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Osteoprotegerin Inhibits Cartilage Degradation Through an Effect on Trabecular Bone in Murine Experimental Osteoarthritis

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Objective. **To characterize bone microarchitectural changes and to test the hypothesis that disrupting local cytokine equilibrium could modify cartilage degradation in a murine model of experimental osteoarthritis (OA).**

Methods. **Ten-week-old male C57BL/6 mice underwent medial meniscectomy of their right knees and a sham operation of their left knees. The mice received intraperitoneal injections of osteoprotegerin (OPG) (10 mg/kg), interleukin-1 receptor antagonist (IL-1Ra) (100 mg/kg), or phosphate buffered saline for 6 weeks. The microarchitecture of the trabecular bone, the OA score, and expression of ADAMTS-4 and ADAMTS-5 were assessed. Proteoglycan release was measured in cartilage explant cultures in the presence of IL-1Ra and OPG.**

Results. **In the meniscectomized knees, bone volume/tissue volume (BV/TV) was lower, whereas trabecular separation, the OA score, and aggrecanase expression were higher than in the sham-operated knees. After treatment with OPG, BV/TV was significantly increased and trabecular separation was reduced in the knees that underwent meniscectomy. The OA score and the number of ADAMTS-positive cells were significantly decreased by treatment with OPG but were not affected by IL-1Ra. Moreover, OPG did not directly reduce the release of proteoglycans from cartilage explant cultures.**

Conclusion. **In an experimental model of OA, meniscectomy induced bone loss and cartilage degradation at 6 weeks. Systemic administration of OPG prevented bone and cartilage degradation in vivo but had no effect on cartilage in vitro. These data collectively indicate that bone could be a contributor in the early stages of OA pathogenesis. They further suggest that disruption of RANKL/OPG balance might result in the degradation of cartilage subjected to mechanical loading. Specific targeting of the bone cytokine network might help to prevent OA.**

Osteoarthritis (OA) is a slowly progressive joint disease characterized by breakdown of the cartilage matrix, which can ultimately lead to a complete loss of joint cartilage. Many factors contribute to the onset of OA, including both metabolic and biomechanical mechanisms (1). Cartilage matrix breakdown is mediated by several metalloprotease enzymes, the main ones being ADAMTS aggrecanases such as ADAMTS-4 and ADAMTS-5. For example, mice in which the ADAMTS-5 gene was deleted were protected against cartilage degradation in a joint instability model (2). Under physiologic conditions, the balance between the breakdown and synthesis of the extracellular matrix is maintained by cytokines and growth factors. Indeed, metalloprotease expression is increased in response to stimulation of the inflammatory cytokines (3). Interleukin-1 (IL-1) is a cytokine that plays a major role in inflammatory responses and is also believed to play a central role in cartilage destruction (4). Furthermore, inhibiting IL-1 reduces both metalloprotease synthesis and cartilage degradation in vivo (5).

Modifications of the subchondral bone are hallmarks of OA. The possible contribution of the bone beneath the cartilage to the initiation and progression of OA was first suggested by Radin and Rose (6). The integrity of cartilage could depend on the subchondral bone, which confers specific mechanical properties and

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influences cartilage remodeling. More recently, evidence showing changes in the structure of bone beneath damaged cartilage in confirmed OA in both humans and animals was obtained, suggesting that underlying bone, and not only subchondral bone, might contribute to the development of OA. Indeed, such bone modifications might influence the transmission of mechanical forces and thus the progression of cartilage damage. In late-stage OA, the bone volume of the subjacent bone is high (7,8) and reflects a late adaptive response of bone (9). In contrast, surgical models of joint instability in OA in animals have suggested that early OA is associated with an increase in bone resorption (1,10–12). It has therefore been speculated that increased bone resorption might be responsible in part for initiating cartilage destruction under stressful conditions (13). Indeed, alendronate has been shown to diminish metalloprotease expression and to reduce cartilage changes (10). This suggests a close link between bone and cartilage and further indicates that cell crosstalk via bone cytokines could be an initiator of cartilage destruction.

RANKL and osteoprotegerin (OPG) are the main factors that regulate bone remodeling in osteoporosis related to estrogen deficiency and inflammation (14). OPG interacts with RANKL and inhibits osteoclast differentiation. Because the surrounding bone is important for cartilage metabolism (10), we speculated that disrupting the RANKL/OPG balance could allow us to determine how bone cytokines interact with cartilage. Moreover, IL-1 might act directly on cartilage breakdown on bone, both in vivo and in vitro (15). In this study, we used a murine joint instability model of OA to, first, characterize the early microarchitectural changes in the underlying trabecular bone and, second, test the hypothesis that reducing RANKL or IL-1, both of which are cytokines involved in bone and cartilage metabolism, might modify cartilage degradation.

MATERIALS AND METHODS

Animals. Ten-week-old intact male C57BL/6 mice were purchased from Harlan France (Gannat, France). The mice were anesthetized, and knee joint instability was induced surgically by medial meniscectomy, which was performed using microscopic lenses (11). The right knee was opened, and the meniscotibial ligament was sectioned to access the medial meniscus. After being freed, the medial meniscus was gently pulled out using surgical forceps and sectioned using an ophthalmologic scalpel. Muscles and skin were then stitched up to restore muscle integrity. The left knees of the mice underwent a sham operation (a single cutaneous incision) and were then stitched up. The day after surgery, 7 mice received intraperitoneal injections of OPG (10 mg/kg), 7 mice received intraperitoneal injections of IL-1 receptor antagonist (IL-1Ra; 100 mg/kg), and 10 control mice received phosphate buffered saline (PBS). The injections were administered twice weekly for 6 weeks, and the mice were then killed. The experiments complied with the Guidelines for Animal Experimentation issued by the local Ethics Committee on Animal Care and Experimentation.

Preparation of joint specimens. At the time when the mice were killed, whole knee joints were dissected free of soft tissue. Specimens were fixed with 4% paraformaldehyde (pH 7.4) for 48 hours at 4°C and then decalcified with 1% paraformaldehyde–0.2*M* EDTA (pH 7.4) at 4°C for 2 weeks; the medium was changed twice weekly. The specimens were then dehydrated using increasing concentrations of ethanol before being embedded in paraffin. Each knee was embedded in a specific orientation, the medial knee being marked with a thread, to allow the knees to be sectioned in the meniscectomized area. Five-micrometer–thick serial sagittal sections were obtained.

Histochemical analysis. *S*ections were deparaffinized by 2 successive 20-minute immersions in a xylene bath. The sections were then hydrated in 3 successive alcohol baths at decreasing concentrations of 100% to 70% to 40% for 1 minute, before being washed twice by immersing for 5 minutes in distilled water baths. The sections were stained using Mayer's hemalum stain for 5 minutes to color the nuclei, and the sections were counterstained with 0.125% fast green for 10 minutes to visualize bone tissue. The sections were rinsed in 2 successive baths of 1% acetic acid, then stained in 0.5% Safranin O in the same bath, and rinsed in 95% ethanol. All of the stained sections were then rinsed with distilled water.

Histologic scores for the tibial plateaus were recorded. Two observers, under blinded conditions, used a modified Mankin version (16) of a semiquantitative scoring system to rate the cartilage destruction, as follows: 0 = no damage, 1 = irregular cartilage surface, 2 = loss of Safranin O staining without structural changes, 3 = loss of surface <20%, 4 = severe loss of joint cartilage (>80%), and 5 = erosion down to the bone.

Immunohistochemical analysis. Immunohistochemistry was performed on decalcified serial sections using the PK-6101 stain kit (Vector, Abcys, France). The manufacturer's protocol was applied, and the sections were then counterstained with toluidine blue. Immunohistochemistry was performed using primary polyclonal antibodies for ADAMTS-4 and ADAMTS-5 (RP1-ADAMTS-4 and RP1-ADAMTS-5, respectively; Triple Point Biologics, Forest Grove, OR) at a dilution of 1:100 and the secondary antibodies provided in the staining kit. Positive cells were counted on the same defined area (magnification × 25) and expressed as a percentage of the total cell count.

Histomorphometric assessment of trabecular bone. Modifications of the bone were measured on the same slides that were used for the protease measurements. Microarchitectural indices of the underlying bone were assessed in the total epiphysis by an image analyzer (Microvision, Evry, France), using specially designed software (BonoLab; Microvision). On each slide, the underlying trabecular zone between the cartilage and the growth plate was identified. The following parameters were measured: bone volume/tissue volume (%), trabecular thickness (μm) as a parameter of bone formation,

Table 1. Effects of meniscectomy on tibial cartilage and trabecular bone indices*

Index	Sham-operated knees (n = 10)	Meniscectomized knees (n = 10)
OA score	0.50 ± 0.85	1.90 ± 1.29
BV/TV, %	53.5 ± 12	38.70 ± 10
TbTh, μm	33.1 ± 4.9	28.51 ± 4.7
TbSp, μm	30.5 ± 14.1	52.9 ± 24.3
OcN/BV	0.004 ± 0.002	0.007 ± 0.001

* Values are the mean ± SEM. $P < 0.05$ for all comparisons between sham-operated knees and meniscectomized knees. OA = osteoarthritis; BV/TV = bone volume/tissue volume; TbTh = trabecular thickness; TbSp = trabecular separation; OcN = osteoclast number.

trabecular separation (μm) as a parameter of bone resorption, and osteoclast number/bone volume.

Osteoclasts were detected by enzyme histochemistry in serial decalcified sections, using tartrate-resistant acid phosphatase (TRAP) staining. Briefly, sections were stained for acid phosphatase using naphthol ASTR phosphate as substrate in the presence of 50 m*M* tartrate with hexazotized pararosaline and were counterstained with methyl green. TRAP-positive cells were counted in the whole epiphysis (magnification × 25) and expressed as the number of osteoclasts per bone volume.

Cartilage explant cultures. Femoral heads were harvested from 10-week-old C57BL/6 mice, and the cartilage was separated from the underlying subchondral bone. The cartilage samples were cultured as explants at 37°C in a humidified atmosphere of 5% CO_2 and 95% air in Dulbecco's modified Eagle's medium (DMEM) containing 1% antibiotic/antimycotic solution (Sigma, Saint-Quentin Fallavier, France). All cartilage explants weighed ~0.5 mg. After prestimulation with IL-1 or RANKL for 1 day, the cartilage explants were washed 3 times and cultured for 72 hours in serum-free DMEM with IL-1Ra (50 μg/ml) or OPG (10 ng/ml), after the explants had been prestimulated with IL-1 and RANKL, respectively. After 72 hours, the supernatants were collected, and their proteoglycan content was measured by a colorimetric assay using dimethylmethylene blue with shark chondroitin 6-sulfate as a standard, according to a previously described procedure (17). Each condition was tested in triplicate wells, and the experiments were performed at least 3 times.

Statistical analysis. Results are expressed as the mean ± SEM. Statistical analysis was performed using StatView version 5 software (SAS Institute, Cary, NC). Parameters were compared by analysis of variance (ANOVA), followed by Fisher's least significant difference test when *P* values by ANOVA were less than 0.05.

RESULTS

Effect of meniscectomy on trabecular bone volume. Assessment of the effects of meniscectomy on the underlying trabecular bone and cartilage revealed significant differences between the underlying trabecular bone of the meniscectomized knees and that of the sham-operated knees (Table 1). Both bone volume and trabecular thickness were significantly reduced in meniscectomized knees, whereas trabecular separation was increased. The osteoclast count was significantly higher in meniscectomized knees, indicating increased bone resorption in the underlying bone of the meniscectomized knees.

We confirmed that meniscectomy induces cartilage damage, as previously reported. In PBS-treated mice, the OA score was significantly greater in the meniscectomized knees than in the sham-operated knees (Figure 1 and Table 1). These data indicated that meniscectomy induced cartilage degradation, and that this was associated with increased resorption of bone underneath the cartilage. Moreover, the number of ADAMTS-4– and ADAMTS-5–expressing cells was greater in the meniscectomized knees than in the sham-operated knees (mean ± SEM 64.6 ± 4.8% versus

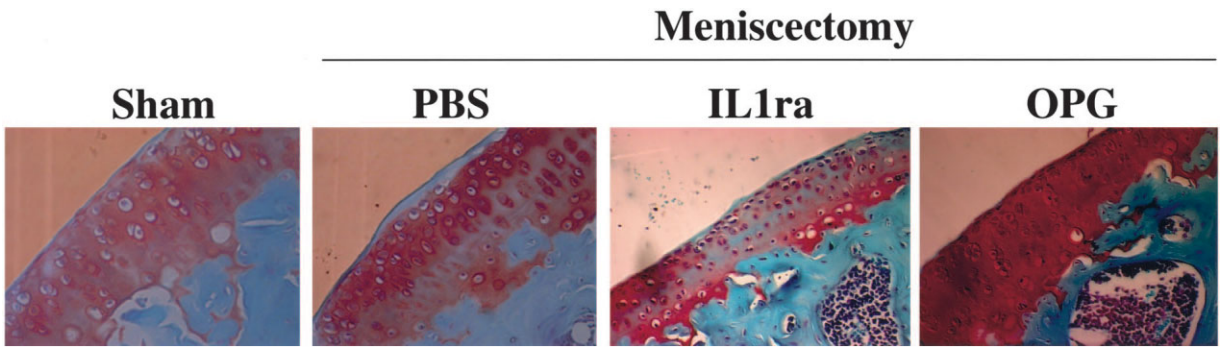

Figure 1. Cartilage degradation induced by meniscectomy. Cartilage degradation was assessed 6 weeks after meniscectomy in 10 mice that received phosphate buffered saline (PBS), 7 mice that received osteoprotegerin (OPG), and 7 mice that received interleukin-1 receptor antagonist (IL-1Ra). Meniscectomized knees in the PBS and IL-1Ra groups displayed a loss of Safranin O staining (original magnification × 20).

Table 2. Expression of ADAMTS-4–positive and ADAMTS-5–positive cells in mice treated with PBS, OPG, or IL-Ra*

	ADAMTS-4–positive cells		ADAMTS-5–positive cells	
Treatment	Sham (n = 7)	Mnx (n = 7)	Sham (n = 7)	Mnx (n = 7)
PBS	35.9 ± 3.6	64.6 ± 4.8†	47.3 ± 4.4	78.9 ± 6.2†
OPG	38.6 ± 7.9	52.6 ± 18.2	42.9 ± 6.2	53.86 ± 10.1†
IL-1Ra	35.5 ± 6.8	52.8 ± 12.5†	46.2 ± 5.9	64.27 ± 6.9†

* Values are the mean ± SEM percent of cells. PBS = phosphate buffered saline; OPG = osteoprotegerin; IL-1Ra = interleukin-1 receptor antagonist; Mnx = meniscectomy.
† $P < 0.05$.

35.9 ± 3.6% [$P < 0.05$] and 78.9 ± 6.2% versus 47.3 ± 4.4% [$P < 0.05$], respectively) (Table 2).

Effect of anticytokines on the microarchitectural changes in underlying trabecular bone induced by meniscectomy. To compare the effects of treatment under stressful conditions, we analyzed the microarchitectural indices for underlying trabecular bone in the meniscectomized knees of mice in the 3 treatment groups (Figure 2). Bone volume was significantly greater after systemic treatment with OPG than after treatment with PBS (mean ± SEM bone volume/tissue volume 56 ± 14% versus 39 ± 10%), as was trabecular thickness (35.64 ± 6.85 μm versus 28.51 ± 4.74 μm), whereas trabecular separation in OPG-treated mice was lower than that in PBS-treated mice (29.07 ± 11.51 μm versus 52.89 ± 24.26 μm) ($P < 0.05$ for all comparisons). Moreover, trabecular bone displayed fewer osteoclasts in the OPG-treated mice than in the PBS-treated mice ($P < 0.001$). In contrast, bone indices and osteoclast numbers in meniscectomized knees were not different in the IL-1Ra– and PBS-treated groups. These data suggest that OPG protected the knees against early bone loss and the microarchitectural changes induced by meniscectomy, whereas IL-1Ra did not.

As expected, the systemic administration of OPG induced effects on bone in both meniscectomized knees and sham-operated knees. Following treatment with OPG, the bone indices for meniscectomized and sham-operated knees were similar in terms of bone volume (mean ± SEM 56 ± 14% versus 49 ± 17%), trabecular separation (29.1 ± 11.5 μm versus 36.1 ± 14.1 μm), and trabecular thickness (35.6 ± 6.8 μm versus 33.8 ± 11.1 μm), and the P value was never significant. Although a few osteoclasts were observed in the meniscectomized knees, none at all were observed in the sham-operated knees.

Effect of anticytokines on protease expression and cartilage degradation. Cartilage parameters in meniscectomized knees were compared with those in sham-operated knees after treatment with the 2 anticytokines. Among OPG-treated mice, the OA score was dramatically reduced in the meniscectomized knees, although OA could still be scored, whereas the OA score was reduced to zero in the sham-operated knees (mean ± SEM 0.17 ± 0.41 versus 0; $P < 0.05$). Among IL-1Ra–treated mice, meniscectomy induced cartilage degradation as assessed by the OA score (2.29 ± 2.21 versus 0; $P < 0.05$). Following treatment with either OPG or IL-1Ra, the number of ADAMTS-5–positive cells was

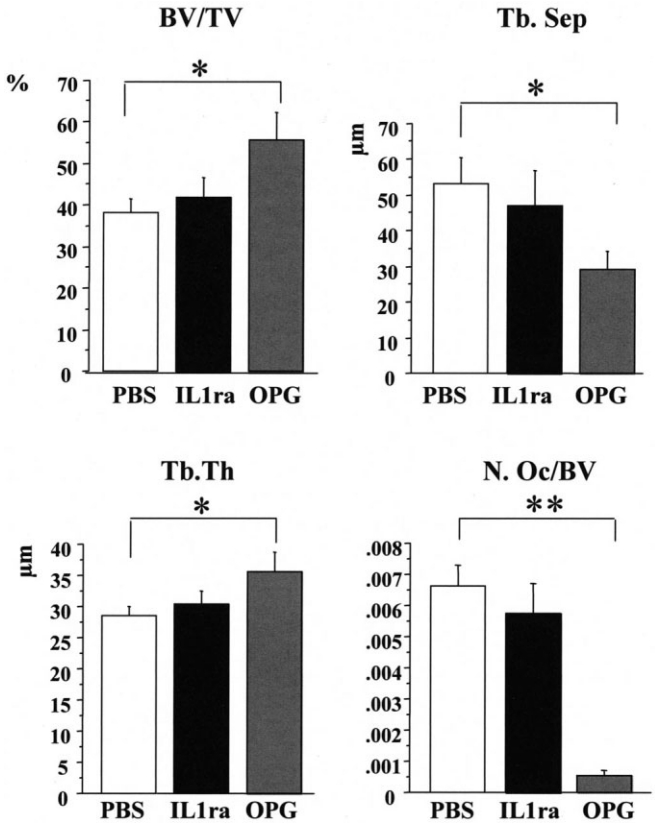

Figure 2. Modifications in trabecular bone after treatment with OPG and IL-1Ra. Trabecular bone parameters were measured at the epiphysis in meniscectomized knees of 10 mice treated with PBS, 7 mice treated with OPG, and 7 mice treated with IL-1Ra. Compared with PBS-treated mice, mice treated with OPG displayed increased bone volume/tissue volume (BV/TV), increased trabecular thickness (Tb.Th), and decreased trabecular separation (Tb.Sep), whereas the bone indices for PBS-treated mice and IL-1Ra–treated mice were similar. Treatment with OPG resulted in a significantly lower number of osteoclasts per bone volume (N.Oc/BV) compared with PBS treatment. Values are the mean and SEM. * = $P < 0.05$; ** $P < 0.001$. See Figure 1 for other definitions.

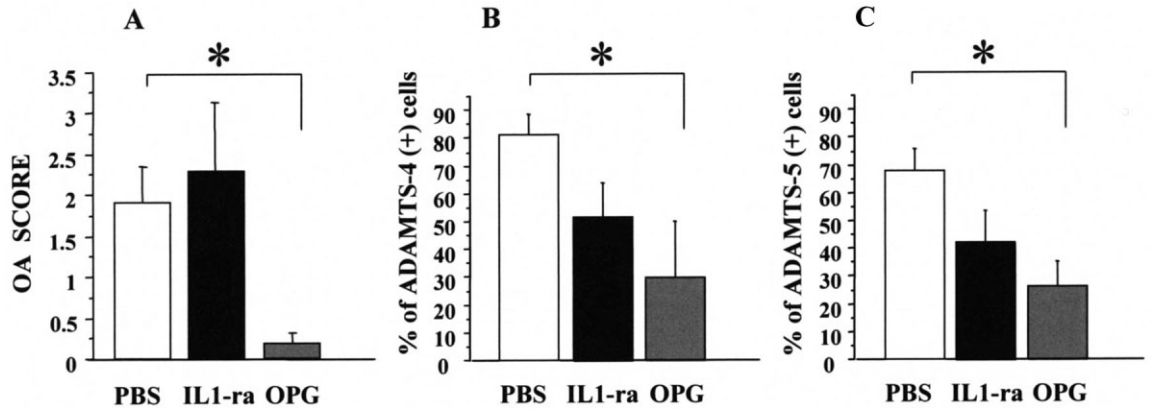

Figure 3. Percent increase in ADAMTS-4– and ADAMTS-5–expressing cells after treatment with osteoprotegerin (OPG) and interleukin-1 receptor antagonist (IL-1Ra). The osteoarthritis (OA) score and results of immunohistochemistry were quantified in 10 mice treated with phosphate buffered saline (PBS), 7 mice treated with IL-1Ra, and 7 mice treated with OPG. **A,** The OA score, which was quantified using Safranin O staining, was significantly reduced by OPG treatment, while it was unaffected by treatment with IL-1Ra. **B** and **C,** OPG treatment significantly reduced the number of ADAMTS-4– and ADAMTS-5–expressing cells. Values are the mean and SEM. $* = P < 0.05$.

significantly higher in meniscectomized knees than in sham-operated knees (Table 2). Among meniscectomized knees, the OA score was lower after OPG treatment than after PBS treatment (0.17 ± 0.41 versus 1.90 ± 1.29; $P < 0.05$) but was not significantly modified by treatment with IL-1Ra (2.29 ± 2.21; P not significant [NS] versus PBS treatment), showing that OPG prevented cartilage degradation after meniscectomy, whereas IL-1Ra did not.

To further investigate the role of meniscectomy in the presence of anticytokines, we determined the numbers of ADAMTS-4–positive and ADAMTS-5–positive cells in meniscectomized mice compared with sham-operated mice following treatment with PBS, OPG, and IL-1Ra (Figure 3). The percentage of ADAMTS-4–positive cells was significantly lower after treatment with OPG than after treatment with PBS but was not significantly lower in the IL-1Ra–treated group. The percentage of ADAMTS-5–positive cells was significantly reduced in the OPG-treated mice compared with the PBS-treated mice ($P < 0.05$) but was not significantly reduced in the IL-1Ra–treated group. These findings indicate that OPG partially inhibits protease expression, which remains present in meniscectomized knees because of mechanical loading.

Effect of OPG on cartilage explants in vitro. Treatment with IL-1 induced an increase in proteoglycan release (mean ± SEM 1.63 ± 0.29 μg/ml versus 0.89 ± 0.20 μg/ml in controls; $P < 0.05$), and this increase was reduced by treatment with IL-1Ra (0.93 ± 0.17 μg/ml) (Figure 4). In contrast, after exposure to RANKL alone, proteoglycan release from cartilage explants was the same as that from controls (1.02 ± 0.09; P NS versus controls). Treatment with OPG did not increase proteoglycan release compared with controls (0.86 ± 0.14; P NS versus controls). This indicated that OPG has no direct effect on cartilage degradation.

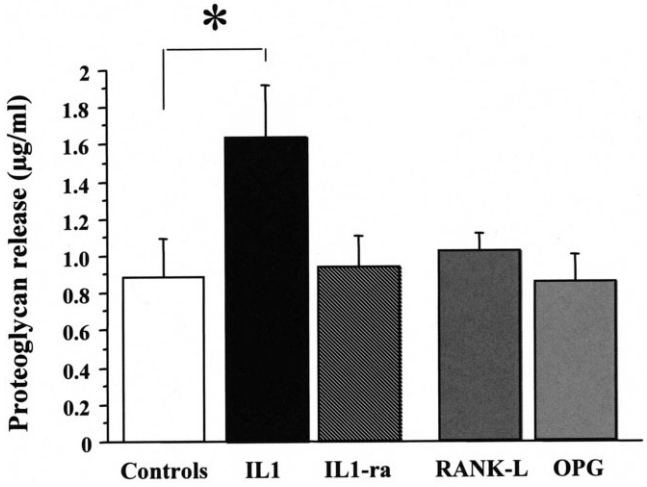

Figure 4. Effects of IL-1Ra and OPG in cartilage explants. Cartilage explants were cultured in the presence of IL-1 or RANKL for 24 hours. The explants were then washed before being cultured with IL-1Ra or OPG, respectively, for 72 hours. The proteoglycan content was measured in the supernatant. IL-1 induced an increase in proteoglycan release, which was reduced by treatment with IL-1Ra. RANKL did not affect proteoglycan release, and OPG had no direct effect. Values are the mean and SEM results from 3 experiments performed in triplicate. $* = P < 0.05$. See Figure 3 for definitions.

DISCUSSION

Several models have been used to induce cartilage damage in mice in order to mimic human OA (10,11,18). Here, we surgically induced knee instability by destabilizing the medial meniscus. This model provided sufficient sensitivity to show early significant modifications of cartilage and OA progression and to permit the assessment of early bone changes as well (18). Preliminary experiments carried out while the method was being developed allowed us to define the appropriate time points at which to kill the mice (data not shown). At 6 weeks, the biochemical changes attributable to cartilage damage were noticeable but remained moderate. Therefore, this time point appeared suitable for trying to understand the early events during disease progression. Indeed, a recent study provided results that are consistent with our findings (18).

In addition to providing information about the pathophysiology of early joint damage, this model also makes it possible to track early changes in the underlying trabecular bone. Because most lesions are located on the central portion of the medial joint, all sagittal sections were obtained through this zone in order to provide pertinent findings in the different groups. In this model, we observed that meniscectomy resulted in cartilage degradation along with an enhanced number of ADAMTS-4– and ADAMTS-5–expressing cells, which were mainly localized in the superficial layer of the joint cartilage. Both of these aggrecanases had been identified as being potentially important in aggrecanolysis and cartilage destruction (19), although ADAMTS-5 seems to play a major role, because gene deletion prevented cartilage degradation in a joint instability model (2). We also observed that the expression of ADAMTS-4 is increased under stressful conditions. Although ADAMTS-4–knockout mice were not susceptible to OA in the same surgical model (20), we cannot exclude the possibility that this aggrecanase may contribute to structural damage (21).

The role of bone in OA has aroused great interest in recent years (13), but the changes affecting both subchondral and underlying bone in OA remain obscure. In animal models, bone microstructure is assessed in frontal sections, with the intention of offering a parallel with radiologic hallmarks in humans. However, the bone damage cannot be assumed to be restricted to the frontal zone of the joint and bone, because mice are not bipedal. Therefore, we measured bone indices in sagittal sections. Bone changes affected both subchondral and trabecular bone, either of which could be responsible for altered transmission of biomechanical forces, which in turn might influence bone cell activity and the release of cytokines. We observed that trabecular bone was decreased by meniscectomy. Bone loss was associated with an increased number of osteoclasts and increased trabecular separation, both of which indicate increased bone resorption.

In this study, we demonstrate that increased bone activity is not limited to subchondral bone but also affects the underlying trabecular bone, which is responsible for microarchitectural changes. Our data are consistent with previous reports suggesting that bone loss is observed in the subchondral and trabecular region in rats, dogs, and guinea pigs in which OA has been induced by sectioning the anterior cruciate ligament or by meniscectomy (1,10,22). Moreover, microfocal computed tomography analysis revealed a reduction in trabecular bone volume along with increased trabecular separation in mice with collagenase-induced OA (23). Our data further show that microstructural changes are related to increased bone resorption. We also observed a concomitant increase in the expression of ADAMTS-4 and ADAMTS-5. This latter observation suggests that it could be linked to a direct effect of mechanical loading on cartilage or to indirect effects of changes in bone cell activity.

We then investigated whether inhibiting local factors involved in bone balance would have any effect on cartilage alterations. Because cartilage degradation is influenced by the stimulation of local cytokines and/or mechanical stress, we tested the hypothesis that modifying bone cell balance might result in changes in protease expression. We used OPG in an attempt to disrupt bone balance, because RANKL is known to play a crucial role in bone homeostasis. RANKL expression has been demonstrated in human joint chondrocytes (24) and in murine epiphysis chondrocytes (25), but the specific effects of OPG have been little reported. We observed that OPG prevents meniscectomy-related bone loss and is associated with the inhibition of bone resorption. In addition, OPG reduces the OA score and ADAMTS-4 and ADAMTS-5 expression following meniscectomy. We therefore conclude that OPG reversed the cartilage degradation induced by mechanical loading and suggest that this might be linked to an effect on bone.

We speculated that meniscectomy led to an increase in the mechanical load on bone, which in turn induced the release of local factors from the bone microenvironment and might subsequently have influenced cartilage remodeling. In a recent study, daily local injections of OPG were shown to prevent cartilage

degradation (26). In that study, Shimizu et al showed that OPG reduces chondrocyte apoptosis directly via a mechanism involving TRAIL binding. In the present study, we observed no effect of OPG on proteoglycan release in cartilage explants, which could indicate an effect through bone. These findings also suggest the contribution of bone close to cartilage and possible interaction via bone cytokines. The prevention of mechanically induced cartilage degradation might be related to a direct effect on bone.

In order to test the specificity of the action of OPG, a group of mice was treated with IL-1Ra, based on the premise that blockade of IL-1 might reduce both bone remodeling and aggrecanase expression (27). We observed that IL-1Ra did not protect against either OA or bone loss induced by meniscectomy despite a slight reduction in aggrecanase expression. Indeed, the role of IL-1 in experimental OA is unclear. Local IL-1Ra gene therapy suppressed experimental OA in dogs (4), whereas OA was aggravated in IL-1–knockout mice 4 weeks after meniscectomy (28). Our data show that systemic administration of IL-1Ra might not prevent cartilage and bone degradation under stressful conditions at the dose and schedule used in the current study.

In conclusion, we have shown that increased bone remodeling is present in the early stages of OA, and that OPG might prevent cartilage degradation via its action on bone. We propose that modulating RANKL/OPG equilibrium in the early stages of OA might contribute to the prevention of cartilage destruction. These findings also provide evidence suggesting the potential value of anti-RANKL therapy in OA.

AUTHOR CONTRIBUTIONS

Dr. Cohen-Solal had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Kadri, Ea, Bazille, Hannouche, Cohen-Solal.
Acquisition of data. Kadri, Ea, Bazille, Hannouche, Cohen-Solal.
Analysis and interpretation of data. Kadri, Ea, Lioté, Cohen-Solal.
Manuscript preparation. Kadri, Ea, Lioté, Cohen-Solal.
Statistical analysis. Kadri, Lioté, Cohen-Solal.
In vivo experiment for meniscectomy. Hannouche.

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