一方，IL-1は破骨細胞の
分化を誘導しないが，RANKLと同様に成熟破
骨細胞に直接作用し，その骨吸収活性を誘導する
ことが示された[9,11]（図2）。

　TNFファミリーに属するサイトカインのシグ
ナルは，それぞれの受容体の細胞内ドメインに会
合するTNF receptor-associated factor
（TRAF）を介して伝達される[12]。それぞれの受
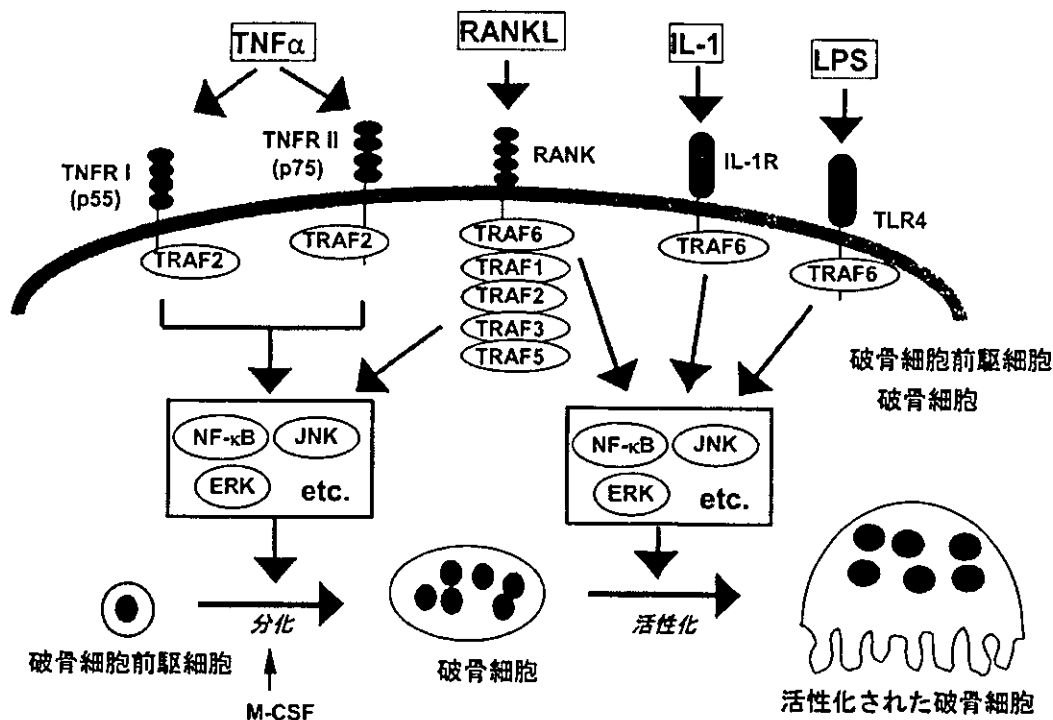容体には，異なったパターンでTRAFが会合す
る。TNF受容体にはTRAF2が，RANKには
TRAF1，TRAF2，TRAF3，TRAF5，

図 2 　破骨細胞の分化と機能を誘導する TNF 受容体，RANK，IL-1 受容体および TLR 4 のシグナル系

TRAF 6 が会合する。一方，IL-1 は TNF ファミリーには属さないが，IL-1 受容体を介するシグナルは TRAF 6 を活性化する。以上の知見は，破骨細胞の分化には TFAF 2 を介するシグナルが，そして破骨細胞の活性化には TRAF 6 を介するシグナルが重要であると思われる（図2）。一方，TRAF 6 欠損マウスは典型的な大理石骨病を呈することから，骨吸収を調節する TRAF 6 のシグナルの重要性が注目されている[13,14]。TRAF 6 欠損マウスから得られた破骨細胞前駆細胞は RANKL あるいは TNFα によっても破骨細胞への分化が著しく抑制されているという[14]。また，TNFα が誘導する破骨細胞形成を少量の RANKL が強く促進することも報告された。これらの知見は，破骨細胞の分化誘導にも TRAF 6 からのシグナルが極めて重要であることを示唆するものである。骨吸収に関与する RANKL，IL-1，TNFα などサイトカイン間におけるシグナル伝達系のクロストークの解明が今後急速に進むと思われる。

3. 細菌菌体成分を認識する Toll 様受容体とそのシグナル

近年，マクロファージや樹状細胞は細菌を構成する病原性分子（pathogen-associated molecular patterns：PAMP）を Toll 様受容体（Toll-like receptor：TLR）を介して認識し，免疫反応を起こすことがわかってきた[15]。TLR はショウジョウバエにおいて真菌の感染防御に関与する Toll のホモログであり，ヒトで10種類，マウスで 9 種類知られている。これら TLR が認識する細菌を構成する抗原性分子もこの数年間で次々と明らかにされた。TLR に関する知見をまとめると，(1) TLR はそれぞれ特異的な細菌細胞成分と結合し，シグナルを細胞内に伝達する。(2) TLR の細胞内ドメインは IL-1 受容体（IL-1 R）ファミリーの属する受容体のそれと類似し，ともに MyD 88 と IRAK（IL-1 receptor-associated kinase），さらに TRAF 6 を介してシグナルが伝達される。(3) TRAF 6 を介するシグナルは，さらに MAPK(mitogen activated protein kinase) の活性化や NF-κB，AP-1 が活性化を誘導する。(4) TLR 4 からのシグナルは MyD 88

依存性経路とともに TIRAP (Toll-IL-1 receptor domain-containing adapter protein) を介する MyD 88 非依存性経路が報告された[16]。この MyD 88 非依存性経路は TLR 4 以外の他の TLR /IL-1 R には認められない。このように，この数年間で LPS をはじめとする PAMP の受容機構とシグナル系路が急速にと明らかにされた。また，PAMP の破骨細胞前駆細胞と破骨細胞に対する効果も詳細に解析されつつある。

4. LPS と IL-1 の破骨細胞誘導作用：OPG 発現抑制の重要性

マウス骨髄細胞と骨芽細胞の共存培養系において，破骨細胞形成に及ぼす LPS ならびに IL-1 の作用を検討したところ，LPS と IL-1 は破骨細胞形成を促進した。実際に LPS と IL-1 は骨芽細胞の RANKL の発現を促進し，OPG の発現を抑制した[17]。また，COX 2 (cyclooxygenase 2) の阻害薬である NS 398 は $1,25(OH)_2D_3$ が誘導する破骨細胞形成は抑制しなかったが，LPS と IL-1 による破骨細胞の形成を完全に阻害した。このことは，LPS と IL-1 は PGE_2 の産生を介して，RANKL の発現亢進と OPG の発現抑制を惹起するものと推測された。さらに，OPG 遺伝子欠損マウス由来の骨芽細胞と骨髄細胞を用いた共存培養系における LPS と IL-1 による破骨細胞形成が解析された。興味深いことに，OPG 欠損マウスから得られた細胞を用いた共存培養において，NS 398 は LPS と IL-1 による破骨細胞形成を全く抑制しなかった[17]。この共存培養系に OPG を添加すると LPS による分化が完全に抑制された。さらに，正常マウスの骨芽細胞培養系で，NS 398 は $1,25(OH)_2D_3$ とともに LPS と IL-1 による RANKL の発現誘導を全く阻害しなかった。また，恒常的に発現している OPG mRNA は，$1,25(OH)_2D_3$，LPS あるいは IL-1 処理によって減少した。NS 398 を更に添加すると LPS あるいは IL-1 によって誘導された OPG の発現抑制のみが回復した[17]。これらの知見は，共存培養系での LPS と IL-1 による破骨細胞形成は，次の二つの事象により引き起こされること
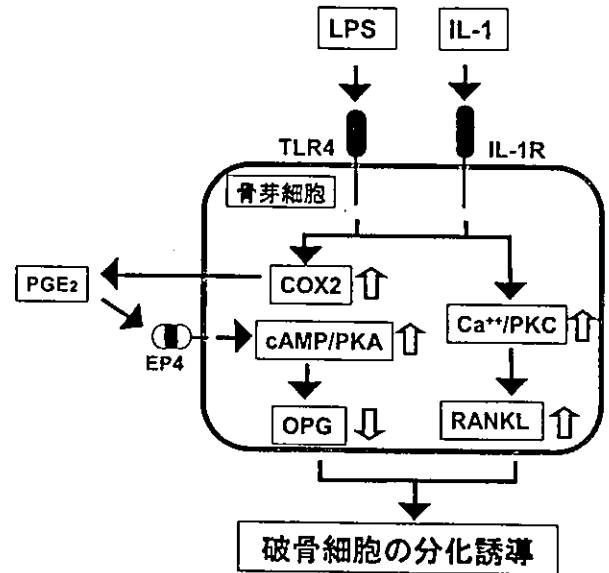

図3　骨芽細胞における RANKL と OPG の発現に対する LPS と IL-1 の作用

を示す。第一に LPS と IL-1 は RANKL を誘導するが，この RANKL 誘導に PGE_2 は関与しない。第二に LPS と IL-1 は PGE_2 の産生亢進を介して OPG の発現を抑制する。この OPG の発現抑制は LPS と IL-1 による破骨細胞形成にとって極めて重要である。NS 398 の抑制効果は，OPG 欠損マウスでは認められない。すなわち，LPS または IL-1 は，骨芽細胞に作用し直接 RANKL の発現を誘導するとともに，PGE_2 の産生を介して OPG 産生を抑制し，破骨細胞の分化誘導するものと考えられた（図3）。さらに，IL-1 と LPS による RANKL 発現誘導には関わるシグナル系が解析され，細胞内 Ca 濃度の上昇により活性化された PKC が関与するものと考えられた。

5. PAMP の破骨細胞前駆細胞と破骨細胞に対する直接作用

破骨細胞の前駆細胞である骨髄マクロファージは，TLR 1 から TLR 9 まで全ての TLR を発現している。骨髄マクロファージは RANKL と M-CSF の存在下で培養すると破骨細胞に分化する。Takami ら[18]は LPS，ペプチドグリカン，2 本鎖 RNA [Poly(I：C)]，CpG DNA など

PAMP は破骨細胞前駆細胞に作用し，RANKL と M-CSF が誘導する破骨細胞分化を著しく抑制することを報告した。この知見は，菌体成分はマクロファージから破骨細胞への分化を抑制し，骨組織への細菌侵入の防御に働いている可能性を示している。一方 IL-1 は，骨髄マクロファージの p38 MAPK を活性化するにもかかわらず（IL-1 受容体は機能するにもかかわらず），RANKL 誘導性の破骨細胞形成を全く抑制しない[9,19]。IL-1 R と TLR のシグナル伝達系の類似性を考えると，破骨細胞分化に対する IL-1 と PAMP の作用の違いは興味深い。

成熟破骨細胞に対する PAMP の作用も解析された。成熟破骨細胞は TLR 2 と TLR 4 の mRNA を発現しているが，他の TLR は発現していない。実際に，LPS は，IL-1 と同様に破骨細胞の延命を促進した[20,21]。また，LPS は IL-1 と同様に破骨細胞の骨吸収活性を促進した[20]（図3）。LPS による破骨細胞延命効果は，C 3 H/HeJ マウス由来の細胞では起こらないことから，PAMP による破骨細胞の延命促進作用は TLR を介するものと推察される。前述したように，TLR 4 からのシグナルには MyD 88 依存性と非依存性に経路が存在する。今後の研究で，LPS と IL-1 の作用に違いが明確化されることが期待

される。

前述したように，骨髄マクロファージの培養系に LPS など PAMP を添加すると，RANKL と M-CSF によって誘導される破骨細胞形成は強力に抑制される[18]。しかし，共存培養系においては PAMP は破骨細胞形成を促進する。この矛盾は現段階では説明できないが，骨芽細胞は破骨細胞前駆細胞における PAMP のシグナルを抑制する因子を産生しているのかもしれない。

おわりに

1997 年の OPG の発見とそれに続く RANKL のクローニングにより，破骨細胞の形成と分化を調節するシグナル伝達が分子レベルで説明できるようになった。また，リウマチや炎症時に産生されるサイトカインは骨芽細胞の RANKL の発現を促進するのみならず，破骨細胞前駆細胞や成熟破骨細胞へ直接作用し，活性化や延命を調節する機構もわかってきた。さらに，1997 年細菌の菌体成分を認識する受容体のヒトおよびマウスのホモログ（TLR）が同定されて以来，菌体成分による骨吸収調節機構が急速に解明されつつある。以上の知見をまとめると，PAMP はそれぞれの受容体を介して，(1) 骨芽細胞の RANKL の発
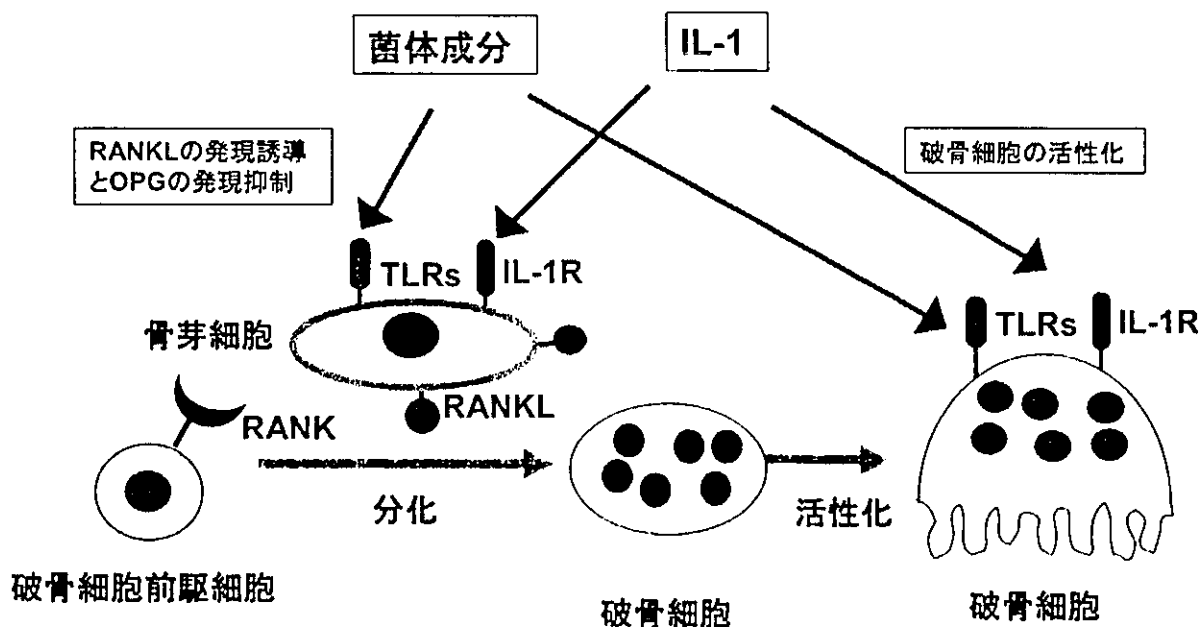

図 4 骨芽細胞と破骨細胞に対する IL-1 と細菌菌体成分の作用

現を誘導し，OPG の発現を抑制すること，(2)
成熟破骨細胞に作用して，その延命と骨吸収活性
を誘導する，という2つの作用を有している（図
4）。今後の研究で破骨細胞の分化と活性化におけ
る炎症性サイトカインと菌体成分からのシグナル
系の *in vitro* と *in vivo* 両面での詳細な解析は，
炎症性骨吸収の治療薬の開発や治療指針の確立に
おおいに貢献するであろう。

文　献

1) Yasuda H, Shima N, Nakagawa N, et al.:
Osteoclast differentiation factor is a ligand for
osteoprotegerin/osteoclastogenesis-inhibitory
factor and is identical to TRANCE/RANKL.
Proc Natl Acad Sci USA 95: 3597-3602, 1998

2) Wong BR, Josien R, Lee SY, et al.: The
TRAF family of signal transducers mediates
NF-xB activation by the TRANCE receptor.
J Biol Chem 273: 28355-28359, 1997

3) Anderson DM, Maraskovsky E, Billingsley
WL, et al.: A homologue of the TNF receptor
and its ligand enhance T-cell growth and
dendritic-cell function. Nature 390: 175-179,
1997

4) Suda T, Takahashi N, Udagawa N, et al.:
Modulation of osteoclast differentiation and
function by the new members of the tumor
necrosis factor receptor and ligand families.
Endocr Rev 20: 345-357, 1999

5) Tsuda E, Goto M, Mochizuki S, et al.: Isola-
tion of a novel cytokine from human fibrob-
lasts that specifically inhibits osteoclas-
togenesis. Biochem Biophys Res Commun
234: 137-142, 1997

6) Simonet WS, Lacey DL, Dunstan CR, et al.:
Osteoprotegerin: a novel secreted protein
involved in the regulation of bone density. Cell
89: 309-319, 1997

7) Takami M, Takahashi N, Udagawa N, et al.:
Intracellular calcium and protein kinase C
mediate expression of receptor activator of
NF-xB ligand and osteoprotegerin in osteob-
latsts. Endocrinology 141: 4711-4719, 2000

8) Jimi E, Akiyama S, Tsurukai T, et al.: Osteo-
clast differentiation factor acts as a multifun-
ctional regulator in murine osteoclast
differentiation and function. J Immunol 163:
434-442, 1999

9) Kobayashi K, Takahashi N, Jimi E, et al.:
Tumor necrosis factor alpha stimulates osteo-
clast differentiation by a mechanism indepen-
dent of the ODF/RANKL-RANK interaction.
J Exp Med 191: 275-286, 2000

10) Azuma Y, Kaji K, Katogi R, et al.: Tumor
necrosis factor-α induces differentiation of
and bone resorption by osteoclasts. J Biol
Chem 275: 4858-4864, 2000

11) Jimi E, Nakamura I, Duong L, et al.: Interleu-
kin 1 induces multinucleation and bone-resor-
bing activity of osteoclasts in the absence of
osteoblasts/stromal cells. Exp Cell Res 247:
84-93, 1999

12) Inoue J, Ishida T, Tsukamoto N, et al.:
Tumor necrosis factor receptor-associated
factor (TRAF) family: adapter proteins that
mediate cytokine signaling. Exp Cell Res 254:
14-24, 2000

13) Lomaga MA, Yeh WC, Sarosi I, et al.:
TRAF 6 deficiency results in osteopetrosis and
defective interleukin-1, CD 40, and LPS signa-
ling. Genes Dev 13: 1015-1024, 1999

14) Naito A, Azuma S, Tanaka S, et al.: Severe
osteopetrosis, defective interleukin-1 signal-
ling and lymph node organogenesis in TRAF
6-deficient mice. Genes Cells 4: 353-362, 1999

15) Akira S, Takeda K, Kaisho T: Toll-like rece-
ptors: critical proteins linking innate and
acquired immunity. Nat Immunol 2: 675-680,
2001

16) Yamamoto M, Sato S, Hemmi H, et al.:
Essential role for TIRAP in activation of the
signalling cascade shared by TLR 2 and
TLR 4. Nature 420: 324-329, 2002

17) Suda K, Udagawa N, Takami M, et al.:
Lipopolysaccharide stimulates osteoclast pro-
motion via RANKL expression independent of
PGE$_2$ production. In preparation

18) Takami M, Kim N, Rho J, et al.: Stimulation by toll-like receptors inhibits osteoclast differentiation. J Immunol 169: 1516-23, 2000

19) Li X, Udagawa N, Itoh K, et al.: p 38 MAP kinase-mediated signals are required for inducing osteoclast differentiation but not for osteoclast function. Endocrinology 143: 3105-3113, 2002

20) Suda K, Woo JT, Takami M, et al.: Lipopolysaccharide supports survival and fusion of preosteoclasts independent of TNF-α, IL-1, and RANKL. J Cell Physiol 190: 101-108, 2000

21) Itoh K, Udagawa N, Kobayashi K, et al.: LPS promotes the survival of osteoclasts via Toll-like receptor 4, but cytokine production of osteoclasts in response to LPS is different from that of macrophages. J Immunol 170: 3688-3695, 2003

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