IL-1RI participates in normal growth plate development and bone modeling

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The pro-inflammatory cytokine interleukin-1 (IL-1) is produced primarily by macrophages and monocytes, but it is also synthesized by epidermal, epithelial, lymphoid, and vascular tissues. IL-1 expression is upregulated after infection, injury, or antigenic challenge and is highly elevated in chronic inflammatory diseases such as inflammatory bowel disease (11), Crohn’s disease (28), and rheumatoid arthritis (22). When IL-1 gains access to the circulation, it acts like a hormone and induces a broad spectrum of systemic changes in neurological, metabolic, hematological, and endocrinological systems (6, 7). The two forms of IL-1, IL-1α and IL-1β, share ~30% structural homology and are both produced as 33-kDa precursors that can be cleaved to a 17-kDa “mature” form by intracellular or extracellular proteases.

Both IL-1α and IL-1β transduce signals by binding to the 80-kDa plasma membrane receptor IL-1 receptor type I (IL-1RI) (37). This binding facilitates the interaction with IL-1 receptor accessory protein (45, 46), which together induce downstream signaling pathways. The second receptor, IL-1 receptor type II, also binds IL-1, but it lacks the intracellular domain; therefore, it cannot transduce signals and is considered a decoy receptor (5).

In the bone microenvironment, IL-1 is produced by osteoblasts and osteoclasts (17), and it is also expressed in joints (16) under physiological conditions. IL-1RI is expressed by both osteoblasts and osteoclasts (19), and in the growth plate it is expressed by chondrocytes in the resting, proliferative, and hypertrophic zone (30). Little is known about the effect of absence or excess of IL-1 signaling on the physiological development of the growth plate and bone. Studies that focused on the absence of IL-1 signaling showed inconclusive data. Lorenzo et al. (25) reported on normal trabecular bone volume and cortical width in 8-wk-old IL-1RI knockout (KO) mice. Bajayo et al. (2) showed shorter bones and low bone mass phenotype in the distal femoral metaphysis and L3 vertebral body in 15-wk-old IL-1RI KO mice accompanied with increased osteoclast numbers. On the other hand, Lee et al. (24) reported increased trabecular and cortical bone mass together with decreased osteoclast number in 8-wk-old IL-1α and IL-1β KO mice. Several studies showed the inhibitory effect of excess IL-1β on chondrocyte differentiation and bone growth (21, 27, 29, 30). Recently, we have shown that direct injection of IL-1β to the knee joint disturbs the normal sequence of events in the growth plate, resulting in increased proliferation and widening of the proliferative zone, whereas the hypertrophic zone becomes disorganized, with impaired matrix structure and increased apoptosis and osteoclast activity. In vitro we have shown that IL-1β interferes with the major regulatory pathways of the growth plate, in particular fibroblast growth factor receptor 3, thus causing increased proliferation and a G1-to-S phase shift in the cell cycle of chondrocytes, which is followed by delayed cell differentiation, as well as decreased expression of extracellular matrix (ECM) genes and proteoglycan synthesis (38).

Due to the dramatic effect of IL-1β on the growth plate both in vivo and in vitro, we decided to examine the role of IL-1RI in normal bone and growth plate development. We used several histological tools and performed structural analysis using microcomputed tomography (μCT) scans; we found that IL-1RI KO mice have narrower growth plates due to a narrower hypertrophic zone, significantly reduced osteoclast numbers, and higher trabecular and cortical bone mass together with increased density and superior mechanical properties. These results suggest that, under physiological conditions, IL-1RI is involved in normal growth plate development and ECM homeostasis and that it has a significant role in the physiological process of bone modeling.

MATERIALS AND METHODS

Materials. Levamisole, eosin, and detection kit for acid phosphatase in the presence of tartrate [tartrate-resistant acid phosphatase
(TRAP) were purchased from Sigma Chemical (St. Louis, MO). Digoxigenin dUTP was purchased from Enzo (Mannheim, Germany). Digoxigenin-RNA labeling mix, NBT (4-nitroblue tetrazolium), and BCIP (5-bromo-4-chloro-3-indolyl-phosphate) were purchased from Roche (Wiesbaden, Germany).

**Animals.** Homozygous interleukin-1 receptor type I knockout mice (IL1r1tm1Imx) were purchased from Jackson Laboratories. The background of the mice is C57BL/6. The stock number is 003245. For all experiments, 30-day-old male mice were used. As controls, C57BL/6 mice at the matching age were used. All mice were housed under specific pathogen-free conditions in an environmentally controlled clean room. All procedures were approved by the Hebrew University Animal Care Committee.

**Histological staining and in situ hybridization of growth plate sections.** Growth plates were fixed overnight in 4% paraformaldehyde (Sigma) at 4°C, followed by 3 wk of decalcification in 0.5 M EDTA (pH 7.4). The samples were then dehydrated, cleared in histoclear (Bar-Naor, Ramat-Gan, Israel), and embedded in paraplast, and 5-μm sections were prepared. For Masson’s trichrome staining, the following working solutions were used: Weigert’s Iron Hematoxylin solution [mix equal parts of stock A (1 g of hematoxylin in 100 ml of 95% ethanol) and stock B (4 ml of 29% Ferric chloride in water, 1 ml of HCl in 95 ml of double-distilled water)], Biebrich Scarlet-Acid Fuchsin solution (90 ml of 1% Biebrich scarlet, 10 ml of 1% acid fuchsin, and 1 ml of acetic acid), phosphomolybdic-phosphotungstic acid solution (25 ml of 5% phosphomolybdic acid and 25 ml of phosphotungstic acid), aniline blue solution (2.5 g of aniline blue and 2 ml of acetic acid in 100 ml of distilled water), and 1% acetic acid solution (1 ml of Aacetic acid in 99 ml of distilled water). In brief, slides were stained in Weigert’s Iron Hematoxylin solution for 10 min, followed by rinsing in running warm tap water for 10 min, and washed with distilled water. Next, they were stained in Biebrich Scarlet-Acid Fuchsin solution for 15 min and washed in distilled water. They were then incubated in phosphomolybdic-phosphotungstic acid solution and transferred directly to aniline blue solution for 5 min, followed by a quick rinse in distilled water and 1% acetic acid solution for 2 min. For safranin O staining, the sections were stained in Mayer’s hematoxylin, followed by Fast Green FCF Yellowish, and rinsed in 1% acetic acid. The slides were then placed in safranin O for 30 min and rinsed in water and 95% ethanol. Hybridizations were performed as described by Simsa and Ornan (39). In brief, the sections were deparaffinized in xylene, rehydrated, and digested with proteinase K. After digestion, slides were fixed in 10% formaldehyde, blocked in 0.2% glycine, and rapidly dehydrated. The sections were then hybridized with digoxigenin-labeled antisense probes for collagen (Col) II or X (probes were kindly provided by Dr. Elazar Zelzer from the Weizmann Institute). Probes were detected using a polyclonal anti-digoxigenin antibody attached to alkaline phosphatase that, when reacting with its substrate (NBT + BCIP), produces a color response. Endogenous ALP was inhibited with levamisole (39). In all hybridizations, no signal was observed with sense probes, which were used as controls. TRAP staining was performed using a detection kit for acid phosphatase in the presence of tartrate (Sigma).

**Measurement of growth plate width and cell number.** The widths of the whole growth plates, proliferative zones, and hypertrophic zones were measured at six different points along the growth plate (GP), averaged for each GP, and then averaged with measurements from 10 other GP samples in each group. The number of cells per column in the proliferative zone was counted and averaged in slides from 10...
different mice in each group. In each slide, 10 different columns were counted.

The number of TRAP-positive cells was counted and averaged within a defined region of 2.28 mm² in four different slides from four mice in each group.

Quantification of safranin O staining and Col X in situ hybridization. Quantification was done using Image J software. For quantification, three different slides from three different animals were used.

RNA isolation, reverse transcription, and real-time PCR. Tibiae from 30-day-old C57BL/6 and IL-1RI KO mice were suspended in TRI Reagent (Sigma) and homogenized. Total RNA was extracted according to the manufacturer’s protocol. The RNA samples were prepared from pools of three bones from each group. RNA (1 µg) was reverse-transcribed using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). Relative quantification real-time PCR was performed using platinum SYBR Green, 1 µl of cDNA template, and a Col type X specific primer set (forward: ACTCTACACGTGCATGTGAA; reverse: ACTGCCCTGAGCGCTGATCCCA).

µCT. Whole femora (n = 12) and tibiae (n = 10) were scanned with a Skyscan 1174 X-ray computed microtomograph scanner (Skyscan, Aartselaar, Belgium), with a charge-coupled device detector. Images were obtained with 50 kV X-ray tube voltage and 800 µA current. Specimens were scanned using a 0.25-mm aluminum filter. Exposure time was 2,500 ms for femora and 4,000 ms for tibiae. Image pixel size was 13.81 µm for femora and 16.85 µm for tibiae. For each specimen, a series of 900 projection images was obtained with a rotation step of 0.4°, two-frame averaging, for a total 360° rotation. Flat field correction was performed at the beginning of each scan for a specific zoom and image format. A stack of two-dimensional X-ray shadow projections was reconstructed to obtain transverse images using NRecon software (Skyscan) and subjected to morphometric analysis using CTA. Whole femora (n = 12) and tibiae (n = 10) were scanned with a Skyscan 1174 X-ray computed microtomograph scanner (Skyscan, Aartselaar, Belgium), with a charge-coupled device detector. Images were obtained with 50 kV X-ray tube voltage and 800 µA current. Specimens were scanned using a 0.25-mm aluminum filter. Exposure time was 2,500 ms for femora and 4,000 ms for tibiae. Image pixel size was 13.81 µm for femora and 16.85 µm for tibiae. For each specimen, a series of 900 projection images was obtained with a rotation step of 0.4°, two-frame averaging, for a total 360° rotation. Flat field correction was performed at the beginning of each scan for a specific zoom and image format. A stack of two-dimensional X-ray shadow projections was reconstructed to obtain transverse images using NRecon software (Skyscan) and subjected to morphometric analysis using CTA. Whole femora (n = 12) and tibiae (n = 10) were scanned with a Skyscan 1174 X-ray computed microtomograph scanner (Skyscan, Aartselaar, Belgium), with a charge-coupled device detector. Images were obtained with 50 kV X-ray tube voltage and 800 µA current. Specimens were scanned using a 0.25-mm aluminum filter. Exposure time was 2,500 ms for femora and 4,000 ms for tibiae. Image pixel size was 13.81 µm for femora and 16.85 µm for tibiae. For each specimen, a series of 900 projection images was obtained with a rotation step of 0.4°, two-frame averaging, for a total 360° rotation. Flat field correction was performed at the beginning of each scan for a specific zoom and image format. A stack of two-dimensional X-ray shadow projections was reconstructed to obtain transverse images using NRecon software (Skyscan) and subjected to morphometric analysis using CTA. Whole femora (n = 12) and tibiae (n = 10) were scanned with a Skyscan 1174 X-ray computed microtomograph scanner (Skyscan, Aartselaar, Belgium), with a charge-coupled device detector. Images were obtained with 50 kV X-ray tube voltage and 800 µA current. Specimens were scanned using a 0.25-mm aluminum filter. Exposure time was 2,500 ms for femora and 4,000 ms for tibiae. Image pixel size was 13.81 µm for femora and 16.85 µm for tibiae. For each specimen, a series of 900 projection images was obtained with a rotation step of 0.4°, two-frame averaging, for a total 360° rotation. Flat field correction was performed at the beginning of each scan for a specific zoom and image format. A stack of two-dimensional X-ray shadow projections was reconstructed to obtain transverse images using NRecon software (Skyscan) and subjected to morphometric analysis using CTA. Whole femora (n = 12) and tibiae (n = 10) were scanned with a Skyscan 1174 X-ray computed microtomograph scanner (Skyscan, Aartselaar, Belgium), with a charge-coupled device detector. Images were obtained with 50 kV X-ray tube voltage and 800 µA current. Specimens were scanned using a 0.25-mm aluminum filter. Exposure time was 2,500 ms for femora and 4,000 ms for tibiae. Image pixel size was 13.81 µm for femora and 16.85 µm for tibiae. For each specimen, a series of 900 projection images was obtained with a rotation step of 0.4°, two-frame averaging, for a total 360° rotation. Flat field correction was performed at the beginning of each scan for a specific zoom and image format. A stack of two-dimensional X-ray shadow projections was reconstructed to obtain transverse images using NRecon software (Skyscan) and subjected to morphometric analysis using CTA.

Mechanical testing. Mechanical properties of the femora (n = 12) and tibiae (n = 10) were determined by three-point bending tests, using a custom-made micromechanical testing device. Each bone was placed on two supports with rounded profiles (0.5 mm diameter) such that the supports were located equidistant from the ends of the bone and at the maximum feasible distance from each other (34). The distance between the supports was set to 5 mm. Each bone was loaded on its lateral aspect. Monotonic loading was then conducted at a constant rate of 600 µm/min. The following whole bone biomechanical parameters were derived from the load-displacement curves: bone stiffness, yield load, force to fracture (F fracture), and maximal force (F max). Young’s modulus of the bone material was approximated using beam theory and the relationship

\[
E = \frac{S \times L^3}{48 \times I}
\]

where E is the effective Young’s modulus (N/mm²), S is the slope of the linear portion of the load-displacement curve (N/mm), L is the support span (mm), and I is the cross-sectional moment of inertia (mm⁴) determined at the area of fracture by µCT. It should be noted that Young’s moduli values are likely to be underestimated due to the aspect ratio of the bones, which results in shear participating in beam deflection during the tests (40).

Statistical analysis. Statistical analyses were performed by using Student’s t-test. P < 0.05 was considered significant.

RESULTS

IL-1RI KO mice have narrower GPs and higher proteoglycan content. IL-1RI KO mice were viable and appeared healthy. They bred normally and possessed normal size litters. No differences were observed in body mass, size, or bone length between IL-1RI KO mice compared with wild-type (WT) mice. IL-1RI is expressed in all of the zones of the GP (30), and excess IL-1 signaling has a dramatic effect on GP morphology and bone growth (38). For these reasons, we were interested in examining the GPs of IL-1RI KO mice. Histological examination showed that the GP of KOs is narrower than that of the WTs: 275.22 ± 18.09 vs. 255.63 ± 16.05 µm (in the WTs and KOs, respectively). The width of the proliferative zone was not different between the two mice (142.32 ± 18.75 vs. 142.71 ± 15.56 µm), nor was the number of cells in the proliferative zone (13.23 ± 1.86 vs. 14.28 ± 1.79). However, the hypertrophic zone was narrower in the KO (129.02 ± 16.5 vs. 111.28 ± 10.54 µm; Fig. 1, A and B). Safranin O staining was used to evaluate the amount of ECM in the growth plate (Fig. 1, C and D). The intensity of safranin O staining is directly proportional to the proteoglycan content in normal

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<th>C57BL/6</th>
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<td>GP</td>
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Fig. 2. Decreased osteoclast no. in IL-1RI KO mice. Tibiae from 30-day-old C57BL/6 and IL-1RI KO mice were subjected to histological analysis. Tartrate-resistant acid phosphatase staining for the detection of osteoclasts (red spots) in the chondro-osseous junction (A), trabecular bone (TB; B), and cortical bone (CB; C).
cartilage (4). It can be seen by the intensity and quantification of the stain that the GPs of the IL-1RI KO mice have higher proteoglycan content compared with the WTs (Fig. 1, C and D). In situ hybridization for the expression of Col II, the major collagenous protein in the proliferative zone, shows the same pattern and width of expression in the WTs and KOs (Fig. 1E). On the other hand, in situ hybridization for the expression of Col X, the major collagensous protein in the hypertrophic zone, shows a narrower zone of expression in the KOs (Fig. 1F).

Quantification of this analysis (Fig. 1G) together with quantification of Col X mRNA in bones (Fig. 1H) confirmed that the expression of Col X is lower in IL-1RI KO mice. These results demonstrate that lack of IL-1 signaling alters the structure of the GP and affects ECM homeostasis.

**IL-1RI KO mice have decreased osteoclast number.** TRAP staining was used as an osteoclast-specific marker (2, 10, 31). The staining revealed TRAP-positive cells at the chondro-osseous junction (Fig. 2A), trabecular bone (Fig. 2B), and cortical bone (Fig. 2C) of the C57BL/6 mice. Interestingly, the bones of the IL-1RI KO mice have significantly decreased TRAP-positive cells in all of those areas (Fig. 2, A–C). Counting of average TRAP-positive cells per defined area shows that the WT mice have 27.2 ± 7.7 osteoclasts/2.28 mm², whereas the KO has 10.22 ± 4.9 osteoclasts/2.28 mm² (P = 0.000286). These results suggest that osteoclast numbers are reduced in IL-1RI KO mice and may result in impaired bone resorption and modeling.

**IL-1RI KO mice have increased cortical thickness and mass.** We used μCT scanning to evaluate whether the lack of IL-1R influences the geometric parameters of the bones. Our results indicate that the cortical thickness of IL-1RI KO mice is larger than that of C57BL/6 mice; in the femora it was elevated by 17%, from 0.12 to 0.14 mm (Fig. 3A), and in the tibia by 20%, from 0.15 to 0.18 mm (Fig. 3B). In the femora, the cortical cross-sectional area was also significantly larger in the KOs compared with the WTs (0.57 vs. 0.49 mm²; Fig. 3A), whereas the total cross-sectional area inside the periosteal envelope was not different between the two strains of mice in either the femora (Fig. 3A) or tibiae (Fig. 3B), indicating a smaller medullary area in the KO mice, a trend that was indeed observed but was not statistically significant. Analysis of cortical bone mineral density (BMD) showed an 8.2% increase in the BMD of the femora (Fig. 3A) and an 11.25% increase in the BMD of the tibiae (Fig. 3B). These results indicate that the IL-1RI KO mice have either elevated bone formation and mineralization, lower resorption rate, or both.

**IL-1RI KO mice have increased trabecular bone volume fraction.** Examination of the trabecular bone microarchitecture revealed 34% higher bone volume per total volume (BV/TV) in the femora of the IL-1RI KO compared with the WT mice (35.74 vs. 26.68%; Fig. 4A) and 66% higher BV/TV in the tibiae (24.95 vs. 14.99%; Fig. 4B). This is due primarily to higher trabecular number, which is 35 and 61% higher in the femora (Fig. 4A) and tibiae (Fig. 4B), respectively, of the KO mice. The trabecular thickness is not significantly different, but a trend toward thicker trabeculae in the KO mice is evident. In the knockouts’ femora, a corresponding 30% decrease in trabecular separation is observed (Fig. 4A), with no differences in this parameter in the tibiae.

**Femora of the IL-1RI KO mice have superior mechanical characteristics.** To evaluate whether the differences observed in the morphology and mineral density are accompanied by

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<td>Tt.Ar (mm²)</td>
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**Fig. 3.** IL-1RI KO mice have increased cortical thickness and mass. Femora (A and B) and tibiae (C and D) from 30-day-old C57BL/6 and IL-1RI KO mice were subjected to μCT scan using 13.81 μm pixel size resolution for femora and 16.85 μm for tibiae. Exposure times were 2,500 ms for femora and 4,000 ms for tibiae. A and C: cortical morphological parameters for femora (A) and tibiae (C); total cross-sectional area inside the periosteal envelope (Tt.Ar), cortical cross-sectional area (Ct.Ar), cortical area fraction (Ct.Ar/Tt.Ar), medullary area (Ma.Ar), cortical thickness (Ct.Th), and bone mineral density (BMD). Results are reported as means (SD). *Parameters that were found to differ significantly (P < 0.05). B, image I, and D, image II: 2-dimensional image of whole femora (B, image I) and whole tibiae (D, image II) and tibiae (D, image II). Note the elevated cortical thickness in the IL-1RI KO mice.
changes in the mechanical properties of the femora and tibiae, we used a three-point bending test (34). The femora (Fig. 5, A and B) of the IL-1RI KO mice had higher stiffness, yield load, Fmax, F fracture, and Young’s modulus. To our surprise, despite the profound differences in bone morphology and BMD, the mechanical properties of the tibiae from the IL-1RI KO mice were not different from those of the WT mice (Fig. 5, C and D).

**DISCUSSION**

In this study, we demonstrate that signaling through IL-1RI is involved in normal growth plate development and ECM homeostasis and that it is significant in the physiological process of bone modeling.

Young and healthy IL-1RI KO mice have normal bone length despite having a narrower growth plate, shorter hypertrophic zone, and higher proteoglycan content. Therefore, we conclude that, despite the morphological alterations, the basic function of the growth plate that is the “engine” of bone elongation and will determine final bone length is normal in IL-1RI KO mice. The decreased size of the hypertrophic zone, together with reduced Col X expression, indicates that IL-1 signaling through IL-1RI participates in the processes of hypertrophic differentiation and ECM regulation. This is in line with our previous findings showing that 1) IL-1β’s-induced growth inhibition is mediated by IL-1RI and 2) excess amounts of IL-1β inhibit hypertrophic differentiation and matrix production both in vivo and in vitro (38). Here, we show that lack of IL-1 signaling also inhibits hypertrophic differentiation. The changes in matrix content are of greater importance than simple alterations in the extracellular scaffold; it is well known that ECM proteins influence cell behavior by sequestering signaling molecules such as growth factors and growth factor-binding proteins and by acting as ligands for cellular adhesion receptors such as integrins that transduce signals into the cell (41). Therefore, changes in ECM may influence many cellular processes within the growth plate. Together, these findings demonstrate that both absence and overactivation of the pathway result in growth plate impairment, indicating that IL-1RI,

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<td>Bv/Tv (%)</td>
<td>26.68(1.74)</td>
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<td>Tb.Sp (mm)</td>
<td>0.22(0.03)</td>
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**Fig. 4.** IL-1RI KO mice have increased trabecular bone volume. Femora (A and B) and tibiae (C and D) from 30-day-old C57BL/6 and IL-1RI KO mice were subjected to μCT scan using 13.81 μm pixel size resolution for femora and 16.85 μm for tibiae. Exposure times were 2,500 ms for femora and 4,000 ms for tibiae. A and C: trabecular bone morphological parameters: bone volume per total volume (BV/TV), trabecular no. (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp). Results are reported as means (SD). *Parameters that were found to differ significantly (P < 0.05). B, image I, and D, image I: 2-dimensional image of the trabecular region of the femora (B, image I) and tibiae (D, image I). B, image II, and D, image II: 3-dimensional images of trabecular bone from femora (B, image II) and tibiae (D, image II). Note the increased trabecular volume in the IL-1RI KO mice.

**Fig. 5.** Femora of the IL-1RI KO have superior mechanical characteristics. Three-point bending was used to measure the biomechanical properties of femora (A and B) and tibiae (C and D). A and C: representative load-displacement curves. B and D: biomechanical parameters derived from load-displacement curves. Results are reported as means (SD). *Parameters that were found to differ significantly (P < 0.05).

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although not essential, participates in normal growth plate development and matrix homeostasis. Having said that, one must remember that once a gene is fully knocked out, compensatory pathways “take over” some of its functions, which may result in normal-appearing development. Hence, to better understand the exact mechanism by which IL-1RI contributes to growth plate development, a conditional knockout should be used. However, this is beyond the scope of this work.

IL-1RI KO mice have increased cortical and trabecular bone mass; the cortical thickness and BMD as well as trabecular number and volume were increased in the femora and tibiae from the KO compared with the WT mice. As shown by us and others, C57BL/6 mice have very few trabeculae in both the distal femur (12) and proximal tibia (14); therefore, our findings demonstrating such a large increase in BV/TV and trabecular number in the IL-1RI KO mice are very significant. These changes, together with the superior mechanical properties of femora, suggest that IL-1RI has an important role in bone metabolism.

Among tissues, bone is the most sensitive to IL-1 (32). IL-1 is a potent cytokine for bone resorption; its bone resorbing activity was shown both when infused in vivo (3, 35) and in vitro organ cultures (13, 26). Moreover, it takes a significant part in the pathological bone destruction associated with multiple myeloma, rheumatoid arthritis, and osteoporosis (8) through involvement in multiple steps of osteoclastogenesis (1, 15, 19, 20, 24, 33). IL-1RI is also important for osteoclast formation; IL-1RI deficiency resulted in a 90% reduction of osteoclast formation in cocultures of spleen cells with IL-1R KO osteoblast (23). Here, we show that IL-1RI KO mice have a significantly lower number of TRAP-positive cells in the chondro-osseous junction and the trabecular and cortical bone areas. In contrast to our results, Bajayo et al (2) reported double the amount of osteoclasts in IL-1RI KO mice. However, in line with our results, Lee et al. (24) reported that mice lacking either IL-1α, IL-1β, or both (double knockout) have fewer numbers of active osteoclasts, demonstrating that IL-1 signaling is essential for osteoclast formation and activity. Since IL-1RI is considered the only receptor mediating IL-1 signaling, it is reasonable to expect that similar phenotypes would characterize the knockouts of both the ligand and its receptor. We suggest that IL-1RI deficiency resulted in impaired osteoclast formation; consequently, proteoglycans accumulate in the growth plate, and decreased bone resorption led to a final outcome of increased cortical thickness, smaller medullary area, and increased trabecular bone volume. This may also lead to an increase in BMD since there is less resorption of the “older” layers of the endosteam that are more mineralized.

In the femora of the IL-1RI KO mice, improved architecture of femora, suggest that IL-1RI has an important role in bone metabolism. Moreover, the bones from the KO mice had greater structural strength manifested by higher yield load and higher maximal and fracture loads, suggesting that the KO femora are more resistant to fracture than those of the WT mice. Interestingly, this was not the case in the tibiae, and no differences were observed between the mechanical characteristics of the bones of the KO compared with those of the WT mice. This can result from either differences in bone geometry between the femora and tibiae or different material properties. The bending test we performed was performed on whole bones and yielded the structural stiffness and the structural strength of the entire bone (9, 42). These two parameters are influenced by the material from which the structure is composed (the tissue material properties) as well as how and where that material is distributed (the geometric form of the tissue) (43).

To conclude, we show that IL-1RI is involved in the physiological process of growth plate and bone development. It is well known that excess amounts of IL-1 signaling, as in cases of chronic inflammatory diseases and obesity, inhibit bone growth and increase bone degradation (21, 27, 29, 30, 38). However, we show for the first time that IL-1RI deficiency also results in a narrower growth plate and abnormal proteoglycan content. On the basis of these results, we suggest that IL-1 signaling and IL-1RI are not essential but important to normal growth plate and bone development.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

S.S.-M. and E.M.-O. contributed to the conception and design of the manuscript. S.S.-M., J.Z., and Y.K. performed the experiments; S.S.-M. and R.S. analyzed the data; S.S.-M. and A.R. interpreted the results of the experiments; S.S.-M. prepared the figures; S.S.-M. drafted the manuscript; S.S.-M. edited and revised the manuscript; R.S. and E.M.-O. approved the final version of the manuscript.

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