Overexpression of the Human Interleukin 1α Gene Causes Osteopenia in Mice

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ABSTRACT. ***Objective.*** Osteoporosis is a major complication of chronic inflammatory diseases such as rheumatoid arthritis (RA). We describe disordered bone metabolism in transgenic mice that overexpress human interleukin 1α (hIL-1α).

Methods. Bone mineral density (BMD), microcomputed tomography (μCT), histomorphometry, and blood biochemical data of hIL-1α transgenic mice and littermate wild-type mice were examined.

Results. The femoral BMD of transgenic mice was decreased by 27.7% compared with wild-type mice. μCT revealed a marked reduction in the trabecular bone, and cortical thinning with an enlarged cavity was observed in femora of transgenic mice. Histomorphometric analysis revealed inhibition of several measures of bone formation, while the serum alkaline phosphatase level was reduced in transgenic mice; however, their indices of bone resorption were not elevated.

Conclusion. Overexpression of hIL-1α causes osteopenia in mice. It was suggested that the systemic osteopenia in these transgenic mice occurred primarily as a result of decreased bone formation, with a reduction of bone mineralization rather than increased osteoclastic bone resorption. This may be one aspect of bone metabolism in RA that results in disease complications. (J Rheumatol 2005;32:320–4)

Key Indexing Terms:
INTERLEUKIN 1 OSTEOPENIA TRANSGENIC MICE RHEUMATOID ARTHRITIS

Patients with chronic arthritis, such as rheumatoid arthritis (RA), are known to have a reduced bone mineral density (BMD) and are at risk of pathological fractures[1-4]. Although the etiology of generalized bone loss in RA is unknown, changes in circulating levels of interleukin 1 (IL-1) and tumor necrosis factor-α (TNF-α) have been implicated in the pathogenesis of RA associated systemic bone loss[5-7]. As these proinflammatory cytokines are highly expressed in this systemic chronic inflammatory disorder, they are believed to play an important role in development of the disease[8-10].

The role of IL-1 has been investigated[9,11], but its effect upon bone metabolism, especially on bone formation, is still controversial. It has been reported that IL-1 plays an important role in the bone resorption that may accompany inflammatory disease[11]. Subcutaneous injection of IL-1 increases osteoclast number and stimulates osteoclast activity[12-14], but there have been no reports regarding the effects of general overexpression of IL-1 *in vivo*. Recently we generated transgenic (Tg) mice overexpressing the human IL-1α gene and reported macrophage- and neutrophil-dominant polyarthritis in Tg mice[15]. Our Tg mice showed bilateral symmetrical polyarthritis, which started with the ankles at 3–4 weeks of age. The arthritis extended to the proximal joints with cartilage destruction, and a complete loss of joint movement was observed around 14 weeks of age[15]. In the present study, we examined the bone density and structure of IL-1 Tg mice that presented with general arthritis and littermate wild-type (Wt) mice in order to delineate the specific effects of IL-1 upon bone metabolism.

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MATERIALS AND METHODS

Animals. The generation of human hIL-1α Tg mice has been described[15]. Briefly, human IL-1α cDNA was ligated into a plasmid containing the cytomegalovirus enhancer/ß-actin promoter. The resulting construct was used for microinjection, and 2 mouse lines, Tg 1705 and 1706, were established.

Seven- to nine-week-old Tg mice (n = 7) and nontransgenic littermate Wt mice (n = 6) of the Tg 1706 line (C3H background) were used. All Tg mice were in the stage of progressing polyarthritis, but joint movement was still retained. Mice were maintained under specific pathogen-free conditions after birth. Animals were maintained according to the protocol approved by the Committee on the Ethics of Animal Experiments at the National Defense Medical College.

Blood analysis. Blood samples were collected by cardiac puncture at sacrifice. Serum concentrations of calcium (Ca) and alkaline phosphatase (ALP) were determined with the AU5232 chemistry analyzer (Olympus Inc., Tokyo, Japan), while the serum phosphate level (Pi) was determined with the AU800 analyzer (Olympus).

Radiography, computerized tomography, BMD. The femora were extracted

from Tg and Wt mice and fixed with 70% ethanol after removal of the soft tissues. Bone radiographs of the whole body and the excised femora were taken with a soft x-ray apparatus (Type NSP1005, Sofron, Tokyo, Japan). Microcomputed tomographic (CT) analysis of the femoral metaphysis and diaphysis of femora was performed with a composite x-ray analyzing system (NS-ELEX, Tokyo, Japan). Femoral BMD was measured by dual-energy x-ray absorptiometry using a DCS-600R analyzer (Aloka, Tokyo, Japan).

Histomorphometric measurements. The tibiae were removed from both Tg and Wt mice, fixed with 70% ethanol, and embedded in glycol methacrylate without decalcification. Serial sections (3 µm thickness) were cut using a microtome (model 2050; Reichert Jung, Buffalo, NY, USA), and underwent ALP and titrate-resistant acid phosphatase (TRAP) staining to identify the cellular components. For double fluorescent labeling studies, all mice were subcutaneously injected with calcein (16 mg/kg body weight) 11 and 4 days before sacrifice.

Histomorphometric analysis of trabecular bone was performed in an area 1.8 mm long from 0.1 mm below the growth plate at the proximal tibial metaphysis using a semiautomated system (Osteoplan II; Carl Zeiss, Thornwood, NY, USA) with measurements made at ×400 magnification. Nomenclature, symbols, and units are those recommended by the American Society for Bone Mineral Research (ASBMR) Histomorphometry Nomenclature Committee[16].

Statistical analysis. Results are presented as mean value ± standard error of the mean (SEM). Statistical comparisons were performed using Student's t test for unpaired data. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Radiographic analysis. Plain radiographs and µCT images revealed decreased femoral bone density in the Tg mice. Destructive joint changes were observed in general, especially in the hip joints, where the femoral heads had collapsed and disappeared. Cortical thinning with an enlarged cavity was also observed in the diaphysis of the femora (Figure 1).

BMD of whole femora of Tg mice was decreased by 27.7% compared with Wt mice (Figure 2A). The femora were divided longitudinally into 20 equal regions and the BMD of each region measured (Figure 2B). BMD of the metaphysis was decreased more than that of the diaphysis, meaning that trabecular bone was more affected than cortex bone.

Histological analysis. Histological examination of the femora of Tg and Wt mice revealed a reduction in cortical thickness and trabecular volume (Figure 3).

Histomorphometric analysis revealed a significant reduction of trabecular bone volume (BV/TV) in Tg mice. Inhibition of several measures of bone formation, including mineral apposition rate (MAR), bone formation rate (BFR/BS), mineralizing surface (MS/BS), and mineralized bone volume (Md.BV/TV), was statistically significant compared to Wt mice, but osteoblast surface (Ob.S/BS) was not significantly different. Indices of bone resorption, including osteoclast number (N.Oc/B.Pm), eroded surface (ES/BS), and osteoclast surface (Oc.S/BS), were not significantly different. There also were no significant differences observed in osteoid thickness (O.Th) and osteoid surface (OS/BS) (Table 1). These data suggest that decreased bone formation, especially a reduction of bone mineralization, rather than increased bone resorption is the prime cause of the osteopenia evident in Tg mice.

Blood analysis. Serum Ca and Pi levels of transgenic mice were not so different from those in Wt mice, although the serum ALP level was lower in Tg mice compared to Wt mice (Table 2).

DISCUSSION

The cytokine IL-1 plays an important role in inflammation and inflammatory disease, such as RA, together with other cytokines such as TNF-α or IL-6[8,9]. We investigated bone metabolism and structure in Tg mice that were in the progressing stage of joint arthritis.

Our radiography and BMD results indicated a markedly reduced bone volume of femora of the Tg mice compared with Wt mice. µCT images and BV/TV of histomorphometry also revealed osteopenia in the Tg mice. We thereby confirmed for the first time that IL-1 induces osteopenia *in vivo*. Histomorphometric analysis revealed inhibition of MAR, BFR/BS, MS/BS, and Md.BV/TV in Tg mice, but their indices of bone resorption, including N.Oc/B.Pm, ES/BS, and Oc.S/BS, were not significantly different from those of Wt mice. These data suggest that the systemic osteopenia induced by overexpression of IL-1α resulted primarily from decreased bone formation, indicating that bone loss in Tg mice is due to a negative remodeling process rather than increased bone turnover.

Two functions of IL-1 with bone resorption are known. One is progressive differentiation of osteoclasts. It is reported that IL-1 induces osteoblasts to produce receptor activator of nuclear factor-κB ligand (RANKL), which is one of the essential factors required to differentiate osteoclast precursors and to activate osteoclasts. This means that IL-1 does not act directly on osteoclast precursors, but indirectly through RANK/RANKL interactions. On the other hand, there are some factors that can inhibit this process. For instance, it is known that granulocyte macrophage-colony stimulating factor (GM-CSF) inhibits osteoclast differentiation through reduction of c-Fos expression[17]. GM-CSF concentrations in sera and supernatants from synoviocytes and bone marrow macrophages of our Tg mice were found to be higher than those from littermates[15]. Moreover, the synovium of our Tg mice accumulated IL-1, TNF, IL-8, and matrix metalloproteinases[15]. The balance of some cytokines and factors that control the number of osteoclasts might normalize the differentiation of osteoclasts in our Tg mice. Another function of IL-1 is the activation and elongation of the life of osteoclasts. IL-1 can directly extend the life of osteoclasts, and induces the multinucleation and pit-forming activity of osteoclasts[18,19]. Further, the precursor form of IL-1 in the nucleus may act as an intracrine activator in osteoclasts[20]. This might be the reason behind the apparent

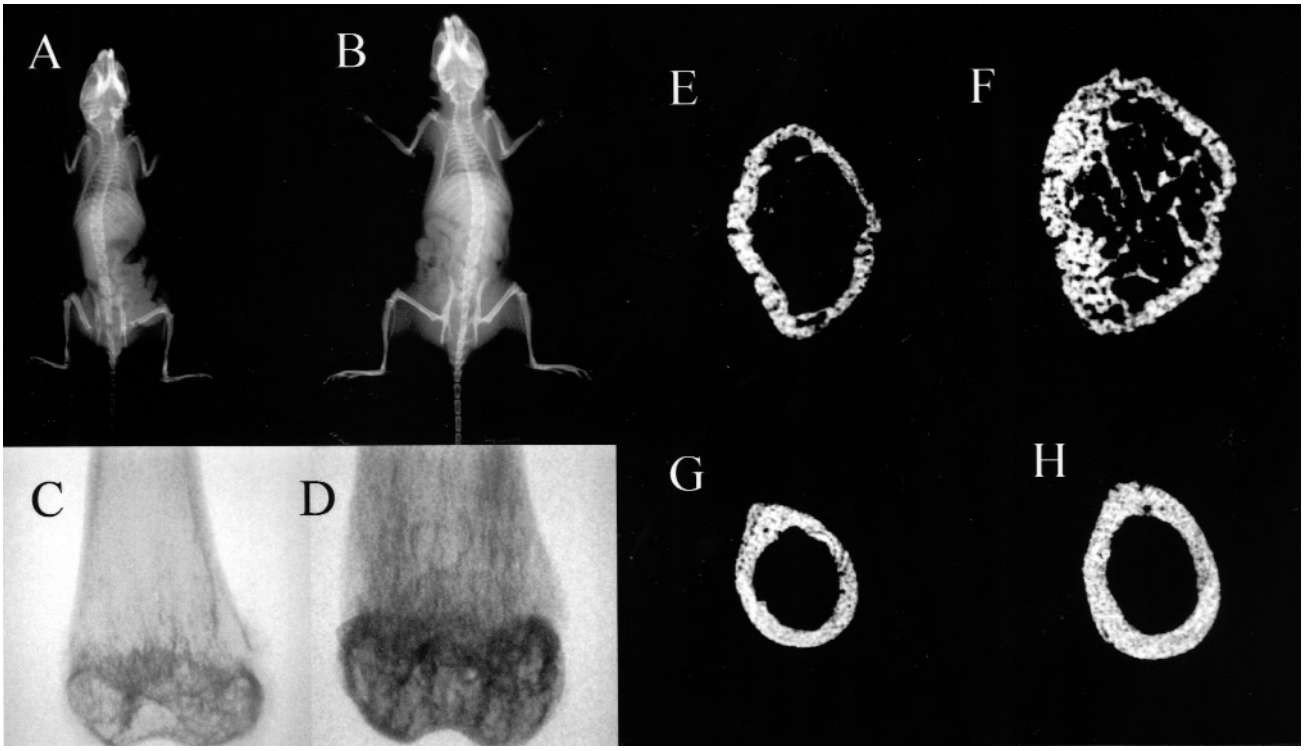

Figure 1. Plain radiographs and CT images of the femora of 7-week-old Tg and Wt mice. A. Radiographs of Tg mice; B. Wt mice. C. Distal femur of Tg mice; D. Wt mice. E. Transverse CT sections from the femoral epiphysis and diaphysis (F) of Tg and Wt mice (G, H).

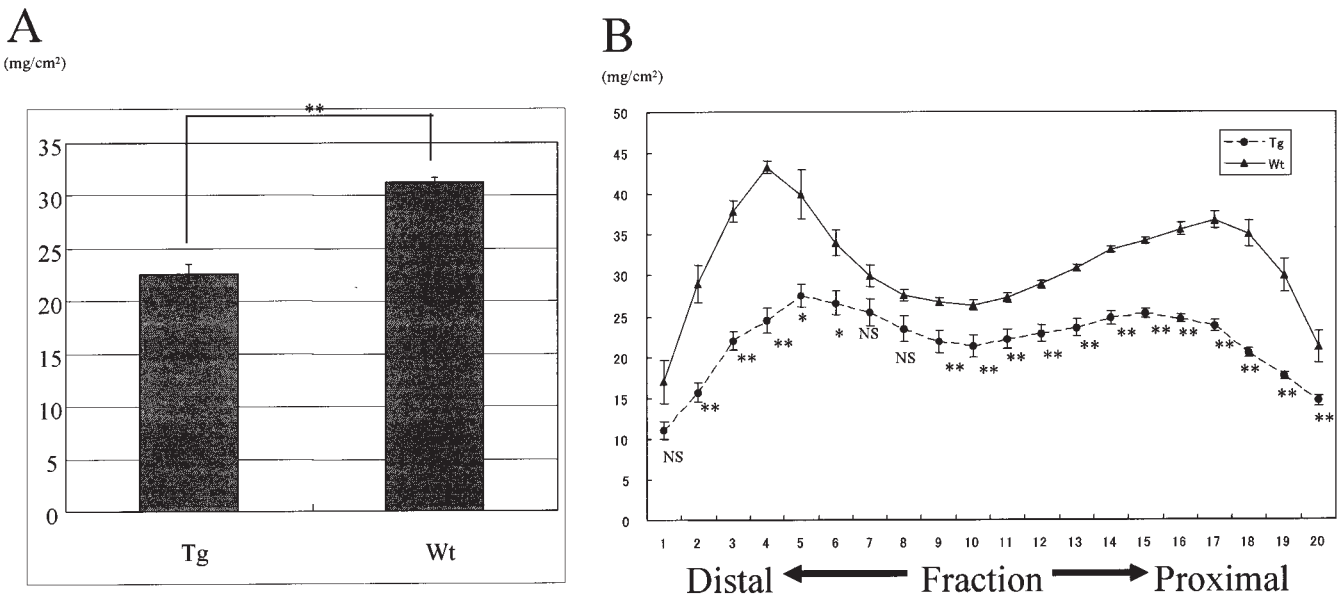

Figure 2. A. BMD of the whole femora. B. BMD of each of 20 equal longitudinal divisions of femora. *p < 0.05; **p < 0.01; NS: nonsignificant.

contradiction that bone volume is reduced in Tg mice despite the fact that the osteoclast number is not increased.

The function of IL-1 in bone formation is still unclear, but generally it is thought that IL-1 inhibits bone formation[21-23], which is consistent with our results. The mechanisms of inhibition of bone formation are suppression of the production of type I collagen[21] and suppression of ALP activity[22], and inhibition of parathyroid hormone-responsive adenylate cyclase in osteoblastic cells[23]. Little is known about the molecular mechanisms of bone mineralization,

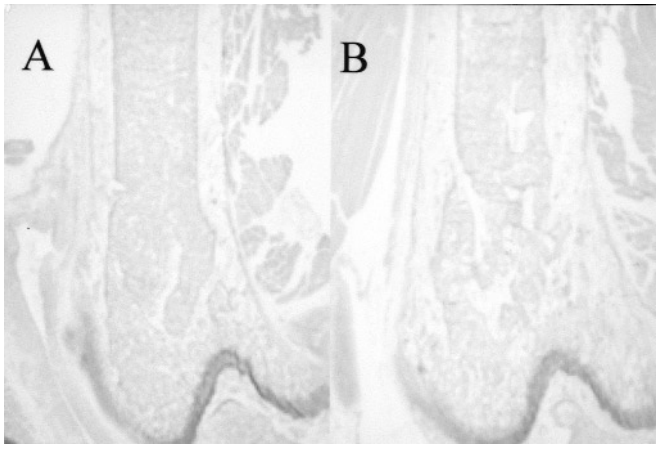

Figure 3. Histological features of femora of Tg (A) and Wt (B) mice (toluidine blue stain).

although it is apparent that ALP plays an important role[24,25]. The suppression of bone mineralization might, at least in part, be a consequence of the lower serum ALP present in Tg mice.

Our Tg mice expressed general joint arthritis and we assume that our mice can be used as a model of general chronic arthritis, such as RA. There have been many reports on bone metabolism in patients with RA. Generally, they conclude that the physiology of osteoporosis is increased bone resorption[26]. However, it is uncertain whether the general osteoporosis results from the disease itself or from related factors such as corticosteroids[27] and reduced physical activity[28,29] in patients with RA.

In our Tg mice, immobilization due to arthritis should be considered, since the osteopenia might have been caused by disuse atrophy. Some investigators have used animal models for analyzing disuse osteopenia; Weinreb, *et al*[30] reported that osteopenia caused by unilateral sciatic neurotomy is due to increased bone resorption and decreased bone formation. According to Rantakokko, *et al*[31], unilateral cast immobilization of the leg in mice induced reduced bone formation and increased bone resorption, resulting in osteopenia. The findings of osteopenia in our Tg mice are different from those of the immobilization models, and we therefore assume that the bone changes in our Tg mice are not caused by disuse due to arthritis.

Some reports have indicated the presence of low bone turnover with reduced bone formation in non-steroid-treated RA[32,33], which is not contradictory with our results indicating that chronic arthritis reduces bone formation. Moreover, it is reported that osteoclast functional activity rather than osteoclast formation is more likely to play a role in the generalized bone loss that occurs in RA[34]. These reports support our hypothesis that the decreased bone volume in Tg mice may be accounted for by stimulation of osteoclast bone-resorbing activity and enhanced osteoclast survival, rather than an increase in osteoclast formation.

It is certain that many complicated factors are involved in bone metabolism in RA that cannot be explained with a single cytokine. Further examination will be required to clarify the role of IL-1 and other cytokines on bone metabolism in RA.

Table 2. Results of serum biochemical analyses. Serum calcium (Ca), phosphorus (Pi), and alkaline phosphatase (ALP) levels of Tg and Wt mice. Data are expressed as mean ± SEM, n = 3 mice per group.

	Ca, mg/dl	Pi, mg/dl	ALP, IU/l
Wt	11.73 ± 0.12	12.33 ± 1.2	581.3 ± 9.8
Tg	12.27 ± 0.24	11.77 ± 1.3	390.3 ± 12.1

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Table 1. Histomorphometry of trabecular bones of tibiae. Trabecular bone samples were measured in an area 1.8 mm long from 0.1 mm below the growth plate at the proximal tibial metaphysis. Data expressed as mean ± SEM for 6 bones of Wt mice and 7 bones of Tg mice.

	BV/TV (%)	Md.BV/TV (%)	MAR (/day)	BFR/BS ($mm^3/cm^2/y$)	O.Th (m)	Ob.S/BS (%)	MS/BS (%)	ES/BS (%)	N.Oc/B.Pm (/100 mm)	Oc.S/BS (%)
Wt	6.44 ± 0.51	6.01 ± 0.60	1.89 ± 0.11	9.72 ± 1.3	3.81 ± 0.52	18.1 ± 4.4	14.4 ± 2.0	9.48 ± 1.6	545.6 ± 44.9	5.02 ± 0.72
Tg	2.72 ± 0.60**	2.55 ± 0.50**	1.36 ± 0.19*	4.23 ± 1.6*	3.46 ± 0.52	16.1 ± 3.8	7.1 ± 2.0*	9.05 ± 1.5	515.3 ± 72.6	5.36 ± 0.78

Significantly different from Wt mice: * $p < 0.05$, ** $p < 0.01$.

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