INTRODUCTION: The remodeling of bone is carried out in a highly coordinate fashion, and disturbance of this process results in an imbalance between resorption and formation and is responsible for most metabolic bone diseases including osteoporosis. The pathophysiology of osteoporosis can be classified into two, i.e., high-turnover type like post-menopausal osteoporosis, and low-turnover type like senile osteoporosis as characterized by a decrease in bone formation that exceeds the decrease in bone resorption. The cellular and molecular mechanism of these are not fully understood, since many molecules are considered to be involved.

We already reported that macrophage migration inhibitory factor (MIF), which has recently been re-evaluated as a mediator in various inflammatory diseases, is exclusively expressed in murine calvarial osteoblasts, and that MIF up-regulates mRNAs for MMP-9 and 13 in rodent osteoblasts; however, its precise role in the development or remodeling of bone tissues remained unclear. The purpose of this study is to clarify the role of MIF in osteoporosis by investigating the cellular and molecular abnormalities in the bone of MIF- transgenic (Tg) mice using molecular, three-dimensional, and histomorphometric analyses.

METHODS: Animals: Male wild-type and MIF-Tg (C57Bl/6) mice were used in this study. These mice were sacrificed at 4, 8, 12, and 16-week-old, and the sera were stored at -80°C until analysis. Right femora were fixed in 10% formaldehyde after removal of soft tissues.

Northern blot analysis: Total RNA isolated from femora of 8-weeks-old mice were electrophoresed, and hybridized with a probe for mouse MIF.

Microcomputed Tomography (µCT): The femoral cancellous bone of the distal femur was analyzed three-dimensionally by the µCT system (Hitachi Medical Corporation, Japan). Using 200 two-dimensional CT images of 8-μm thickness, a three-dimensional microstructural image was reconstructed to calculate trabecular bone volume (bone volume/ tissue volume; BV/TV), number, separation, and thickness. These parameters were quantified by an image analyzer (KGT, Japan).

Assays of serum and urine parameters: The serum contents of parathyroid hormone (PTH), blood urea nitrogen (BUN), and osteocalcin were measured by enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturers’ procedure. The urine deoxypyridinol (DPD)/creatinine levels were also measured by ELISA.

Histology and immunohistochemistry: Femora or tibiae from 4 and 8-weeks-old mice were decalcified, embedded in paraffin, and were stained for hematoxyline-eosine (HE), tartrate-resistant acid phosphatase (TRAP), alkaline phosphatase (ALP), and osteopontin (OPN).

Statistical analyses: The data were evaluated for statistical significance by two-way ANOVA followed by Fisher PLSD analysis for multiple comparison.

RESULTS: MIF mRNA expression and µCT analysis: Northern blot analysis showed a higher level of MIF mRNA expression in MIF-Tg femur than in wild-type femur (Fig. 1a). µCT examination of distal femoral trabecular bones revealed that in 8-weeks-old, the bone volumes were severely decreased in MIF-Tg compared with wild-type (Fig. 1b). Quantification of the three-dimensional trabecular bone volume in the femora indicated that the bone volume of the wild-type, expressed as BV/TV, decreased in association with aging (Fig. 2). In MIF-Tg, severe reduction of bone volume was already evident in as early as 8-weeks-old, and this tendency continued until 12-weeks-old. The difference of BV/TV were significant between wild-type and MIF-Tg (p<0.0001). In MIF-Tg, trabecular bone numbers were also decreased, and trabecular separation was also increased about 1.8 fold than that of wild-type (Fig. 2b).

Histological and immunohistochemical observation: We could identify enlarged hypertrophic zone in the growth plate in tibiae and femora in 4-weeks-old MIF-Tg. Using histochemical staining for TRAP, we found that the number of osteoclasts in the metaphysis were decreased. We also found that in MIF-Tg, the shape of osteoclasts in the metaphysis was not cuboidal but rather flattened, OPN-positive cement lines were decreased, and immuno-reactivity to ALP were also decreased.

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