interleukin-1 (IL-1) acts directly on osteoclasts to induce bone-resorbing activity (8).

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by arthritis affecting multiple joints and the progressive destruction of cartilage and bone (9). Osteoclasts activated by inflammatory cytokines are involved in bone destruction in RA. Recent studies have suggested that a progressive synovial expansion called pannus at sites of bone destruction plays important roles in osteoclastic bone resorption (10–12). In addition, osteoclasts formed from circulating precursors obtained from patients with RA have an increased bone-resorbing activity compared with those obtained from normal control subjects (13). However, the etiology of RA and the mechanism of bone destruction induced by RA have not yet been elucidated completely.

Nurse cells were first recognized in cell suspensions of dissociated thymus (14). Thymic nurse cells supported the differentiation and maturation of T cells. When bone marrow–derived T cell precursors were cocultured with thymic nurse cells, the T cell precursors crawled beneath the thymic nurse cell layers and differentiated into mature thymocytes. This phenomenon, known as pseudoemperipolesis, is peculiar to nurse cells and has been used to identify nurse-like cells (NLCs) in various tissues (15–17). We have established NLC lines from the synovium and bone marrow of patients with RA (16,17). NLCs showed characteristics similar to those of fibroblast-like synoviocytes. NLCs promoted the activation and differentiation of both B and T lymphocytes in coculture. It was also shown that stromal cell–derived factor 1 and CD106 (vascular cell adhesion molecule 1) were involved in the formation and maintenance of B cell pseudoemperipolesis by RA fibroblast-like synoviocytes (18).

We recently showed that NLCs promoted the survival of peripheral blood monocytes as well as B cells (19). Monocytes supported by NLCs possessed tartrate-resistant acid phosphatase (TRAP; a marker enzyme of osteoclasts) activity and differentiated into osteoclast-like multinucleated cells in response to some cytokines, including RANKL. However, it is not clear how fibroblast-like synoviocytes are involved in bone destruction in RA. In the present study, we examined the ability of monocytes supported for 4 weeks by NLCs to differentiate into osteoclasts in comparison with the ability of freshly isolated peripheral blood monocytes to do so. We also examined how NLCs support the survival of osteoclast precursors for a long period of culture.

MATERIALS AND METHODS

Chemicals. Recombinant human M-CSF (Leukoprol) was obtained from Kyowa Hakko Kogyo (Tokyo, Japan), recombinant soluble RANKL and OPG from PeproTech (London, UK), and recombinant human TNF and neutralizing antibody against human M-CSF from Genzyme (Minneapolis, MN). We purchased 1 ,25-dihydroxyvitamin D_3 (1 ,25$[OH]_2D_3$) and prostaglandin E_2 (PGE_2) from Wako (Osaka, Japan). Human parathyroid hormone 1–34 (PTH 1–34) was obtained from Peptide Institute (Osaka, Japan). A monoclonal antibody against vitronectin receptors (VNRs; human CD51/61 complex) (23C6) was purchased from Serotec (Oxford, UK). A monoclonal antibody against human CD68 (KP1) and polyclonal antibodies against human M-CSF were from Dako (Glostrup, Denmark) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively.

Cells and the coculture system. CD14 monocytes were isolated from peripheral blood using anti-CD14 antibody–coated beads, as described previously (19). NLCs were established from synovium and bone marrow obtained from patients with RA. NLCs were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Gaithersburg, MD) supplemented with 10% fetal calf serum (FCS; Hyclone, Logan, UT). Half of the medium was replaced weekly with the fresh medium. SaOS-4/3 cells were established from the SaOS-2 human osteosarcoma cell line by transfection with human PTH/PTH-related protein receptor complementary DNA (cDNA) (20). SaOS-4/3 cells support human osteoclast formation in response to PTH in cocultures with human peripheral blood mononuclear cells (20,21).

CD14 monocytes (5 10^5 cells/well) were cocultured with NLCs (4 10^4 cells/well) or with SaOS-4/3 cells (4 10^4 cells/well) in the presence or absence of PTH ($10^{-8}M$) for 4 weeks in DMEM supplemented with 10% FCS in 12-well plates. Neutralizing antibodies against human M-CSF (final concentrations 50 ng/ml, 500 ng/ml, and 5,000 ng/ml) were added to some cocultures with NLCs. The culture medium was replaced every 3 days with the fresh medium. The number of CD14 monocytes recovered from the coculture with NLCs or SaOS-4/3 cells was counted every week. After coculture for 4 weeks, the floating or weakly adherent CD14 monocytes were harvested as NLC-supported CD14 cells, or NCD14 monocytes, by gently washing the culture with DMEM supplemented with 10% FCS. The ability of NCD14 monocytes and CD14 monocytes to differentiate into osteoclasts was compared as described below.

Osteoclast formation assay. NCD14 monocytes (1 10^4/well) and freshly isolated CD14 monocytes (3 10^4/well) were cultured in the presence or absence of M-CSF (25 ng/ml), RANKL (40 ng/ml), TNF (20 ng/ml), or OPG (100 ng/ml) in -minimum essential medium (-MEM; Gibco) supplemented with 10% FCS in a 96-well plate. NCD14 monocytes (2 10^4/well) were also cocultured with SaOS-4/3 cells (1 10^4/well) in 48-well plates in -MEM supplemented with 10% FCS in the presence or absence of PTH ($10^{-8}M$). Some cultures were treated with OPG (100 ng/ml). After a specific period of time, cells were fixed and stained for TRAP using a TRAP staining kit obtained from Hokudo (Hokkaido, Japan).

For immunohistochemical staining, cells were fixed with cold methanol:acetone (50:50 volume/volume) for 10

Table 1. Sequences of polymerase chain reaction primers*

	Primers (5 –3)		
	Sense	Antisense	Expected product size, bp
CD14	TCCCCACAAGTTCCCGGCCATC	TCCACCTCGGGCAGCTCGTCAG	315
CD68	TTCCCCCACGCAGCAAAGTGGA	ACCCCAAACCCCCTCAGTGCCC	362
CTR	TCCATGGACCTGTCATGGCGGC	TTGGCGCTTCACGGTGGTTTGG	314
M-CSF	GCTTTGCTGAATGCTCCAGC	CAGAGGGACATTGGACAAACG	308, 1,201
OPG	CCGCCTCCAAGCCCCTGAGGTT	ACACGCGGTTGTGGGTGCGATT	400
RANK	CTTCGCGTCTGTGGCCCTGGTG	CCTGGCATCTTCGCCTTGTGCG	319
RANKL	GCATGGCCCCAACGGTACACGA	TCAGCTGCGAAGGGGCACATGA	237
TNFRI	TTCTTGCCCCCACCCGTCCATC	CCAGCCATCCAGGGCCACCTTC	359
GAPDH	TGCTCTTGCTGGGGCTGGTGGT	TGCCAAGGCTGTGGGCAAGGTC	400

* CTR calcitonin receptor; M-CSF macrophage colony-stimulating factor; OPG osteoprotegerin; TNFRI tumor necrosis factor receptor I.

minutes and incubated with a monoclonal antibody against VNR, an osteoclast-associated antigen. The bound antibodies were visualized with biotinylated secondary antibodies, avidin–biotin–conjugated peroxidase, and an aminoethylcarbazole substrate kit (Histofine; Nichirei, Tokyo, Japan).

For the pit-formation assay, NCD14 and CD14 monocytes were cultured on dentin slices in -MEM supplemented with 10% FCS in 48-well plates (1 slice/well) in the presence or absence of several of the factors described above. After 21 days, dentin slices were stained with Mayer's hematoxylin solution to detect resorption pits.

Reverse transcription–polymerase chain reaction (RT-PCR) analysis. Total RNA was extracted from CD14 (5 10^6 cells) and NCD14 (5 10^5 cells) monocytes using an RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's directions. After treatment with DNase I (Life Technologies, Rockville, MD), single-stranded cDNA was synthesized using 2 g of each RNA sample, 100 ng of random primers, and 4 units of Omniscript reverse transcriptase (Qiagen) in a total reaction volume of 20 l. Amplification was performed with 0.5 units of Ex *Taq* (Takara, Shiga, Japan) in a total reaction volume of 20 l containing 1 reaction buffer, 200 *M* of each dNTP, and 10 pmoles of each primer. The PCR conditions were as follows: initial denaturation for 2 minutes at 94°C, then 30 cycles of 30 seconds each at 94°C and 72°C (annealing at 72°C).

To determine the expression of calcitonin receptor (CTR) messenger RNA (mRNA), NCD14 monocytes (4 10^5/well) were cocultured with SaOS-4/3 cells (2 10^5/well) in 6-well plates with or without PTH ($10^{-8}M$) and with or without PTH ($10^{-8}M$) plus OPG (100 ng/ml). Total RNA extracted from the coculture was subjected to RT-PCR analysis of CTR mRNA expression.

To determine the expression of RANKL, OPG, and M-CSF mRNA, NLCs (5 10^6/dish) or SaOS-4/3 cells (5 10^6/dish) were cultured for 3 days in a 10-cm culture dish in the presence or absence of PTH ($10^{-8}M$), PGE_2 ($10^{-6}M$), or 1 ,25$(OH)_2D_3$ ($10^{-8}M$). Total RNA was then extracted from the cells and subjected to a RT-PCR analysis of RANKL, OPG, and M-CSF mRNA expression.

A fragment of GAPDH cDNA was amplified as a control in all reactions. Aliquots of the PCR products were subjected to electrophoresis on 1.5% agarose gels and visualized by staining with ethidium bromide. In all cases, reproducibility was confirmed in triplicate experiments. The primer sets for CD14, CD68, CTR, M-CSF, OPG, RANK, RANKL, TNF receptor I (TNFRI), and GAPDH are shown in Table 1.

Immunohistochemical and TRAP staining of tissue samples from RA patients. Tissue samples, including the bone–synovium interface, were obtained during total knee arthroplasty in 5 RA patients, after they provided informed consent. The American College of Rheumatology (formerly, the American Rheumatism Association) criteria were used for the diagnosis of RA (22). The clinical features of the RA patients are summarized in Table 2.

Tissue samples were fixed in 4% paraformaldehyde at 4°C for 24 hours and decalcified in 20% EDTA for 2 hours in a microwave oven (H2800 Microwave Processor; Energy Beam Sciences, Agawam, MA) at 50°C and then for 22 hours at 4°C (23). Next, the samples were dehydrated through an ethanol series and embedded in paraffin. Sections (4 m thick) were cut with a microtome and stained with TRAP and immunohistochemical stains. Immunohistochemical staining was performed by the streptavidin–biotin–peroxidase complex technique using a Histofine SAB-PO kit (Nichirei) according to the manufacturer's instructions. Briefly, after blocking endogenous peroxidase and nonspecific antigens, tissue sections were incu-

Table 2. Characteristics of the rheumatoid arthritis patients at the time of surgery*

No. of men/women	0/5
Age, mean (range) years	62.2 (57–68)
CRP, mean (range) mg/dl	1.76 (0.9–4.4)
Disease duration, mean (range) years	20.8 (14–28)
No. taking NSAIDs	5
Treatment during previous 6 months, no. of patients	
Gold salts	0
Bucillamine	0
Methotrexate	4
Prednisolone	3

* CRP C-reactive protein; NSAIDs nonsteroidal antiinflammatory drugs.

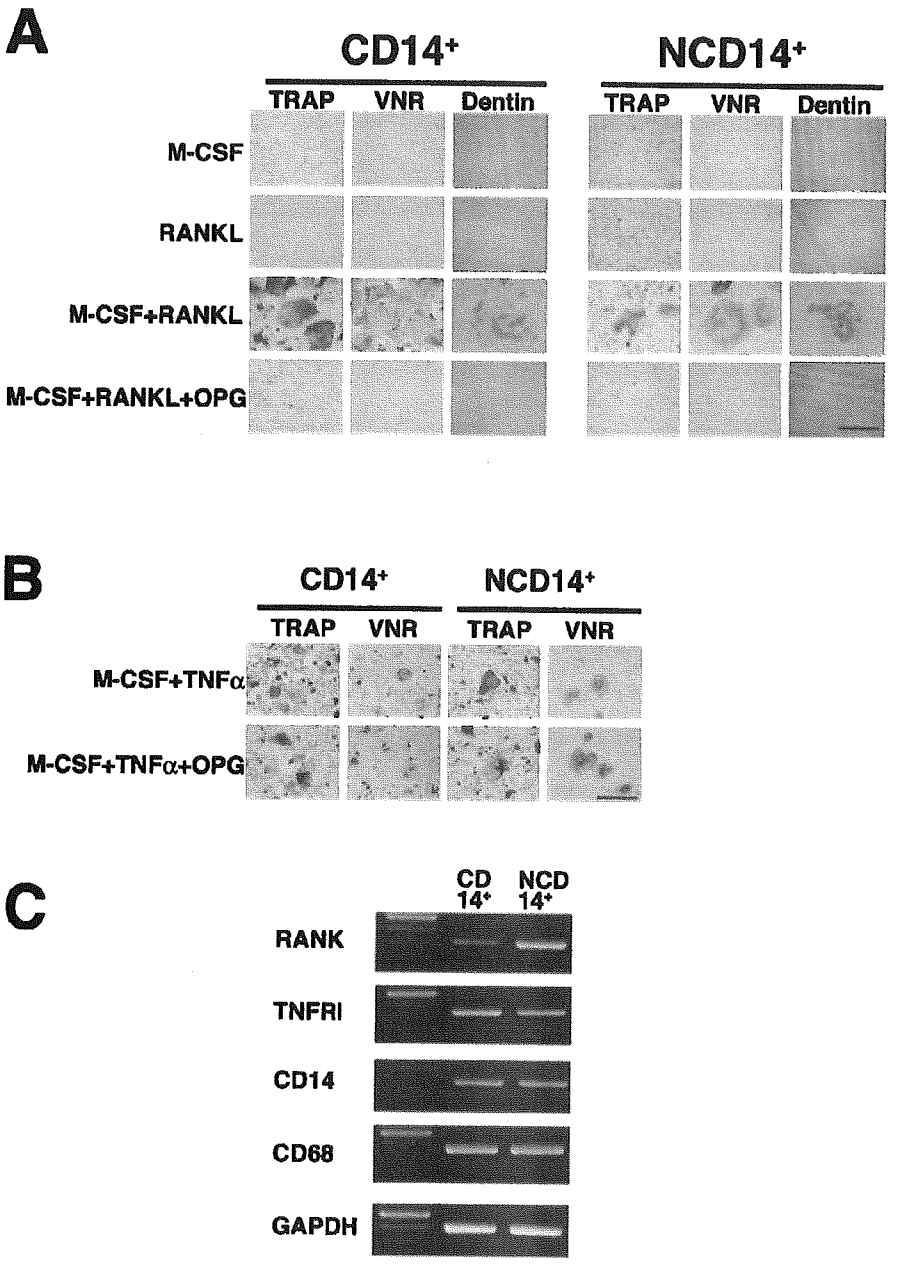

Figure 1. Osteoclast formation from CD14 monocytes and nurse-like cell-supported CD14 (NCD14) monocytes. **A,** CD14 monocytes were cultured for 7 days with or without macrophage colony-stimulating factor (M-CSF; 25 ng/ml), RANKL (40 ng/ml), M-CSF (25 ng/ml) plus RANKL (40 ng/ml), or M-CSF (25 ng/ml) plus RANKL (40 ng/ml) plus osteoprotegerin (OPG; 100 ng/ml). NCD14 monocytes were similarly cultured for 14 days. Cultures were then fixed and stained for tartrate-resistant acid phosphatase (TRAP) or for vitronectin receptor (VNR). For the pit-formation assay, NCD14 and CD14 monocytes were cultured on dentin slices in the presence or absence of several of the factors described above. After 21 days in culture, dentin slices were stained with Mayer's hematoxylin to detect resorption pits. Bar 100 m. **B,** CD14 and NCD14 monocytes were cultured for 7 and 14 days, respectively, with M-CSF (25 ng/ml) plus tumor necrosis factor (TNF ; 20 ng/ml) in the presence or absence of OPG (100 ng/ml). Cultures were stained for TRAP or VNR. Bar 100 m. **C,** Total RNA was extracted from CD14 and NCD14 monocytes, and the expression of mRNA for RANK, TNF receptor I (TNFRI), CD14, CD68, and GAPDH was analyzed by reverse transcription–polymerase chain reaction.

bated with primary antibodies against M-CSF and CD68 for 24 hours at 4°C. The sections were washed with phosphate buffered saline and incubated with the secondary antibody, followed by peroxidase-conjugated streptavidin (Nichirei). After a wash with phosphate buffered saline, the sections were incubated with 3,3 -diaminobenzidine tetrahydrochloride (Dojindo, Kumamoto, Japan) to detect peroxidase activity and then counterstained with hematoxylin. TRAP was detected using a TRAP staining kit (Hokudo).

Statistical analysis. The statistical significance of differences was analyzed using Student's *t*-test. *P* values less than 0.05 were considered significant. All results are representative of at least 3 individual experiments.

RESULTS

Cytokine-induced osteoclast formation from CD14 and NCD14 monocytes. CD14 monocytes were prepared from peripheral blood, and NCD14 monocytes were prepared from CD14 monocytes and NLCs that had been cocultured for 4 weeks. CD14 monocytes were cultured for 7 days with or without M-CSF, RANKL, M-CSF plus RANKL, or M-CSF plus RANKL plus OPG. Multinucleated cells positive for both TRAP and VNR were formed in the presence of M-CSF plus RANKL (Figure 1A). When CD14 monocytes were cultured on dentin slices, resorption pits on the slices were observed only in the culture treated with M-CSF plus RANKL. Addition of OPG, a decoy receptor for RANKL, to the CD14 culture treated with M-CSF plus RANKL inhibited the formation of TRAP and VNR multinucleated cells. Pit formation on dentin slices induced by M-CSF plus RANKL was inhibited by the addition of OPG to the CD14 culture.

Similarly, NCD14 monocytes differentiated into TRAP and VNR multinucleated cells in response to M-CSF plus RANKL (Figure 1A). The formation of TRAP and VNR multinucleated cells from NCD14 monocytes was completely inhibited by the addition of OPG. However, a longer culture period was required to induce the multinucleated cells in NCD14 cultures (7–14 days) than to induce them in CD14 cultures (7 days). Resorption pits were also detected on dentin slices on which NCD14 monocytes had been cultured in the presence of M-CSF plus RANKL.

Both CD14 and NCD14 monocytes differentiated into TRAP and VNR multinucleated cells in response to TNF instead of RANKL in the presence of M-CSF (Figure 1B). OPG failed to inhibit the formation of TNF -induced TRAP and VNR multinucleated cells in CD14 and NCD14 cultures. RT-PCR analysis showed that CD14 and NCD14 monocytes expressed similar levels of mRNA for the monocyte/macrophage-associated antigens CD14 and CD68 (Figure 1C). Both cell populations expressed mRNA for RANK (the receptor for RANKL) and TNFRI (the type I TNF receptor).

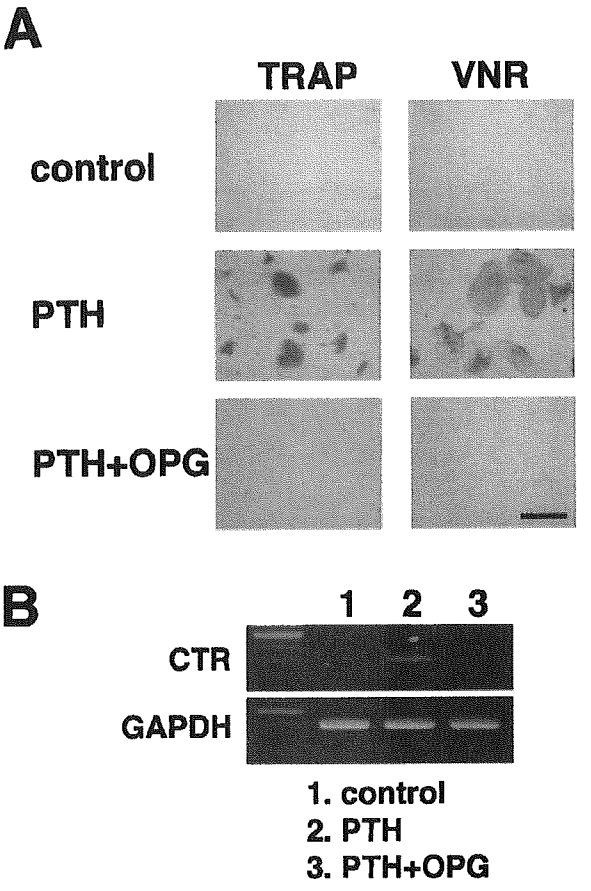

Figure 2. Osteoclast formation from nurse-like cell–supported CD14 (NCD14) monocytes in coculture with SaOS-4/3 cells. **A,** NCD14 monocytes were cocultured with SaOS-4/3 cells in the presence or absence of parathyroid hormone (PTH; $10^{-8}M$). Osteoprotegerin (OPG; 100 ng/ml) was added to some cocultures treated with PTH. After 14 days, cells were fixed and stained for tartrate-resistant acid phosphatase (TRAP) or vitronectin receptor (VNR). Bar 100 m. **B,** NCD14 monocytes and SaOS-4/3 cells were cocultured for 14 days with vehicle (control) (lane 1), PTH ($10^{-8}M$) (lane 2), and PTH ($10^{-8}M$) plus OPG (100 ng/ml) (lane 3). Total RNA was extracted from the cocultures, and the expression of mRNA for calcitonin receptor (CTR) and GAPDH was analyzed by reverse transcription–polymerase chain reaction.

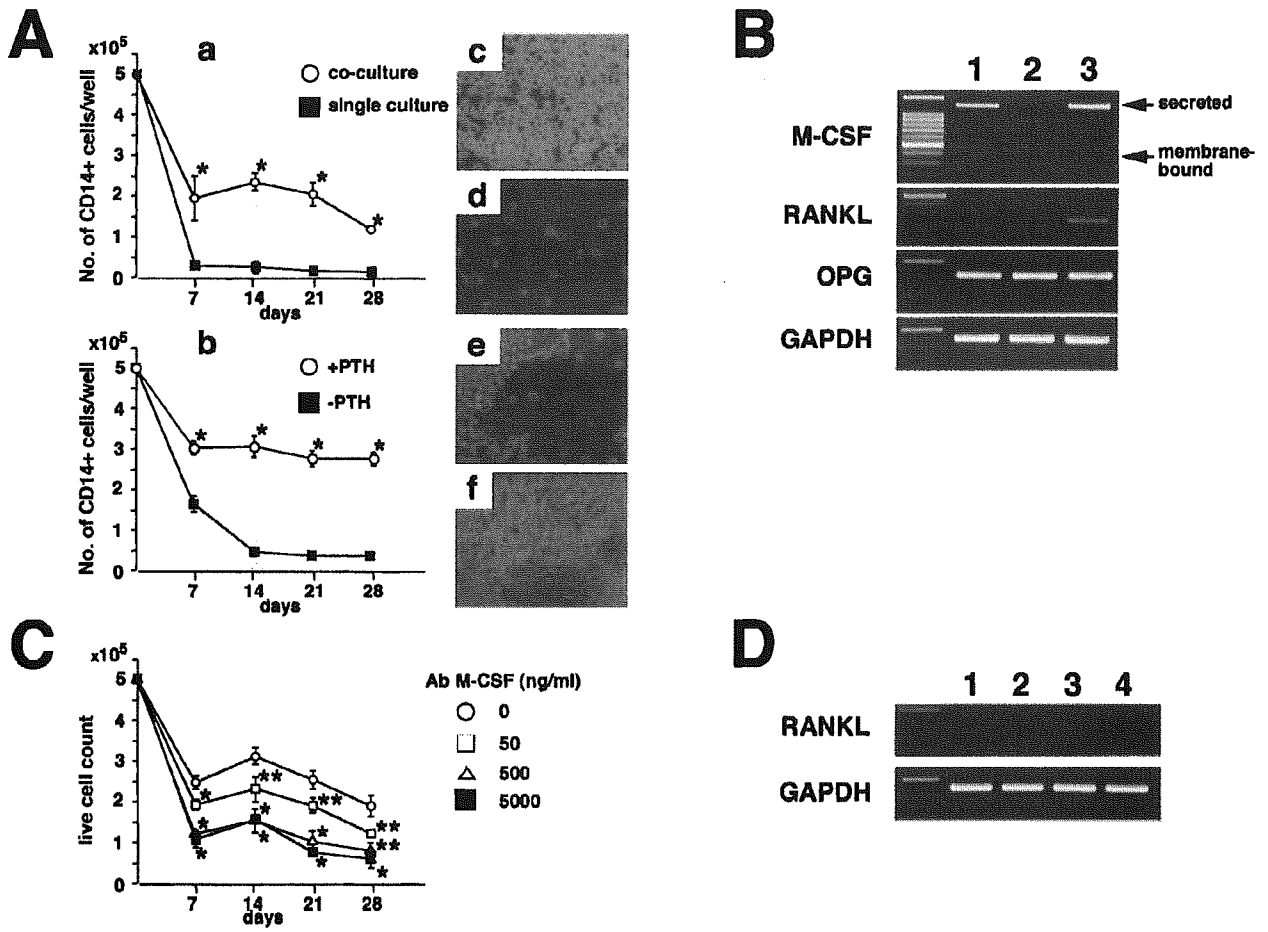

Figure 3. Importance of monocyte colony-stimulating factor (M-CSF) produced by SaOS-4/3 cells and nurse-like cells (NLCs) in the maintenance of CD14 monocytes. **A,** Survival of CD14 monocytes in cultures with or without NLCs or SaOS-4/3 cells. **a,** CD14 monocytes were cultured with and without NLCs for 28 days, and the number of CD14 monocytes recovered each week was counted. **b,** CD14 monocytes were cultured with SaOS-4/3 cells in the presence and absence of parathyroid hormone (PTH; $10^{-8}M$), and the number of CD14 monocytes recovered each week was counted. P 0.01. **c** and **d,** Monocyte cultures with and without NLCs, respectively. **e** and **f,** Monocyte cultures with SaOS-4/3 cells in the presence and absence of PTH, respectively. (Original magnification 40.) **B,** Total RNA was extracted from NLCs (lane 1) and from SaOS-4/3 cells treated for 3 days with (lane 2) or without (lane 3) PTH ($10^{-8}M$), and the expression of mRNA for M-CSF (secreted and membrane-bound forms), RANKL, osteoprotegerin (OPG), and GAPDH was analyzed by reverse transcription–polymerase chain reaction (RT-PCR). **C,** CD14 monocytes were cocultured with NLCs in the presence of increasing concentrations of neutralizing antibodies (Ab) against human M-CSF, and the number of CD14 monocytes recovered each week was counted. P 0.01; P 0.05 versus cultures without antibodies. **D,** NLCs were treated with vehicle (control) (lane 1), PTH ($10^{-8}M$) (lane 2), prostaglandin E_2 ($10^{-6}M$) (lane 3), and 1 ,25-dihydroxyvitamin D_3 ($10^{-8}M$) (lane 4). After 23 days, total RNA was extracted from NLCs, and the expression of mRNA for RANKL and GAPDH was analyzed by RT-PCR.

Osteoclast formation from CD14 and NCD14 monocytes in coculture with SaOS-4/3 cells. When NCD14 monocytes were cocultured for 7 days with SaOS-4/3 cells, TRAP and VNR osteoclasts formed in the presence of PTH (Figure 2A). This osteoclast formation was completely inhibited by the addition of OPG. The mRNA for CTR, one of the most reliable signals for osteoclast differentiation, was detected in the coculture treated with PTH, but not in the coculture treated with PTH together with OPG (Figure 2B). Thus, NCD14 monocytes differentiated into osteoclasts through their interaction with specialized stromal cells

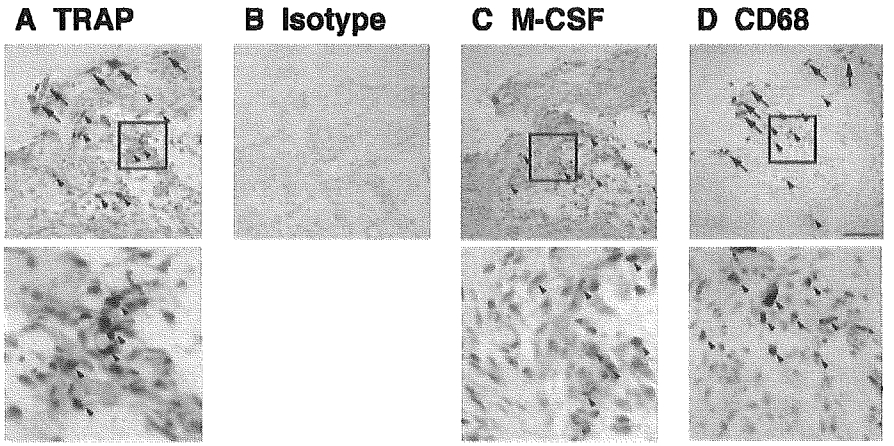

Figure 4. Localization of cells positive for tartrate-resistant acid phosphatase (TRAP), macrophage colony-stimulating factor (M-CSF), and CD68 in areas adjacent to the interface between bone and the progressive expansion of synovium in patients with rheumatoid arthritis. **A,** Some specimens stained positive for TRAP. **Arrows** indicate TRAP multinucleated cells; **arrowheads** indicate TRAP mononuclear cells. The other specimens were immunohistochemically stained with **B,** isotype control, **C,** antibodies against human M-CSF, and **D,** antibodies against CD68. **Arrowheads** in **C** indicate M-CSF synovial cells. **Arrowheads** in **D** indicate CD68 monocytes; **arrows** in **D** indicate CD68 multinucleated osteoclasts. Boxed areas in **A, C,** and **D** are shown at higher magnification underneath the respective panels (original magnification 100). Bar 100 m.

that possess the ability to support osteoclast differentiation.

Involvement of M-CSF in the NLC-supported survival of CD14 monocytes. When CD14 monocytes were cultured without NLCs, almost all of the CD14 monocytes disappeared within a week (Figure 3A, parts a and d). Approximately 40% of CD14 monocytes survived for 4 weeks in coculture with NLCs (Figure 3A, parts a and c). SaOS-4/3 cells could not promote the survival of CD14 monocytes in the absence of PTH (Figure 3A, parts b and f). However, treatment of the coculture with PTH enhanced the survival of CD14 monocytes (Figure 3A, parts b and e). Furthermore, multinucleated cells were observed after a 4-week culture in the PTH-treated coculture (Figure 3A, part e). In contrast, multinucleated cells were not detected in cocultures with NLCs (Figure 3A, part c). These results suggested that PTH induced the expression of a cytokine(s) that promoted the survival of CD14 monocytes and their resultant osteoclast formation.

We previously reported that treatment of SaOS-4/3 cells with PTH stimulated the expression of mRNA for both the membrane-associated and secreted forms of M-CSF (21). RT-PCR analysis showed that NLCs constitutively expressed mRNA for the secreted form of M-CSF and for OPG, but not for RANKL (Figure 3B). In contrast, neither M-CSF mRNA nor RANKL mRNA was expressed in SaOS-4/3 cells in the absence of PTH, but the expression of mRNA for both the membrane-associated and secreted forms of M-CSF as well as for RANKL was induced in PTH-treated SaOS-4/3 cells (Figure 3B). OPG mRNA expression in SaOS-4/3 cells was not affected by PTH.

When cocultures of CD14 monocytes and NLCs were treated with neutralizing antibodies against human M-CSF, the number of CD14 monocytes that survived in the coculture decreased in a dose-dependent manner (Figure 3C). Higher concentrations of the antibody (500 and 5,000 ng/ml) almost completely blocked the survival of CD14 monocytes in the coculture. In contrast to SaOS-4/3 cells, NLCs failed to express RANKL mRNA in response to PTH, PGE_2, or 1 ,25$(OH)_2D_3$, which are known to stimulate RANKL expression in mouse osteoblasts/bone marrow stromal cells (Figure 3D). Osteoclasts were not formed in the coculture of NLCs and CD14 monocytes, even in the presence of such RANKL-inducing factors (data not shown).

Histologic examination of sites of bone destruction in RA patients. We examined the distribution of CD68 monocytes, TRAP osteoclasts, and M-CSF

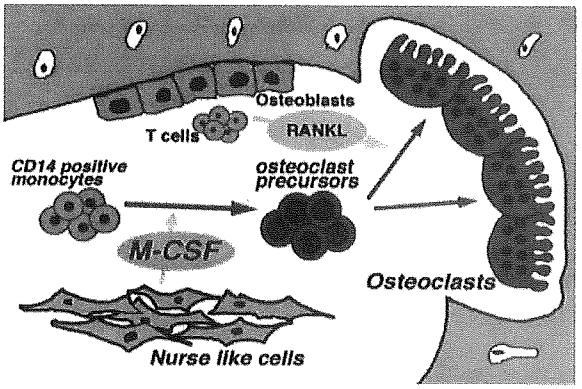

Figure 5. Possible role of nurse-like cells (NLCs) in bone destruction during progressive synovial expansion in rheumatoid arthritis (RA). NLCs show characteristics of fibroblast-like synoviocytes. NLCs support the survival of CD14 monocytes as osteoclast precursors through the production of macrophage colony-stimulating factor (M-CSF). The resultant osteoclast precursors differentiate into osteoclasts that resorb bone in response to RANKL produced by osteoblasts and activated T cells. Thus, fibroblast-like synoviocytes present in RA synovium play important roles in RA-induced bone destruction by maintaining CD14 monocytes that do not lose the capacity to differentiate into osteoclasts.

stromal cells in areas of progressive synovial expansion into bone in RA patients (Figure 4). TRAP multinucleated cells were detected along the surface of the bone and TRAP mononuclear cells were also detected in the pannus surrounding the damaged bone (Figure 4A). M-CSF cells were present in fibroblast-like synoviocytes surrounding areas of bone destruction (Figure 4C). CD68 multinucleated cells and monocytes were detected at the bone–pannus interface and in the pannus surrounding the damaged bone (Figure 4D). Consistent with previous studies (12), we found that some multinucleated osteoclasts were positive for CD68. Thus, CD68 monocytes and M-CSF synoviocytes were colocalized in areas adjacent to destroyed bone in RA patients.

DISCUSSION

In the present study, we demonstrated that NLCs support the survival of monocytes for a long period of time and that the resultant monocytes differentiate into osteoclasts in the presence of M-CSF together with RANKL or TNF . Moreover, we revealed that M-CSF is one of the key molecules needed for NLCs to maintain monocytes without losing their capacity to differentiate into osteoclasts. CD68 monocytes and M-CSF synoviocytes were colocalized in areas adjacent to destroyed bone in RA patients. These results suggest that fibroblast-like synoviocytes play an important role in bone destruction by continuously providing osteoclast precursors (Figure 5).

We compared the capacity of 2 types of cells, CD14 and NCD14 monocytes, to differentiate into osteoclasts. Consistent with the findings of previous studies (24–26), CD14 monocytes differentiated into mature osteoclasts when stimulated with M-CSF and RANKL/TNF . Mature osteoclasts were also generated from NCD14 monocytes by treatment with M-CSF plus either RANKL or TNF . NCD14 monocytes expressed mRNA for RANK and TNFRI, suggesting that these cells could respond to RANKL and TNF . SaOS-4/3 cells supported the differentiation of NCD14 monocytes into osteoclasts in cocultures treated with PTH. Thus, NCD14 monocytes interacted normally with stromal cells to receive RANKL and M-CSF signals. These results suggest that NCD14 monocytes fulfill a phenotype of the osteoclast precursor.

M-CSF is known to induce the proliferation and maturation of monocyte/macrophages. SaOS-4/3 cells maintained CD14 monocytes in the presence, but not the absence, of PTH. SaOS-4/3 cells produced M-CSF in response to PTH. NLCs constitutively expressed mRNA for the secreted type of M-CSF, and neutralizing antibodies against M-CSF strongly inhibited the NLC-supported survival of CD14 monocytes. Using the mice transgenic for human TNF as a model of erosive arthritis, Li et al (27) demonstrated that RANK signaling is not required for an increase in osteoclast precursors but is essential for osteoclast formation. Consistent with their finding, NLCs were able to support osteoclast precursors in the absence of RANKL. The monocyte-supporting activity found in NLCs appears to be a dominant trait of synovial cells. In fact, CD68 monocytes and M-CSF synoviocytes were colocalized adjacent to areas of destroyed bone in RA patients. These results suggest that M-CSF produced by synoviocytes is involved not only in the formation of osteoclasts, but also in the maintenance of osteoclast precursors in the vicinity of RA-induced bone loss (Figure 5).

It is well known that M-CSF is synthesized by mesenchymal cells, including fibroblasts and osteoblasts (28–30). Therefore, we examined whether fibroblastic stromal cells other than SaOS-4/3 cells and NLCs could support the survival of CD14 monocytes. Human skin fibroblasts derived from healthy volunteer donors and from human osteosarcoma MG63 cells supported the survival of CD14 monocytes for 4 weeks, as the NLCs

had done (data not shown). These CD14 monocytes obtained from cocultures with skin fibroblasts or MG63 cells differentiated into TRAP multinucleated cells in the presence of RANKL and M-CSF. These results support the conclusion that M-CSF produced by NLCs plays an essential role in the survival of osteoclast precursors for a long culture period.

The formation of osteoclasts from NCD14 monocytes was observed in cocultures with SaOS-4/3 cells treated with PTH, but not those treated with NLCs. SaOS-4/3 cells expressed RANKL upon treatment with PTH, but NLCs could not express RANKL mRNA in response to any osteotropic factors. NLCs established from different patients all supported the survival of CD14 monocytes (16), but none of them supported osteoclast formation in the presence of any osteotropic factors (data not shown). In contrast, several groups of researchers have reported that RA synovial fibroblasts support osteoclast formation in cocultures with peripheral blood mononuclear cells (11,26). Takayanagi et al (26) and Shigeyama et al (12) showed that RA synovial fibroblasts express RANKL in the presence of 1 ,25$(OH)_2D_3$. Gravallese et al (10) reported that the expression of RANKL in synovial fibroblasts is difficult to detect in prolonged cultures. This assertion may support our findings concerning the characteristics of NLCs.

We previously demonstrated that NLCs may play an important role in disease pathogenesis by producing large amounts of cytokines and maintaining infiltrating lymphocytes (16,31). Burger et al (18,32) reported that blood-derived NLCs protect B cells from apoptosis through the production of stromal cell–derived factor 1. They also described the supporting mechanism, pseudoemperipolesis, in detail. Using Transwell culture plates, we confirmed that CD14 monocytes survived for 4 weeks in the coculture with NLCs, even in the absence of the direct contact with NLCs. However, when those cells were further cultured with RANKL plus M-CSF, only a few osteoclasts were formed (data not shown). When cultured with M-CSF, CD14 monocytes survived for 4 weeks, but could not differentiate into osteoclasts in the presence of M-CSF plus RANKL (data not shown). These results suggest that another unidentified stromal factor(s) is required to maintain CD14 monocytes for a long period without the loss of their capacity to differentiate into osteoclasts.

Histologic examinations showed that numerous M-CSF stromal cells were detected in the vicinity of damaged bone in RA patients. Consistent with our results, it was previously shown that RA synovial fibroblasts expressed M-CSF (33) and that the concentration of M-CSF in synovial fluid was higher in RA patients than in patients with osteoarthritis (34). Moreover, a number of CD68 cells and TRAP cells were colocalized in areas adjacent to the same sites of destroyed bone in RA patients. These results suggest that synoviocytes appear to participate in the recruitment and maintenance of osteoclast precursors in the vicinity of RA-induced bone loss (Figure 5).

In conclusion, our study suggests that NLCs are involved in RA-induced bone destruction by maintaining osteoclast precursors in areas of progressive synovial expansion. M-CSF constitutively produced by synovial fibroblasts is essential in the maintenance of osteoclast precursors in the vicinity of damaged bone. Therefore, blocking of the M-CSF pathway as well as the RANKL pathway may be a therapeutic target for the prevention of bone destruction caused by synovial expansion in patients with RA.

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ORIGINAL PAPER

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Comparison between mobile-bearing and fixed-bearing knees in bilateral total knee replacements

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Abstract The purpose of this study was to compare mid-term results of mobile-bearing and fixed-bearing in bilateral total knee arthroplasty (TKA). Twenty-two patients underwent bilateral TKA with a mobile-bearing prosthesis (Rotaglide, Corin, UK) on one side and a fixed-bearing prosthesis (NexGen-CR, Zimmer, USA) on the other. There were 21 female patients, and in 18 patients, the diagnosis was rheumatoid arthritis. The average age was 59.6 (35–78) years. In all procedures, the posterior cruciate ligament was retained and patella re-surfaced. The average follow-up in the mobile-bearing group was 98 (79–107) months and 96 (79–107) months in the fixed-bearing group. At the final follow-up, the knee score was 91.8 points and 91.1 points, respectively, and the function score 65.5 points. The range of motion was similar in the two groups (1.1–106.9°; 0.4–106.9°). Five patients favoured the fixed-bearing prosthesis, but 16 found no difference. In patients with bilateral TKA, there was no difference in the short-term result between mobile-bearing and fixed-bearing prostheses.

Résumé Le but de cette étude était de comparer les résultats à moyen terme des plateaux fixes et des plateaux mobiles dans l'arthroplastie totale du genou bilatérale (TKA). Vingt-deux malades ont eu une arthroplastie bilatérale avec une prothèse à plateau mobile (Rotaglide, Corin, ROYAUME-UNI) d'un côté et une prothèse à plateau fixe (NexGen-CR, Zimmer, USA) de l'autre. Il y avait 21 femmes et, pour 18 malades le diagnostic était polyarthrite rhumatoïde. L'âge moyen était 59.6 (35–78) ans. Dans tous les cas, le ligament croisé postérieur a été conservé et la rotule resurfacée. Le suivi moyen dans le groupe plateau mobile était 98 (79–107) mois et 96 (79–107) mois dans le groupe plateau fixe. Au dernier recul le score du genou était 91,8 points et 91,1 points respectivement et le score fonctionnel de 65,5 points. L'amplitude de mouvement était semblable dans les deux groupes (1.1–106.9° resp. 0.4–106.9°). Cinq malades préféraient la prothèse à plateau fixe mais 16 n'ont trouvé aucune différence. Chez les malades avec une arthroplastie bilatérale du genou il n'y avait aucune différence dans les résultat à court terme entre les prothèses à plateau fixe et celles à plateau mobile.

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Introduction

The mobile-bearing knee is designed to allow antero-posterior (AP) and rotational movement of the knee during flexion while keeping the loaded articular contact area to the maximum; other design goals include reducing wear on the polyethylene insert and improving the long-term performance of the implant. It is not yet clear, however, whether the mobile-bearing knee provides better results than the fixed-bearing knee (Fig. 1). For cases requiring bilateral total knee arthroplasty (TKA), we used a mobile-bearing implant for one knee and a fixed-bearing for the other and compared the short-term results between them under the same conditions of gender, age, body weight, diagnosis, bony quality and activity. In addition, we issued questionnaires to the patients to determine their subjective evaluation of both knee implant types.

Patients and methods

Of the 23 bilateral TKA cases treated from February 1996 to June 1998 for which we used a mobile-bearing implant for one knee and a fixed-bearing implant for the other, we

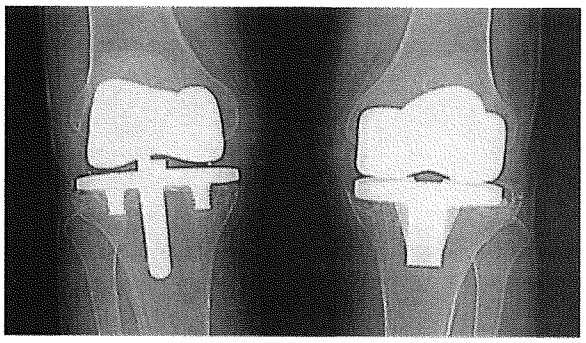

Fig. 1 Radiograph of bilateral total knee prostheses with a Rotaglide mobile-bearing implant in the right knee and a NexGen CR fixed-bearing implant in the left knee.

examined 44 knees of 22 cases (21 women and one man). One patient died. Average patient age at the time of operation was 59.6 (range 35–78) years. Diagnosis was rheumatoid arthritis (RA) in 18 patients (36 knees) and osteoarthritis (OA) in four patients (eight knees). For mobile-bearing knees, we used 22 Rotaglide prostheses (Corin, UK). The polyethylene-bearing insert of the Rotaglide allows 5 mm of AP translation and 25° of axial rotation on a polished metallic tibial tray. For the fixed-bearing knee, we used 22 NexGen CR prostheses (Zimmer, Warsaw, IN, USA). We performed a medial para-patellar approach, retained the posterior cruciate ligament and re-surfaced the patella in all knees. No cases required lateral patellar retinaculum release. The implant was fixed with cement in 40 knees of 20 cases, and we used a hybrid TKA (tibial and patellar fixation with cement and femoral fixation without cement) in four knees of two cases. In four cases, both knees were treated simultaneously and in 18 cases, they were operated on separately. The implant type was randomly selected for each knee.

For evaluation, we determined the American Knee Society knee scores, pain and function scores [4], range of motion (ROM), and joint line displacement pre-operatively and at final follow-up [3]. We also examined the femoro-tibial angle (FTA) pre-operatively and at the final follow-up. The angle of components, occurrence of radiolucent lines (RLL) [2], and complications at the final follow-up were recorded. Furthermore, we investigated the differences in subjective symptoms between the two knee groups based on the questionnaires. We used Mann–Whitney's *U* test for statistical verification.

Results

The average duration of follow-up was 98.6 (range 79–107) months for the Rotaglide group and 96.2 (range 79–107) months for the NexGen group. The average knee scores for the Rotaglide group were 26.1 (range 0–70) points pre-operatively and 91.8 (range 72–100) points at the final follow-up. For the NexGen group, average scores were 28.2 (range 3–70) points pre-operatively and 91.1 (range 72–99) points at the final follow-up. The average pain scores of the Rotaglide group were 5.5 (range 0–30) points pre-operatively and 49.7 (range 45–50) points at the final follow-up. For the NexGen group, average scores were 7.3 (range 0–30) points pre-operatively and 50.0 (range 50–50) points at the follow-up. Average function scores were 15.2 (range 0–55) points pre-operatively and 65.5 (range 0–100) points at the follow-up. For two cases the final function score was 0 points due to polyarthritis caused by RA in one and paralysis as an after effect of cerebral infarction in the other.

Pre-operatively, the average ROM of the Rotaglide group was from 10.5° (range 0–35° fixed flexion) to 113.2° (range 60–140°); that of the NexGen group was from 12.8° (range 0–30° fixed flexion) to 107.8° (range 45–140°). At the final follow-up, the average ROM of the Rotaglide group was from 1.1° (range 0–15°) to 106.9° (range 85–125°); that of the NexGen group was from 0.4° (range 0–5°) to 106.9° (range 85–120°) (Table 1).

Table 1 Summary of results. (*FTA*=Femoro-tibial angle)

		Rotaglide	Range	NexGen CR	Range	*p* value
Pre-operative knee score	Points	26.1	0–70	28.2	3–70	0.30
Pre-operative pain score	Points	5.5	0–30	7.3	0–30	0.74
Pre-operative flexion contracture	Degrees	10.5	0–35	12.8	0–30	0.91
Pre-operative flexion angle	Degrees	113.2	60–140	107.8	45–140	0.64
Pre-operative FTA	Degrees	175.6	156–195	176.6	165–195	0.79
Follow-up period	Month	98.6	79–107	96.2	79–107	0.58
Post-operative knee score	Points	91.8	72–100	91.1	72–99	0.82
Post-operative pain score	Points	49.7	45–50	50.0	50–50	1.00
Post-operative flexion contracture	Degrees	1.1	0–15	0.4	0–5	0.96
Post-operative flexion angle	Degrees	106.9	85–125	106.9	85–120	0.79
Post-operative FTA	Degrees	173.2	165–178	172.5	164–180	0.36
angle	Degrees	96.8	92–102	97.0	93–105	0.60
angle	Degrees	89.3	83–94	89.8	84–94	0.73
angle	Degrees	6.7	0–15	6.3	1–15	0.60
angle	Degrees	82.5	75–89	83.6	80–90	0.47
Change of joint line	Millimeters	4.9	0–8	6.3	2–15	0.57

The average post-operative change of joint line was 4.9 (range 0–8) mm higher for the Rotaglide group and 6.3 (range 2–15) mm for the NexGen group. Pre-operatively, the average FTA was 175.6° (range 156–195°) in the Rotaglide group and 176.6° (range 165–195°) in the NexGen group. At the final follow-up, the average FTA was 173.2° (range 165–178°) in the Rotaglide group and 172.5° (range 164–180°) in the NexGen group (Table 1). Average angles of components determined in the final follow-up were =96.8° (range 92–102°), =89.3° (range 83–94°), =6.7° (range 0–15°), and =82.5° (range 75–89°) for the Rotaglide group and =97.0° (range 93–105°), = 89.8° (range 84–94°), =6.3° (range 1–15°), and =83.6° (range 80–90°) for the NexGen group (Table 1). At the final follow-up, a 1 mm or larger RLL was seen in six Rotoglide knees and three NexGen knees. For the Rotaglide group, the distribution of the lines was five in zone 1 of the frontal view of the tibia, one in zone 2, three in zone 3, and one in zone 2 of the lateral view of tibia; and for the NexGen group, the distribution was two in zone 1 of the frontal view of the tibia, two in zone 2, and two in zone 4. During the follow-up, we found no statistically significant difference ($p>0.05$) between the Rotaglide and NexGen groups with regard to, the knee score, pain score, ROM, and FTA before and after operation. Change of joint line, the angle of components and RLL were similar in both groups. Nor did we recognize any post-operative complication such as infection, wear, instability, dislocation, or patellar disorder.

In the questionnaire, 16/22 patients answered that they did not notice any difference between their two knees. Of the remainder, five claimed that their NexGen knee was better than the other, and one that the Rotaglide was better. Subjective symptoms reported for the Rotaglide knee but not for the NexGen included swelling for three knees, less flexion angle than contra-lateral side for three knees, knocking sound for two knees, minor pain for one knee, sense of discomfort for one knee, and clicking for one knee. Subjective symptoms reported for the NexGen knee but not for the Rotaglide included swelling for one knee and instability for one knee.

Discussion

The mobile-bearing knee was developed to disperse the stress on tibial components and reduce both polyethylene wear and loosening of fixation, but the effects of a mobile insert on the dynamic state inside a living organism, such as the ROM and stability, are unknown. Several reports, compare a mobile-bearing prosthesis for one knee and fixed-bearing one for the other in bilateral TKA cases, under the same conditions of gender, age, body weight, bony quality, and activity of the subjects [1, 5–7]. Kim et al. [5] reported the clinical outcome after more than 6 years of follow-up after simultaneous bilateral posterior-cruciate-retaining TKA cases using LCS (DePuy, Warsaw, IN, USA) meniscal-bearing prosthesis and AMK (DePuy) fixed-bearing prosthesis. Chiu et al. [1] reported short-term results of bilateral TKA cases using LCS (DePuy) rotating-platform prosthesis and AMK (DePuy) posterior-stabilized, fixed-bearing prosthesis. Ranawat et al. [7] reported short-term results of bilateral TKA using PFC Sigma (DePuy) posterior-stabilized rotating-platform and posterior-stabilized fixed-bearing prostheses with the same femoral components. These studies reported that there was no difference between the mobile-bearing and fixed-bearing groups for clinical scores and ROM. Price et al. [6] reported clinical results at 1-year follow-up of bilateral posterior-cruciate-retaining TKA cases using TMK (Biomet, UK) mobile-bearing prosthesis and AGC (Biomet) fixed-bearing prosthesis. This study reported that clinical scores and pain scores for the mobile-bearing group were slightly better than those for the fixed-bearing group but there was no difference in ROM. Our results indicated no difference between the mobile-bearing and fixed-bearing groups for knee scores, ROM, and the occurrence of RLLs.

The questionnaires completed by the patients, however, tended to indicate more subjective symptoms for the mobile-bearing knee. In particular, such symptoms as reduced flexion, knocking sounds, feelings of discomfort, and clicking seemed to be related to the mobile bearing insert mechanism. For the mobile-bearing TKA, more importance is attached to balancing the soft tissue. We assumed that these subjective symptoms occurred as a result of incomplete soft-tissue balancing. The follow up period was not sufficient to evaluate the reduction in wear of inserts and/or loosening of implants, which constitutes a design concept of the mobile-bearing knee. A longer-term observation of the two groups used in this study is necessary to fully evaluate the usefulness of the mobile-bearing knee. We found no statistically significant differences between the mobile bearing and fixed-bearing implants in midterm performance evaluations of bilateral TKA cases. However, the patients tended to notice more subjective symptoms with the mobile-bearing group.

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運動についての評価

小池 達也*

骨粗鬆症および関連する骨折は，高齢化が進行する社会において，重要な問題になっている。骨量が減少した状態である骨粗鬆症においては，目に見えない形で骨の微細構造が破綻しており，骨強度が減少し軽微な外傷により骨折を引き起こす。これまで運動は，骨量を増加維持する有効な手段であると考えられてきた。スポーツ選手を対象とした横断分析では，運動と骨量の間に有意な相関が認められてきたが，もともと体格の大きい人がスポーツ活動を行う傾向があるというバイアスが入り込んでいる可能性がある。一般高齢女性において，身体活動性が高い群に大腿骨頸部骨折が少ないという報告もあるが，骨を増加させるような激しい運動によって得られる骨量増加効果はわずかであり，ビスホスホネート製剤を投与した時よりも劣る。骨粗鬆症の予防法として運動を評価する場合には，転倒防止や骨折予防に焦点を当てるべきである。

Evaluation of Exercise

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Osteoporosis and osteoporotic fractures have become an epidemic in the industrialized world. Osteoporosis, low bone mass, is a silent condition with microarchitectural deterioration of the bone structure leading to decreased bone strength and osteoporotic fractures. Physical activity has been advocated as offering a potential means to increase and maintain bone mineral density. Previous cross-sectional studies showed that there is a strong association between exercise and bone mineral density, especially in athletic individuals. However, there might be a self-selection bias ; i.e. individuals with larger muscles and bones are more likely to choose an athletic lifestyle. Although there is a report that physical activity is associated with a reduced risk for hip fracture among older community-dwelling women, the effects of vigorous exercises building bone mass is modest and considerably less than bisphosphonates. The proper evaluation of exercise as a preventative therapy for osteoporosis should focus on prevention of falls or osteoporotic fractures.

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はじめに

骨粗鬆症は，文明化が進み高齢者人口が増加している国々において，非常に重要な問題になりつつある。最も大きな問題は大腿骨頸部骨折や脊椎変形であり，これらのイベントが発生することにより，高齢者の日常生活動作は極度に制限され，非介護人口の増加に結びつくため，医療経済学的に見ても解決策の確立が急務である。

骨粗鬆症には，他の退行期疾患同様に危険因子が存在し，逆に危険因子に対する予防措置をとることで予防や治療に結びつく可能性がある。その一つが身体活動性である。これまで多くの研究が行われ，横断分析において身体活動性と骨量の正の相関が報告され，骨折との関係も論じられてきた。しかし，骨量増加を目的とした縦断分析においては，緩やかな効果しか報告されておらず，目標設定が誤っているという指摘[1]もある。

本稿では，これまでの運動と骨粗鬆症の関係を論じた研究の変遷をたどってみたい。

骨量に及ぼす運動の影響

不動性は骨量低下の重要な因子であり，運動により骨量が増える程度に比して，非動化による骨量減少はより顕著である。健常人に強制的にベッドレストを行わせても骨量低下は観察されるし[2]，脳卒中や脊髄損傷により片麻痺や対麻痺が生じた場合にも，運動機能を失った四肢には著明な骨量減少が生じる。極端な例では，宇宙空間で無重力状態での生活を行うと，バネやゴムを用いて強力な運動を行っても骨量低下を完全に防止することはできない[3]。

逆に，骨に負荷をかけることによって，骨密度は増加する。多くの横断分析は，荷重負荷運動により，peak bone massが高くなると報告している。20歳前後の男子大学生を対象に我々が行った研究においても，短距離走・野球・バレーボール・柔道などの衝撃力あるいは荷重負荷がかかる運動を日常的に行っている群が，運動を行っていない群よりも高い骨塩量を示した（図1）。

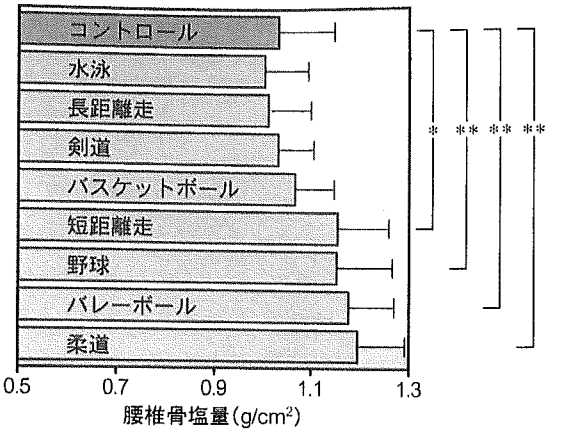

図1　運動種目別の腰椎骨塩量

男子大学生180人(平均年齢20歳)の腰椎骨塩量を種目別に比較。体重補正済み。
*：$p < 0.05$，**：$p < 0.01$，共分散分析。
（筆者作成）

運動効果と年齢の関係

骨粗鬆症は高齢女性において最も大きな問題となるが，低骨塩量は成人前から存在する可能性がある。高いpeak bone massを有する人たちは，その後の骨量減少に耐えうると考えるのは妥当な推論であろう。従って，骨量を高めるような運動をいつから開始すべきであるかという疑問が生じる。初潮前の少女に対して衝撃性の強い運動を10カ月間処方することにより，筋力も骨量も増加し得たとする報告[4]や，思春期前の少年に対する8カ月間にわたる中程度の運動負荷による骨量増加[5]の報告から考えて，成長期の骨は運動負荷に対して感受性が高いと考えられる。しかも，これらの運動効果は成人後も維持されていると考えられる[6]ので，成長期に運動を行うことは，将来の骨粗鬆症を予防する意味で非常に重要であろう。

では，すでに骨粗鬆症の危険年齢域にある閉経前後の女性に対する運動効果は存在するのだろう

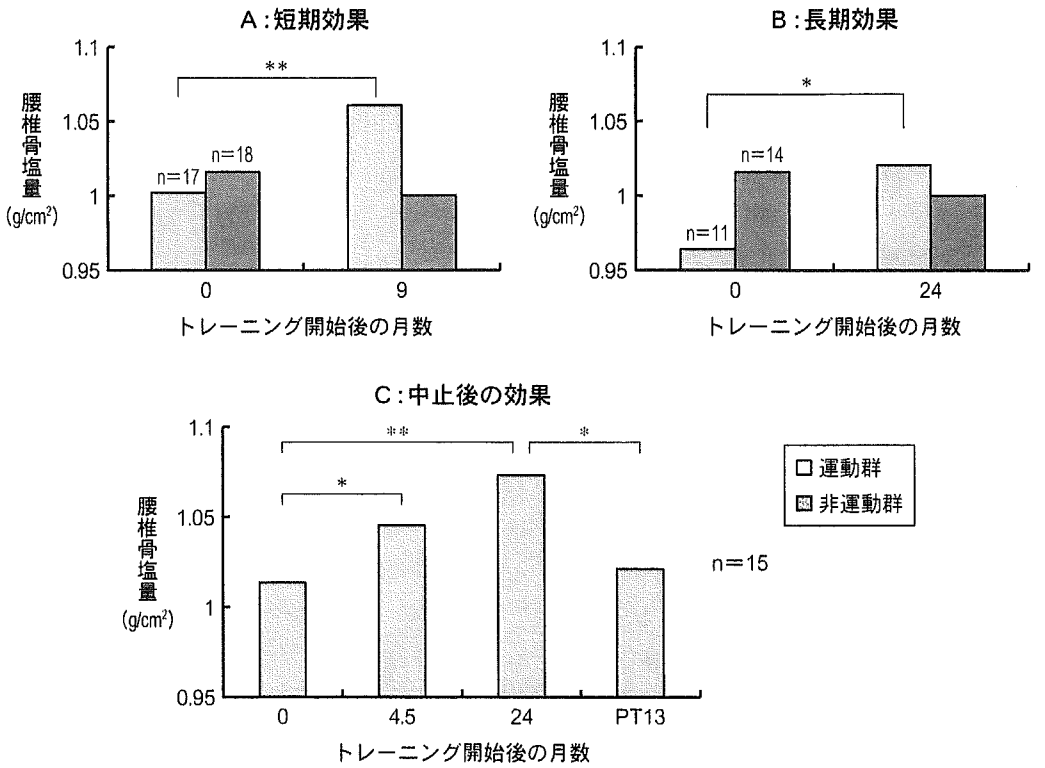

図2　運動効果が腰椎骨塩量に与える影響

55～70歳の特に運動をしていない女性を対象に，最大酸素摂取量の70～90%の負荷となるように，週に3回1時間の荷重運動（歩行，ジョギング，階段昇降）を行わせた。

A：短期効果。運動群は9カ月後に有意に腰椎骨塩量が増加したが，非運動群では有意ではないものの骨塩量の低下を認めた。

B：長期効果。24カ月でも同様の効果を認めた。

C：中止後の効果。運動負荷群のみの経過を見ると，運動負荷後24カ月までは有意に腰椎骨塩量は増加したが，運動を中止すると，その効果は消失した。

PT13：運動負荷終了後13カ月。

*：$p < 0.05$，**：$p < 0.01$

（文献8より筆者作成）

か？Wallaceらによるメタアナリシスの結果[7]では，最も多く研究対象となっていた閉経後女性の腰椎骨塩量に対しては，衝撃性の強い運動（エアロビクスなど）でも，衝撃性のない運動（ウエートトレーニングなど）でも，骨量増加作用が認められた。一方，大腿骨骨塩量に対しては，衝撃性のある運動では骨量減少防止効果が認められたが，衝撃性の少ない運動では，研究の数が少ないこともあって明確な結論は得られていない。

Dalskyら[8]は，人数は少ないが縦断分析で閉経後の女性に対して運動負荷を行い，運動終了後の影響も観察している。彼らの結果を図2に示す。短期であっても長期であっても，荷重負荷運動は腰椎骨塩量を増加させうるが，運動を終了するとその効果は消失するようである。少なくとも，閉経後に始めた骨量維持運動には継続が必須である。

ところで，骨粗鬆症の治療目標は時代とともに

変化しており，疼痛の除去から骨量増加へ，そして骨折予防へと変遷してきた。実際に，近年開発されてきたビスホスホネート製剤などは，はっきりとした骨折抑制効果が証明されている。では，運動にはそのような効果が存在するのだろうか？

骨粗鬆症に伴う骨折のリスクファクターとしての運動の影響

日常生活レベルでの活動性が，脊椎変形に及ぼす影響を観察したヨーロッパでの横断分析の報告[9]がある（図3）。50歳以上の男女14,261人を対象にした研究で，過去の活動レベルを年代ごとに聴取し，脊椎変形との関係を論じている。結果は，男性では激しい活動性を続けた場合に脊椎変形が強く認められたが，女性ではそのような影響は認められなかった。彼らは同時に，現在の屋外での活動性を調査し，女性において屋外で良く歩く女性に脊椎変形が少なかったとも報告している。この結果は，女性と男性において，脊椎変形の機序が異なる可能性を示しており，骨粗鬆症治療の戦略においても男女別の方法を考える必要性があるかもしれない。

身体活動性が，脊椎以外の骨折発生にも影響しているかを調べた研究も存在する。ノルウェーで行われた16,676人（平均年齢男性47.3歳，女性45.1歳）を対象とした大規模調査で，調査期間中に1,435件の非脊椎骨折が生じ，女性ではどの部位の骨折も年齢に伴い増加したが，男性ではそのような傾向は認められなかった。45歳以上の対象者のうち，活動性が最も高い群において荷重骨の骨折が有意に少なかった（相対危険率0.6）が，非荷重骨では活動性と骨折の間に有意な関係は認められなかった。

より高齢の女性（65歳以上）を対象としたコホート研究では，活動性の高い女性において，年齢・栄養・転倒・機能健康状態を補正した上でも，大腿骨頸部骨折の発生頻度が低いことが報告[10]されている（図4）。身体活動性を5分割し，上位2群に限れば，活動性の一番低い群に比して，実に36%の大腿骨頸部骨折発生率の低下を認めている。この低下は，ビスホスホネートの大規模試験の結果[11]に匹敵する。しかし，これらの結果は，脊椎骨折や前腕骨骨折には当てはまらなかった。

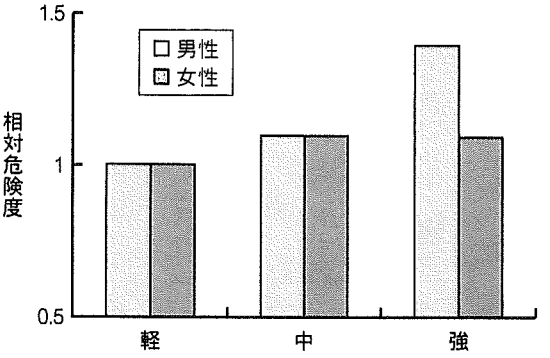

図3　脊椎変形に及ぼす過去の身体活動性の影響

50～79歳の男女14,261人にアンケート調査と脊椎レントゲン撮影を行い，過去の活動性をアンケートから評価し，脊椎変形との関係を調査。活動性は，軽・中・強の三段階に分けている。

（文献9より筆者作成）

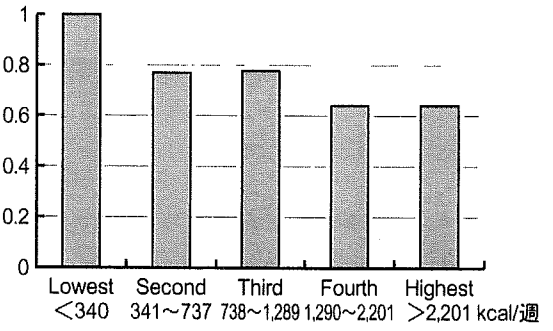

図4　身体活動性別に見た大腿骨頸部骨折発生相対危険度

9,704人の65歳以上の白人女性を対象に行ったコホート研究。週毎の消費カロリーを基準にして活動性を5段階に分けて，各群における大腿骨骨折発生頻度を比較。年齢・栄養・転倒・機能健康状態を補正。

（文献10より筆者作成）

骨粗鬆症に伴う骨折予防に対する運動の効果

高齢者における運動の効果が骨折予防に結びつくとすれば，転倒予防を介しての効果であると考えられる。転倒危険因子に関しても多くの研究があり，それから得られた結果をもとにして，多くの転倒予防プログラムが考案されている。多くの研究は，介入法として運動プログラムを用いているが，その効果は完全には証明されていない。Robertsonらによるメタアナリシス[12]では，筋力強化とバランス改善プログラムにより，転倒は35%減少したが，重度外傷発生に関しては効果がなかったと結論している。

骨折を生じる転倒は，全転倒の10%程度にすぎないこともあり，骨折抑制を目標とした介入試験で有意な結果は出ていない。

おわりに

非運動性が骨量減少や骨折の危険因子として存在することは間違いない。しかし，これまでの研究は，運動処方により骨量が増えること，あるいは維持できることを証明することに焦点を当てすぎてきたきらいがある。骨粗鬆症の薬物治療の目標が，骨量増加から骨折予防に変化してきたように，骨粗鬆症の治療予防戦略の一環として運動プログラムを考えるならば，骨折予防を視野に入れた研究がなされるべきである。その際には，転倒予防がキーワードになるであろう。

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Ⅲ．栄養と運動は骨粗鬆症予防に役立つか

最も有効な骨粗鬆症の運動療法

小池　達也*

骨粗鬆症および関連する骨折は，高齢化が進行する社会において，重要な問題になっている。骨量が減少した状態である骨粗鬆症においては，目に見えない形で骨の微細構造が破綻しており，骨強度が減少し軽微な外傷により骨折を引き起こす。これまで，運動は骨量を増加維持する有効な手段であると考えられてきた。スポーツ選手を対象とした横断分析では，運動と骨量の間に有意な相関が認められてきたが，もともと体格の大きい人がスポーツ活動を行う傾向があるというバイアスが入り込んでいる可能性がある。一般高齢女性において，身体活動性が高い群に大腿骨頸部骨折が少ないという報告もあるが，骨を増加させるような激しい運動によって得られる骨量増加効果はわずかであり，ビスホスホネート製剤よりも劣る。骨粗鬆症の予防法として運動を評価する場合には，転倒防止や骨折予防に焦点を当てるべきである。

The best physical therapy for osteoporosis

Rheumatosurgery, Osaka City University Medical School

Koike Tatsuya

Osteoporosis and osteoporotic fractures have become an epidemic in the industrialized world. Osteoporosis, low bone mass, is a silent condition with microarchitectural deterioration of the bone structure leading to decreased bone strength and osteoporotic fractures. Physical activity has been advocated as offering a potential means to increase and maintain bone mineral density. Previous cross-sectional studies showed that there is a strong association between exercise and bone mineral density, especially in athletic individuals. However, there might be a self-selection bias; i.e. individuals with larger muscles and bones are more likely to choose an athletic lifestyle. Although there is a report that physical activity is associated with a reduced risk for hip fracture among older community-dwelling women, the effects of vigorous exercises building bone mass is modest and considerably less than bisphosphonates. The proper evaluation of exercise as a preventative therapy for osteoporosis should focus on prevention of falls or osteoporotic fractures.

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はじめに

骨粗鬆症は，文明化が進み高齢者人口が増加している国において，認知症と並び非常に重要な問題である。骨粗鬆症によって引き起こされる大腿骨頸部骨折や脊椎変形が問題であり，これらのイベントが発生することにより高齢者の日常生活動作は極度に制限され，被介護人口の増大につながる。

骨粗鬆症には他の退行期疾患同様に危険因子が存在し，それらの危険因子を除去することによって，予防や治療に結びつく可能性がある。危険因子には，遺伝・ライフスタイル・疾患・外傷などがあるが，介入できるとすれば身体活動性のコントロールであろう。

これまで多くの研究が行われ，横断分析において身体活動性と骨量の正の相関が報告され[1)~3)]，骨折との関係も論じられてきた[4)]。しかし，骨量増加を目的とした縦断分析においては緩やかな効果しか報告されておらず[5)]，目標設定が誤っているという指摘もある[6)]。では，現時点で最も有効な骨粗鬆症の運動療法は何で，その目的はどこにあるのだろうか。

不動性と骨代謝

不動性は骨量低下の重要な因子であり，運動により骨量が増える程度に比して，不動化による骨量減少はより顕著である。健常人に強制的にベッドレストを行わせても骨量低下は観察されるし[7)]，脳卒中や脊髄損傷により片麻痺や対麻痺が生じた場合にも，運動機能を失った四肢には著明な骨量減少が生じる。極端な例では，宇宙空間で無重力状態での生活を行うと，バネやゴムを用いて強力な運動を行っても骨量低下を完全に防止することはできない。従って，極端な不動性は骨代謝にとって不利であることは間違いないが，運動を行うことが骨代謝にとってどの程度有利であるのかははっきりしない。

運動種目と骨塩量

運動種目と骨塩量の関係を調査した数多くの研究は，ハイレベルスポーツ[8)]であってもアマチュアレベルスポーツ[9)]であっても，荷重負荷がかかる運動（重量挙げなど）の方が非荷重運動（水泳など）よりも高い骨塩量を獲得できるという結果で一致している。

しかし，これらの研究には大きなバイアスが含まれている。体格が大きいために重量挙げを選択し，身長が高いためにバスケットボールに参加したかも知れないからである。

同一人物の身体各部位の骨塩量を比較することで，この問題の答えを得ようとした研究がある。Huddlestonら[10)]は，比較的高齢まで長期にわたってテニスを続けてきた35名のテニスプレーヤーの利き手側前腕骨骨量が，反対側より有意に高いことを示した。また，Morelら[9)]は全身骨塩量に対する身体各部位の骨塩量の比率を種目別に検討し，サッカー・長距離では足の比率が高く，登山・水泳では腕の比率が高いことを報告している。

これらの事実は，運動による骨量増加作用が身体各部位に特異的に及んでいることを示しており，特定の部位に非日常的負荷がかかることが重要であることが分かる。

運動を始める時期

骨粗鬆症は高齢女性において最も大きな問題となるが，低骨塩量は成人前から存在する可能性がある。高いpeak bone massを有する人たちは，その後の骨量減少に耐え得ると考えるのは妥当な推論であろう。従って，骨量を高めるような運動をいつから開始すべきであるかという疑問が生じる。

初潮前の少女に対して衝撃性の強い運動を10カ月間処方することにより，筋力も骨量も増加し得たとする報告[11)]や，成長期の活動性が骨塩量増

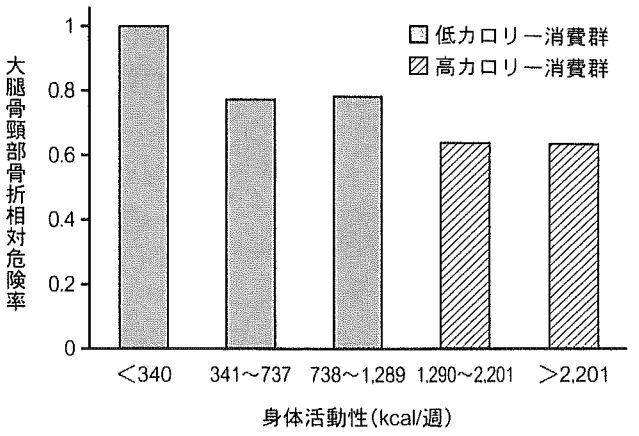

図1　身体活動性別に見た大腿骨頸部骨折相対危険率

9,704名の65歳以上の白人女性を対象に行ったコホート研究。週毎の消費カロリーを基準にして活動性を5段階に分けて，各群における大腿骨骨折発生頻度を比較。最少カロリー摂取群の骨折危険率を基準に，縦軸は相対危険率。年齢・栄養・転倒・機能健康状態を補正。1,290kcal以上の高カロリー消費群のみが，最低カロリー群に比して有意に骨折発生頻度が少ない。

（文献16より作成）

加に作用すること[12]から考えて，成長期の骨は運動負荷に対して感受性が高いと考えられる。しかも，これらの運動効果は成人後も維持されていると考えられるので，成長期に運動を行うことは将来の骨粗鬆症を予防する意味で非常に重要であろう。

では，既に骨粗鬆症の危険年齢域にある閉経前後の女性に対する運動効果は存在するのだろうか。Wallaceらによるメタアナリシスの結果[13]では，最も多く研究対象となっていた閉経後女性の腰椎骨塩量に対しては，衝撃性の強い運動（エアロビクスなど）でも衝撃性のない運動（ウエートトレーニングなど）でも，骨量増加作用が認められた。

一方，大腿骨骨塩量に対しては，衝撃性のある運動では骨量減少防止効果が認められたが，衝撃性の少ない運動では研究の数が少ないこともあって明確な結論は得られていない。

Dalskyら[14]は，縦断分析で閉経後の女性に対して運動負荷を行い，運動終了後の影響も観察している。短期であっても長期であっても，荷重負荷運動は腰椎骨塩量を増加し得るが，運動を終了するとその効果は消失するようである。少なくとも，閉経後に始めた骨量維持運動には継続が必須である。

骨粗鬆症の治療目標

骨粗鬆症の治療目標は時代とともに変化しており，疼痛の除去から骨量増加へ，そして骨折予防へと変遷してきた。実際，近年開発されてきたビスホスホネート製剤などは，はっきりとした骨折抑制効果が証明されている[15]。

運動を骨粗鬆症の治療法として捉えた場合，骨折を抑制するのに十分なだけ骨量を増加させ得るとは考えにくい。しかし，身体活動性をもとに大腿骨頸部骨折の発生頻度を比較すると，明らかに活動性が高いものほど骨折が少ない（図1）[16]。このことは，運動療法の目的が骨量増加ではなく，