IL-6 Negatively Regulates IL-11 Production in Vitro and in Vivo

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IL-6 and IL-11 are two cytokines that increase osteoclast formation and augment bone resorption. PTH stimulates the production of both cytokines by human osteoblast-like cells. Circulating levels of IL-6 are elevated in patients with states of PTH excess and correlate strongly to markers of bone resorption. In contrast, serum levels of IL-11 were significantly reduced in patients with primary hyperparathyroidism compared with values in euparathyroid controls. Further, after successful parathyroid adenomectomy, circulating levels of IL-6 fell, whereas IL-11 levels increased. Five-day infusions of hPTH-(1–84) in rodents resulted in a significant decline in mean circulating levels of IL-11, whereas IL-6 levels significantly increased. Pretreatment of cells and mice with neutralizing serum to IL-6 enhanced PTH-induced IL-11 production compared with the effect of pretreatment with nonimmune sera. These data indicate that IL-6 negatively regulates IL-11 production in vivo and in vitro. Analysis of steady state mRNA levels in SaOS-2 cells indicated that this effect is posttranscriptional. As both IL-6 and IL-11 stimulate osteoclast formation, down-regulation of IL-11 by IL-6 may help modulate the resorptive response to PTH. (Endocrinology 142: 3850–3856, 2001)

INTERLEUKINS WERE FIRST identified as cytokines that regulate hemopoietic cell function and proliferation (1, 2). Together with leukemia inhibitory factor, oncostatin M, and ciliary neutrophic factor, IL-6 and IL-11 belong to a family of cytokines that shares the use of gp130 for intracellular signaling (2, 3). IL-6 and IL-11 have multiple, partially overlapping functions, including effects on hemopoiesis and modulation of immune responses (2, 4, 5).

Several in vitro experiments have established that both IL-6 and IL-11 also play prominent roles in bone homeostasis and share similar functions in that tissue as well. First, both molecules are produced by osteoblasts in response to osteotropic factors such as PTH and 1,25-dihydroxyvitamin D (6, 7). Transcripts for both the IL-6 and IL-11 receptors are expressed in osteoblast progenitor cells and mature osteoblasts (8). In vitro, IL-6 and IL-11 stimulate osteoclast formation, which appears to be mediated by the signal transducer gp130 (9). In addition, recent data suggest that IL-11 inhibits osteoclast apoptosis (10, 11). In the aggregate, these data suggest a proresorptive action for both cytokines in bone.

Recent studies have also indicated a role for these cytokines in states of disordered mineral homeostasis. Thus, neutralizing IL-6 in vivo prevents the increased osteoclastogenesis that occurs after estrogen withdrawal (12). In states of PTH excess, such as primary hyperparathyroidism, circulating levels of IL-6 are elevated and correlate to markers of bone resorption (13). In vivo, neutralizing IL-6 attenuates PTH-induced bone resorption in mice (14). Reduced expression of IL-11 in the bone marrow of the senescence-accelerated mouse (SAMP6) is associated with diminished bone formation and osteoclastogenesis; the former is thought to explain in part the reduced bone mass seen in these animals (15).

Although both IL-6 and IL-11 appear to have important roles in mediating bone resorption, studies addressing the coordinate regulation of these molecules by osteotropic hormones have not been reported. We therefore explored the responses of both cytokines to alterations in parathyroid function. Our data indicate that IL-6 suppresses PTH-induced IL-11 production. As both IL-6 and IL-11 stimulate osteoclast formation, down-regulation of IL-11 by IL-6 may help modulate the resorptive response to PTH.

Materials and Methods

Human studies

These studies were approved by the Yale University human investigation committee.

Measurement of circulating levels of IL-11 in hyperparathyroidism

Sera were collected from 22 normal volunteers, 29 patients with primary hyperparathyroidism, and 7 patients with hypoparathyroidism. The patients with hypoparathyroidism have been previously described (13).

Effect of parathyroidectomy on cytokine levels

Sera from three patients who underwent parathyroidectomy were obtained before surgery and every 4 h thereafter for 24 h. Mean serum calcium in these patients fell from a preoperative value of 10.7 to 9.7 mg/dl 24 h postoperatively. All patients have subsequently remained eucalcemic. All samples were frozen immediately at −70 C until analyzed.

Animal studies

Animal experiments were approved by the Yale animal care and use committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Baseline levels of IL-6 and IL-11

Six-week-old CD-1 mice (Charles River Laboratories, Inc., Wilmington, MA) were used in all studies. Baseline circulating levels of IL-6 and

Abbreviations: CV, Coefficient of variation; hPTH, human PTH.
IL-11 were determined in samples obtained from eight mice. Six-week-old female IL-6 knockout mice (13) (IL-6−/−; n = 6) and their wild-type littermates (IL-6+/+; n = 6) were anesthetized, and blood was collected by cardiac puncture. Samples were stored at −70°C until analyzed.

**PTH infusion**

**Rats.** Twelve-week-old Sprague Dawley rats (250 g; Taconic Farms, Inc., Germantown, NY) were infused with human PTH(1–84) [hPTH(1–84)] at 12.9 pmol/kg per day for 5 days as previously reported (14). During the infusion, serum samples were collected daily by tail bleed. Animals infused with vehicle were studied in parallel. Samples were stored at −70°C until analyzed.

**Mice.** PTH infusions were performed as previously reported (14). hPTH-(1–84) was infused at a rate of 4.3 pmol/kg per day using osmotic minipumps (Alza Corp., Palo Alto, CA), then mice were anesthetized, and blood was collected by cardiac puncture. Samples were stored at −70°C until analyzed. Just before infusion, five mice were injected with IL-6-neutralizing antibody (1 mg/mouse, ip) as previously described (14). Five mice received an irrelevant isotype-matched antibody (clone RR8-1 to the Vα 11.1 and Vα 11.2 murine T cell receptors, which are not expressed in CD-1 mice). Five mice received hPTH-(1–84) infusions alone.

**Studies in cultured cells**

Human osteoblast-like cells (SaOS-2) were cultured in RPMI medium (Life Technologies, Inc., Gaithersburg, MD) supplemented with 10% FCS (Sigma, St. Louis, MO), 1% penicillin/streptomycin (Sigma), and 1% glutamine. The cells were grown to confluence in six-well plates. At confluence, media were aspirated and replaced with 2 ml medium containing 0.4 mg/ml (final concentration) neutralizing antibody to human IL-6 (R&D Systems, Inc., Minneapolis, MN) or nonimmune serum. The experiment was repeated using the following conditions.

**Cytokine assays**

Concentrations of human IL-6 and IL-11 were measured by solid phase ELISAs (R&D Systems, Inc.). The lower limits of detection for these assays are less than 1 pg/ml for IL-6 and less than 8.0 pg/ml for IL-11. The precision of these measurements in our laboratory is: for IL-6: intraassay coefficient of variation (CV), 4.4%; interassay CV, 5.4%; and for IL-11: intraassay CV, 3.9%; interassay CV, 5.1%. Rat and mouse IL-11 were measured using a murine solid phase ELISA (R&D Systems, Inc.). We routinely increased the antibody incubation time to increase the sensitivity to 3.9 pg/ml. The precision of this measurement in our laboratory is: intraassay CV, 3.2%; and interassay CV, 4.1%.

A murine sandwich ELISA was developed to measure murine IL-11 in cell culture supernatant and serum. Goat antiserum against IL-11 (R&D Systems, Inc.) was diluted to a working concentration of 2 µg/ml in PBS without carrier protein. Ninety-six-well microplates (Costar, Corning, Inc., Corning, NY) were immediately coated with 100 µl/well of the diluted antibody. The plate was sealed and incubated overnight at room temperature or in an incubator at 25°C. Each well was then aspirated and washed with 300 µl wash buffer (0.05% Tween 20 in PBS, pH 7.4). The process was repeated for a total of four washes. The wells were blocked with 300 µl block buffer (1% BSA and 5% sucrose in PBS with 0.05% sodium azide) for a minimum of 1 h at room temperature. The aspiration/wash cycle was then repeated.

A seven-point standard curve using a 2-fold serial dilution of mouse IL-11 (R&D Systems, Inc.) was diluted in reagent diluent: 0.1% BSA and 0.05% Tween 20 in Tris-buffered saline (20 mM Trizma base and 150 mM NaCl), pH 7.3 (0.2 µm pore size filtered). Standards ranged from 7.8–500 pg/ml.

One hundred microliters of the reagent diluent were added to each well, followed by the addition of 100 µl of either standard or sample. After a 2-h incubation, the plate was washed as before. Two hundred microliters of biotinylated goat antiserum against IL-11 (200 ng/ml) were then added, followed by a 2-h incubation at room temperature. The aspiration/wash cycle was repeated as described above. One hundred microliters of streptavidin-horseradish peroxidase (R&D Systems, Inc.) were then added to each well, followed by a 20-min incubation at room temperature. The aspiration/wash cycle was repeated as described above, followed by the addition of 100 µl of equal volumes of hydrogen peroxide and tetramethylbenzidine. After a 30-min incubation at room temperature, the reaction was stopped with 50 µl 2 M sulfuric acid. The OD of each well was determined using a microplate reader set at 450 nm, and at 540 nm for wavelength correction. Calculations are performed using a computer-generated 4PL curve fit. The sensitivity of the assay is 2.9 pg/ml. The intra assay coefficient of variation is 3.7%, and the interassay coefficient of variation is 4.7%.

**PTh assays**

Determination of serum hPTH-(1–84) by immunoradiometric assay and measurement of circulating levels of midmolecule PTH were determined as previously reported (13).

**Northern analysis**

For Northern blot analyses, SaOS-2 cells were grown to confluence in 10-cm diameter tissue culture dishes and treated with 10 nm hPTH-(1–34) for 0, 2, 4, 6, and 8 h to establish the time of maximal PTH-induced increase in IL-11 mRNA expression. After establishing that 2 h was optimal, the experiment was repeated using the following conditions. Cells were treated for 2 h with 1 neutralizing antibody to IL-6 (0.2 mg/ml/final concentration), 2) nonimmune serum (0.2 mg/ml/final concentration), 3) hPTH and neutralizing antibody to IL-6, or 4) hPTH and nonimmune serum. The experiment was repeated four times.

mRNA was isolated using the TRIzol reagent, electrophoresed, and transferred to a nylon membrane using the method of Church and Gilbert (18). The IL-11 probe was provided by Dr. Paul Schendel (Genetics Institute, Boston, MA) (19, 20). Expression of mRNA for murine GAPDH (probe from Ambion, Inc., Austin, TX) was used as a loading control.

**Statistical analyses**

All analyses were performed using the Systat statistical package (version 5.2.1, Systat, Inc., Evanston, IL). Comparisons of data from patients before and after parathyroid surgery and from the rat infusion studies were performed using repeated measures ANOVA. All other comparisons were performed using t test for unpaired data. Data are presented as the mean ± sem.

**Results**

**Circulating levels of IL-11 are suppressed in patients with primary hyperparathyroidism**

As PTH stimulates IL-11 production in vitro (9), we sought to determine whether circulating levels of the cytokine are altered in patients with abnormal parathyroid function.

Mean fasting serum IL-11 values were suppressed in patients with hyperparathyroidism compared with euglycemic controls or subjects with hypoparathyroidism. Thus, in 22 controls the mean circulating IL-11 value was 12.4 ± 1.0 pg/ml, significantly higher than the mean value of 5.7 ± 1.2 pg/ml in 29 patients with primary hyperparathyroidism (P < 0.001). Subjects with hypoparathyroidism had mean serum IL-11 values no different from controls (10.9 ± 1.0
pg/ml). As expected, based on our prior studies, IL-6 levels were elevated in the hyperparathyroid patients and suppressed in the subjects with hypoparathyroidism compared with eucalcemic controls (controls, 1.1 ± 0.1 pg/ml; hyperparathyroid subjects, 12.0 ± 1.4; hypoparathyroid subjects, 0.4 ± 0.04; P < 0.001 controls vs. hyper- and hypoparathyroid subjects). Figure 1A summarizes the pattern of cytokine expression in the three groups of study subjects. In euparathyroid and hypoparathyroid individuals IL-11 circulates in concentrations higher than those of IL-6, whereas in hyperparathyroid subjects the relationship is reversed.

These data suggest that increased circulating levels of PTH are associated with lower serum IL-11 levels. To more closely examine this possible association we regressed values for serum PTH against those of IL-11 in the 29 subjects with primary hyperparathyroidism. Their mean serum calcium level was 10.9 ± 0.6 mg/dl, and the mean midmolecule PTH value was 103 ± 40 nEq/ml (normal, 10–25 nEq/ml). As shown in Fig. 1B, there was a strong negative correlation between circulating levels of PTH and serum IL-11 (r = −0.61; P < 0.001). The higher the serum PTH, the lower the IL-11. There was no correlation between serum calcium concentrations and circulating levels of IL-11.

IL-6 levels fall and IL-11 levels rise after parathyroid adenomectomy

To determine whether the level of circulating IL-11 is influenced by rapid changes in PTH, we measured serum IL-11 and IL-6 every 4 h for 24 h after successful parathyroid adenomectomy in three patients. As illustrated in Fig. 2, adenomectomy resulted in a rapid decline in circulating IL-6 from a preoperative value of 21.5 ± 2.7 pg/ml to a value of 7.1 ± 0.6 pg/ml 24 h after surgery, a decline of 67% (P = 0.03). This was accompanied by a 2-fold rise in mean serum IL-11 levels (mean preoperative value, 4.3 ± 0.7 pg/ml; 24 h postsurgery, 9.9 ± 1.7; P = 0.03), suggesting a reciprocal relationship between circulating levels of IL-6 and IL-11.

Circulating levels of IL-11 decline during PTH infusions in rats

To further explore this relationship, PTH infusions were carried out in rodents. We initially studied rats because serial blood collections can be performed in these animals. PTH infusion for 5 d led to a significant increase in circulating levels of PTH in the infused animals (0 to 120 h, 11.6 ± 1.1 to 347.5 ± 35.7 pg/ml). This was accompanied by a small rise in serum calcium (9.5 ± 0.1 to 10.2 ± 0.1 mg/dl). There was no change in PTH (10.3 ± 0.4 to 10.8 ± 0.3 pg/ml) or serum calcium (9.9 ± 0.1 to 9.8 ± 0.1 mg/dl) in the vehicle-infused animals.

As shown in Fig. 3, PTH infusion resulted in a progressive increase in circulating IL-6 levels, whereas no change in IL-6 levels was observed in vehicle-infused animals [end vs. start of PTH infusion, 20.0 ± 1.6 vs. 5.1 ± 0.6 pg/ml (P < 0.001); vehicle-infused, 4.0 ± 0.1 vs. 3.7 ± 0.3 pg/ml (P = NS)]. In

FIG. 1. A, Mean circulating levels of IL-6 and IL-11 in euparathyroid controls, patients with primary hyperparathyroidism, and patients with hypoparathyroidism (n = 22 for IL-11 and n = 12 for IL-6 values in normal volunteers, n = 29 for patients with primary hyperparathyroidism, and n = 6 for IL-6 and n = 7 for IL-11 levels in patients with hypoparathyroidism). *, P < 0.01; **, P < 0.001 (vs. normal subjects). B, Circulating levels of PTH correlate significantly to serum IL-11 values in patients with primary hyperparathyroidism (r = −0.61; P < 0.001; n = 29).

FIG. 2. Serial measurements of IL-6 and IL-11 before and every 4 h for 24 h after parathyroidectomy (n = 3). ▲, IL-6; ●, IL-11.
compared with the other two groups). The apparent decline
mice pretreated in neutralizing antiserum to IL-6 (anti IL-6, neutralizing antibody to IL-6. nonimmune serum, and 13.4/H11006 6.2/H11006 start of PTH infusion, 5.8/H206 induced increase in circulating IL-11 Neutralizing IL-6 in vivo results in an augmented PTH-
lower than those at the end of the vehicle infusion [end
values were significantly below those at the start and
circulating IL-11 values such that at the end of the infusion
contrast, PTH infusion caused a time-dependent decline in
circulating IL-11 values that such as at the start and
mean values were significantly below those at the end of the
start of PTH infusion, 5.8 ± 0.2 vs. 8.0 ± 0.4 pg/ml (P < 0.05);
end of PTH infusion vs. end of vehicle infusion, 5.8 ± 0.2 vs.
7.5 ± 0.3 pg/ml (P < 0.05).
Neutralizing IL-6 in vivo results in an augmented PTH-
induced increase in circulating IL-11
To determine whether the observed fall in serum IL-11
after PTH infusion was causally related to the increase in
IL-6, CD-1 mice were pretreated with a neutralizing antibody
before being infused with PTH. After IL-6 neutral-
ization, PTH infusion resulted in a significant increase in
circulating IL-11 compared with that in untreated mice or
mice pretreated with nonimmune sera (Fig. 4). Thus, at the
end of the 5-d infusion, mean IL-11 levels in the three groups
of animals were 4.0 ± 0.2 pg/ml in PTH-infused animals,
6.2 ± 0.3 pg/ml in PTH-infused animals pretreated with
nonimmune serum, and 13.4 ± 0.7 pg/ml in PTH-infused
mice pretreated in neutralizing antiserum to IL-6 (P < 0.0001
compared with the other two groups). The apparent decline
in the circulating IL-6 concentration after treatment with
neutralizing antibody to IL-6 is probably an artifact due to
binding of the antibody near or to an epitope recognized by
the antibody used in the ELISA.
Baseline IL-11 values are elevated in IL-6 knockout mice
If IL-6 negatively regulates IL-11 protein expression in
vivo, then IL-11 levels should be higher in mice with targeted
deletion of the IL-6 gene. We measured baseline values of
IL-11 in IL-6−/− mice and in wild-type IL-6+/+ littermates.
Mean serum IL-11 values in IL-6 knockout animals were
more than twice as high as those in wild-type littermates
(IL-6−/−, 12.4 ± 0.6 pg/ml; wild-type controls, 5.1 ± 0.4
pg/ml; n = 7; P < 0.0001; Fig. 5).
Neutralizing IL-6 in vitro enhances PTH-induced IL-11
protein production by bone cells
As PTH induces both IL-6 and IL-11 production by bone
cells (21, 22), we sought to determine whether neutralizing
bone cell-derived IL-6 influenced PTH-induced IL-11 pro-
duction in these cells. In the presence of nonimmune serum,
PTH treatment of SaOS-2 cells induced a 1.7 ± 0.2-fold in-
crase in IL-11, from 235 ± 6 to 405 ± 87 pg/ml. In the
presence of neutralizing antisera to IL-6, however, the
PTH-induced increase in IL-11 levels was significantly aug-
mented to 2.9 ± 0.1-fold (235 ± 6 to 680 ± 78 pg/ml; P < 0.001
for fold increase compared with the fold increase in the

Fig. 3. Circulating levels of IL-6 and IL-11 during 5-d PTH infusions in rats. Blood samples were collected at baseline and every 24 h thereafter until the end of the infusion (n = 5). *, P < 0.005 vs. IL-6 levels in vehicle-infused animals; #, P < 0.05 vs. IL-11 levels in vehicle-infused animals.

Fig. 4. The effect of in vivo neutralization of IL-6 on circulating levels of IL-11 in PTH-infused mice. Six-week-old female CD-1 mice received an ip injection of 1 mg/mouse of either an IL-6-neutralizing antibody or a control antibody, followed by hPTH infusion. Blood samples were collected at the end of the infusion (n = 8 for baseline, n = 5 for neutralizing antibody followed by PTH infusion, and n = 5 for nonimmune serum followed by PTH infusion). *, P < 0.001 vs. baseline; †, P < 0.001 vs. PTH and nonimmune serum. NIS, Nonimmune serum; anti IL-6, neutralizing antibody to IL-6.

Fig. 5. Mean baseline IL-11 values in IL-6−/− mice. Blood was collected from 6-wk-old female IL-6 knockout mice (IL-6−/−; n = 6) and their wild-type littermates (IL-6+/+; n = 6). *, P < 0.0001 vs. wild-type mice.
presence of nonimmune serum; Fig. 6). As SaOS-2 is a malignant cell line, these studies were repeated using primary human and murine osteoblast cultures. Consistent with the findings in the SaOS-2 cells, PTH induced a 2.1 ± 0.1-fold increase in IL-11 levels in the presence of nonimmune serum (64 ± 3 to 134 ± 6 pg/ml) and a 3.0 ± 0.1-fold increase in the presence of neutralizing antiserum to IL-6 (63.7 ± 2.6 to 191.2 ± 5.2 pg/ml) in primary human osteoblast cultures (P < 0.01 for fold increase compared with the fold increase seen in the presence of nonimmune serum). In the primary murine osteoblasts, PTH induced a 2.0 ± 0.1-fold increase in IL-11 levels in the presence of nonimmune serum (39 ± 3 to 77 ± 4 pg/ml) and a 2.9 ± 0.2-fold increase in the presence of neutralizing antiserum to IL-6 (39 ± 3 to 115 ± 9 pg/ml; P < 0.01 for the fold increase compared with the fold increase seen in the presence of nonimmune serum).

Neutralizing IL-6 does not affect PTH-induced IL-11 mRNA expression in SaOS-2 cells

A time-course study was initially performed to determine the time of maximal PTH-induced IL-11 mRNA expression. In agreement with previously reported experiments (22), the peak effect of PTH occurred at 2 h (data not shown). Northern blot analyses were then performed to determine whether IL-6 modulated this effect. The magnitude of PTH-induced IL-6 transcript expression was nearly identical in the presence of neutralizing antiserum to IL-6 or nonimmune sera (Fig. 7). In four separate experiments, PTH-induced IL-11 mRNA expression was not different in the presence of neutralizing antiserum to IL-6 compared with nonimmune serum. The data were evaluated in two ways. First, each IL-11 transcript densitometric measurement was corrected for loading by normalizing to GAPDH transcript expression, and the mean values for the four experiments were compared under the four experimental conditions. These values were: nonimmune serum alone, 0.91 ± 0.32; IL-6-neutralizing antibody alone, 0.75 ± 0.20; PTH plus nonimmune serum, 2.16 ± 1.27; and PTH plus IL-6-neutralizing antibody, 1.78 ± 0.78 (P = 0.27 for the last two groups). In the second method we subtracted the values obtained when cells were treated with only nonimmune serum or anti-IL-6 antibody from the values in the respective PTH-treated cells. These values were: PTH plus nonimmune serum, 1.25 ± 1.05-fold; PTH plus anti-IL-6 antibody, 1.04 ± 0.60-fold (P = 0.49). These data suggest that regulation of IL-11 by IL-6 occurs at a posttranscriptional level.

Discussion

The three principal findings of this study are that: 1) Increased PTH levels in vivo are associated with reduced levels of IL-11. Thus, patients with hyperparathyroidism had significantly reduced serum levels of IL-11 compared with euparathyroid controls or patients with hypoparathyroidism. 2) Circulating IL-11 levels are inversely related to serum IL-6 values. Serum IL-11 levels rose progressively after surgical correction of hyperparathyroidism and mirrored the concomitant decline in IL-6 levels in these patients. Conversely, the induced rise in serum IL-6 in animals infused with PTH was accompanied by a decline in circulating IL-11 values. Consistent with these observations, circulating levels of IL-11 were increased in animals with targeted deletion of the IL-6 gene compared with values in wild-type littermates. There appears to be a threshold for this effect, such that at normal or low circulating levels of PTH, this relationship is not apparent. This would explain the lack of difference in circulating IL-11 values between euparathyroid and hypoparathyroid subjects. 3) Neutralizing IL-6 production augments PTH-induced IL-11 production in vivo and in osteoblasts in vitro. Taken together, these data indicate that IL-6 negatively regulates IL-11 production in vivo and in vitro.

PTH exerts its preresorptive effects principally by stimu-

![Fig. 6. Effect of PTH treatment and IL-6 neutralization on IL-11 protein expression in SaOS-2 cells (two left bars), primary human osteoblasts (two middle bars), and primary murine osteoblasts (two right bars). Cells were grown to confluence and treated with 10 nM hPTH-(1–84) plus nonimmune serum (0.2 mg/ml) or hPTH plus neutralizing IL-6 antibody (0.2 mg/ml). Conditioned media were collected after 24 h for determination of IL-11. Data represent the mean fold increase in IL-11 levels in PTH-treated cultures in the presence of nonimmune serum, open bar or PTH-treated cultures in the presence of neutralizing antibody to IL-6 (filled bar), **P < 0.001; *, P < 0.01 (compared with treatment with nonimmune serum).](https://academic.oup.com/endo/article-abstract/142/9/3850/2989080)

![Fig. 7. Northern blot analysis of PTH-induced IL-11 mRNA expression. SaOS-2 cells were treated with PTH plus nonimmune serum or PTH plus IL-6-neutralizing antiserum for 2 h. RNA was prepared, and Northern analysis was performed. As a control, cells were also treated with antiserum alone for 2 h. Results are representative of those in three other experiments.](https://academic.oup.com/endo/article-abstract/142/9/3850/2989080)
lating the production of soluble and cell surface molecules in osteoblasts and stromal cells. Both IL-11 and IL-6 are produced by osteoblasts in response to PTH and are thought to act principally by enhancing the formation of osteoclast precursors. The physiological importance of the inverse relationship between IL-6 and IL-11 in response to PTH may be in modulating the total resorptive effect of PTH in vivo. Thus, a reduction in IL-11 when IL-6 levels are elevated may restrain the resorptive response to PTH by diminishing osteoclast progenitor recruitment.

Alternatively, this coordinate regulation may be part of the control mechanisms for the overlapping biological function of these two cytokines. Thus, for example, in the absence of IL-6 (e.g. IL-6-/- mice) when the resorptive actions of PTH are impaired (14), these animals are only modestly hypocalcemic (23), suggesting that other factors, such as IL-11, compensate in the absence of IL-6 as downstream effectors for PTH’s resorptive action.

The tissue sites at which this coordinate regulation occurs cannot be identified with certainty from our study. However, our data indicate that at least one site where this interaction occurs is in osteoblasts. Although down-regulation of IL-11 by IL-6 has, to our knowledge, not been previously reported, the converse has been observed. Pretreatment of peritoneal macrophages with IL-11 resulted in a significant (>60%) reduction in lipopolysaccharide-induced IL-6 production (5). In macrophages this inhibition is transcriptionally regulated by induction of IkB family members that inhibit transcriptional activation of NFkB (24). It is postulated that this coordinate regulation restrains the resultant inflammatory response. Thus, IL-11 has been shown to improve survival due to sepsis in two experimental models (25, 26). In bone tissue, the inverse relationship described herein, namely suppression of IL-11 by IL-6, may result in an analogous effect, that is to restrain the net resorbing effect of PTH. Thus, tight coordinate regulation of IL-6 and IL-11 expression in both the resorption and osteoblastic cells is to restrain the net resorbing effect of PTH.

In conclusion, we have established that increased IL-6 production in response to PTH results in decreased circulating IL-11 levels due to down-regulation of IL-11 production. At least one tissue in which this occurs is bone. As both IL-6 and IL-11 promote osteoclast formation, down-regulation of IL-11 by IL-6 may be one way in which the total resorptive activity of PTH is modulated.

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