0013-7227/98/$03.00/0
Endocrinology

Vol. 139, No. 6
Printed in U.S.A.

Mice Lacking the Type I Interleukin-1 Receptor Do Not Lose Bone Mass after Ovariectomy

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Abstract We measured the effects of ovariectomy on the bone mass of mice that lacked type I interleukin-1 receptor (IL-1 R1 -/- mice) in two genetic backgrounds (C57BL/6 x 129/Sv and C57BL/6) to investigate the role of interleukin-1 in the actions of estrogen on bone. At three weeks after surgery, ovariectomized wild-type mice decreased trabecular bone volume in the proximal humerus by 70% in a C57BL/6 x 129/Sv background and 48% in a C57BL/6 background compared to sham-operated controls. In contrast, there was no significant decrease in trabecular bone mass in IL-1 R1 -/- mice after ovariectomy. The estrogen status of all groups was confirmed by measurement of uterine wet weight. These results demonstrate that a functional IL-1 response pathway is required for mice to lose trabecular bone mass after ovariectomy in this model and they imply that IL-1 is an important mediator of the effects of ovariectomy on bone mass. Hence, therapeutic interventions that block the effects of IL-1 on bone may be beneficial for treating diseases of rapid bone loss such as post-menopausal osteoporosis.

Introduction

Estrogens are important regulators of bone turnover (1-2). Withdrawal of estrogen after either a natural or surgical menopause can result in marked decreases in bone mass, which predisposes women to osteoporosis. However, the mechanisms by which estrogen influences bone cell function are not completely understood. Cytokines such as interleukin-1 (IL-1), tumor necrosis factor α (TNF α) and interleukin-6 (IL-6) are likely mediators of this response (1, 3, 4).

The biology of IL-1 is complex (5, 6). Two IL-1 agonists (IL-1 α and IL-1β) and an antagonist (IL-1 receptor antagonist, IL-1 ra) are produced. In addition, there are two IL-1 receptors. Type I receptor (IL-1 R1) appears to mediate all known responses to IL-1α and β (7). In contrast, type II receptor (IL-1 R2) is a "decoy" receptor that has similar extracellular and transmembrane domains to IL-1 R1 but lacks critical intracellular elements that are necessary for post-receptor signaling (8).

We investigated the role of IL-1 in the bone loss that follows estrogen withdrawal by examining the effects of ovariectomy on the bone mass of mice that were genetically engineered to lack IL-1 R1 (IL-1 R1 -/- mice). We have previously found that the bone mass, growth rates and breeding success of these animals is similar to that of wild-type controls (9).

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Received: 01/28/98

Materials and Methods

Animals. IL-1 R1 -/- mice were produced at Immunex Research and Development Corp. (Seattle, WA). They were prepared by gene targeting using a method described previously (10). Briefly, IL-1 R1 -/- mice were generated by homologous recombination in embryonic stem cells using a targeting vector that replaced 2 exons encoding amino acids 4-146 of the IL-1 R1 extracellular domain with a neomycin resistance gene cassette. This mutation deleted sequences critical for IL-1 binding and cells derived from IL-1 R1 -/- mice failed to respond to IL-1.

The original strain of IL-1 R1 -/- mice was in a C57BL/6 x 129/Sv genetic background. Subsequently, mice were backcrossed through greater than 5 generations into a pure C57BL/6 background to create a second strain of IL-1 R1 -/- mice. The genotype of the mutant mice was confirmed by Southern blot analysis of tail genomic DNA.

Control animals for the IL-1 R1 -/- mice in a C57BL/6 x 129/Sv genetic background were F_2 generation mice from Jackson Laboratory (Bar Harbor, ME). Controls for IL-1 R1 -/- mice in a C57BL/6 genetic background were C57BL/6 mice from Charles River Farms (Wilmington, MA). All animal protocols were approved by the Animal Care and Use Committees of the University of Connecticut Health Center, Farmington, CT and the Department of Veterans Affairs Medical Center, Newington, CT.

Treatment Protocol. Wild-type or IL-1 R1 -/- mice (8–9 wks old) were sham-operated (SHAM), or ovariectomized (OVX) and sacrificed 3 weeks after surgery. In all experiments, uteri were isolated and wet uterine weights were measured to confirm the effects of treatment.

Histologic Processing. Mice were sacrificed by CO_2 narcosis followed by cervical dislocation. Humeri were dissected free of surrounding tissues and fixed at 4°C in 4% paraformaldehyde (PFA) and phosphate buffered saline (PBS). Following fixation, bones were decalcified at 4°C in 15% EDTA and 0.5% PFA in PBS for 4 wks, dehydrated in increasing concentrations of ethanol (70-100%), cleared in xylene and paraffin-embedded. Serial 5 μm thick sections were stained for tartrate resistant acid phosphatase (TRAP) (11) and counterstained with Harris hematoxylin (12, 13). Osteoclasts were identified by TRAP staining, characteristic morphology and their association with bone.

Histomorphometry. Measurements were performed under the light microscope (20X magnification) by a blinded observer. The proximal humerus and bone marrow area, excluding the cortical bone, was examined between 0.4 to 1.0 mm distal to the growth plate-epiphyseal junction. The bone marrow area of measurement was 0.75 to 0.85 mm^2. Areas and volumes were determined using a computerized image analysis system (Bioquant-R&M Biometrics, Nashville, TN). The following measurements and calculations (14) were performed: 1) percent trabecular bone volume (TBV), 2) trabecular perimeter, 3) trabecular number, 4) osteoclast number and 5) cortical width. Cortical width was measured 1.1, 1.6 and 2.1 mm distal to the growth plate and the mean was calculated. Measurements were performed on one representative bone section from each animal.

Statistical Analysis. Differences between groups were analyzed by the Student's t test for unpaired samples.

Results

Histomorphometric examination of proximal humeri from wild-type C57BL/6 x 129/Sv mice, 3 weeks after surgery, demonstrated that ovariectomy produced a 70% decrease in the TBV, a measure of trabecular bone mass (Figure 1A). Measurement of additional indices of trabecular bone mass (trabecular area and trabecular perimeter) confirmed a loss of trabecular bone in wild-type animals 3 weeks after ovariectomy (Table 1). In contrast, IL-1 R1 -/- mice in a 129Sv x C57BL/6 genetic background demonstrated no significant effect of ovariectomy on parameters of trabecular bone mass (Table 1 and Figure 1A). Ovariectomy had no significant effect on cortical bone mass, which was measured as cortical bone width, in either IL-1 R1 -/- or wild-type mice (Figure 1B). Osteoclast numbers in the bone distal to the growth plate, where bone mass measurements were

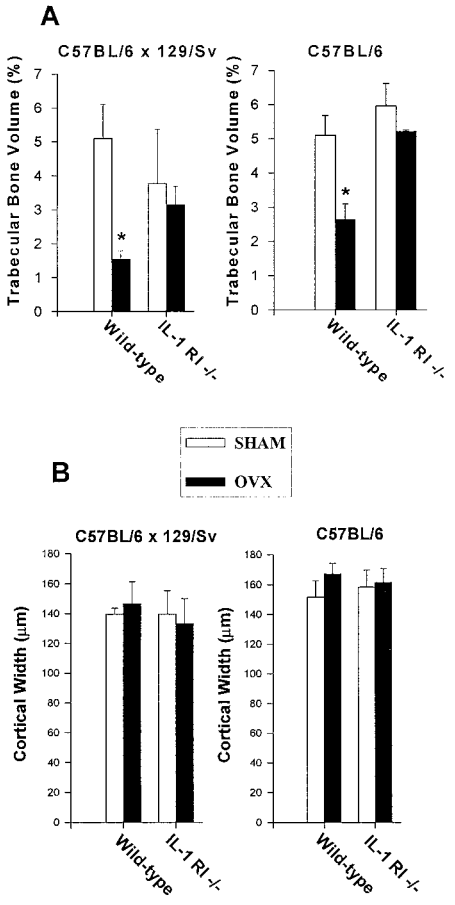

Figure 1. Effects of ovariectomy on trabecular bone volume (A) and cortical width (B) in mice. Values are mean ± SEM for bones from 3-6 animals per group.

* Significantly different from respective sham-operated group $p < 0.05$.

Table 1
Trabecular Bone Mass Indices in C57BL/6 x 129Sv Mice

Group	Trabecular Area ($\mu m^2 \times 10^3$)	Trabecular Perimeter (mm)	Trabecular Number per Bone
Wild-type SHAM	42.8 ± 8.5	3.4 ± 0.6	10.2 ± 1.6
Wild-type OVX	12.6 ± 2.1*	1.4 ± 0.3*	6.3 ± 0.6
IL-1 R1 -/- SHAM	30.9 ± 13.2	2.7 ± 0.9	8.6 ± 2.0
IL-1 R1 -/- OVX	26.1 ± 4.4	2.2 ± 0.3	7.2 ± 1.3

Values are mean ± SEM for bones from 5-6 animals per group.

* Significantly different from respective sham-operated group. $P < 0.05$

Table 2
Trabecular Bone Mass Indices in C57BL/6 Mice

Group	Trabecular Area ($\mu m^2 \times 10^3$)	Trabecular Perimeter (mm)	Trabecular Number per Bone
Wild-type SHAM	41.8 ± 6.9	2.7 ± 0.3	7.5 ± 0.6
Wild-type OVX	22.0 ± 3.3*	1.7 ± 0.2*	5.0 ± 0.3*
IL-1 R1 -/- SHAM	49.2 ± 7.1	3.4 ± 0.5	10.0 ± 1.6
IL-1 R1 -/- OVX	42.8 ± 4.0	3.1 ± 0.1	9.0 ± 1.7

Values are mean ± SEM for bones from 3-6 animals per group.

* Significantly different from respective sham-operated group. $P < 0.05$

made, were small and highly variable (typically 3-20 per section). There were no significant differences in this parameter between SHAM and OVX animals in any of the groups that were examined (data not shown).

Three weeks after ovariectomy, wild-type C57BL/6 mice lost 48% of their TBV. In contrast, mice in whom the IL-1 R1 -/- trait was bred into a C57BL/6 genetic background had no loss of TBV (Figure 1A). The loss of TBV in wild-type but not IL-1 R1 -/- mice was confirmed by other measures of trabecular bone mass (trabecular area, trabecular perimeter and trabecular number, Table 2). As with mice in a C57BL/6 x 129/Sv genetic background, neither wild-type nor IL-1 R1 -/- mice in a C57BL/6 genetic background lost cortical bone width after ovariectomy (Figure 1B).

To confirm the estrogen status of the mice, we measured uterine wet weight in all animals at the time of sacrifice. Ovariectomy decreased this value by 70 to 85% in all groups compared to sham-operated mice (data not shown).

Discussion

Our results demonstrate that IL-1 R1 -/- mice have not lost trabecular bone mass in their proximal humeri 3 weeks after ovariectomy. Hence, bone loss resulting from estrogen withdrawal in this model requires that cells be capable of responding to IL-1. The failure of IL-1 R1 -/- mice to lose trabecular bone mass after ovariectomy was not unique to a single strain since we found this response in mice of two genetic backgrounds. These findings suggest that IL-1 is an important mediator of the effects of estrogen withdrawal on bone mass.

Interestingly, the failure of bones from IL-1 R1 -/- mice to lose mass after ovariectomy appeared to be a selective response since removal of ovaries from IL-1 R1 -/- and wild type mice resulted in similar decreases in uterine weight.

Our inability to detect an increase in osteoclast numbers in bones from wild-type mice after ovariectomy differs from previously described findings (15). This discrepancy most likely reflects the small number of cells in our measurement area, the variability of this index and the limited number of bones that we examined.

In contrast to TBV, cortical bone mass was not lost in wild-type or IL-1 R1 -/- mice after ovariectomy. This effect likely reflects a slower rate of turnover in this type of bone and the relatively short duration of our study. The absence of an action of ovariectomy on cortical bone mass in mice in similar short-term studies was previously demonstrated (15).

IL-1 bioactivity was increased in bone marrow supernatants from ovariectomized mice compared to sham-operated mice or ovariectomized mice that were replaced with estrogen (16, 17). Interestingly, IL-1 levels in the marrow supernatants (measured by ELISA) were not altered by ovariectomy. However, specific inhibitors of IL-1α blocked the increased bioactivity of IL-1 that was seen in the marrow supernatants after ovariectomy. These results imply that changes in the levels of inhibitors of IL-1 were responsible for the changes in IL-1 bioactivity in the marrow supernatants after ovariectomy. It is also likely that changes in IL-1 bioactivity in the bone marrow microenvironment mediate at least some of the actions of estrogen withdrawal on bone. We have consistently failed to find an effect of ovariectomy on the production of IL-1 ra (16). However, recently it was demonstrated that estrogens increase levels of the mRNA for the type II IL-1 receptor (IL-1 R2) in murine bone marrow cells *in vivo* and human osteoclasts *in vitro* (18, 19). This receptor is found on the surface of cells. It can also be released from cell membranes by a cleavage step and circulate in serum as a soluble protein (6). Increases in the expression of IL-1 R2 by cells are associated with decreased responsiveness to IL-1 (20). We hypothesize that changes in the expression of the soluble form of IL-1 R2 may mediate some of the effects of estrogen on IL-1 bioactivity in bone marrow supernatants and the effects of estrogen on bone mass.

Our current finding that mice, lacking a functional IL-1 receptor, do not lose bone mass after ovariectomy supports a role for IL-1 in the effect of estrogen withdrawal on bone mass and implies that inhibitors of this cytokine will have therapeutic value for treating and preventing the clinical syndrome of osteoporosis.

Acknowledgement

Supported by funds from the VA Merit Review program and PHS grant PO1 AR38933

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