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Interleukin-18 Is a Novel Mitogen of Osteogenic and Chondrogenic Cells

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IL-18 was identified due to its ability to induce interferon-γ (IFNγ) production by T cells. It is a pleiotropic factor that shares structural features with IL-1 and functional activities with IL-12. IL-18 has a role in T cell development, where it has been demonstrated to act cooperatively with IL-12 to regulate IFNγ. In bone, IL-18 is mainly produced by macrophages, but is also expressed by osteoblasts and inhibits osteoclast formation through granulocyte-macrophage colony-stimulating factor (GM-CSF) and not IFNγ production by T cells. We have investigated the effects of IL-18 on mature osteoclast activity and for potential actions on osteoblasts or chondrocytes.

The effects of IL-18 on mature osteoclast activity were determined using two assays: isolated mature osteoclast cell culture and neonatal murine calvarial organ culture. IL-18 did not affect bone resorption in either assay system. The actions of IL-18 on osteogenic cells (primary cell cultures of fetal rat and neonatal mouse osteoblasts, as well as neonatal mouse calvarial organ culture) and primary chondrocytes (canine) were assessed by proliferation assays (quantification of cell numbers and thymidine incorporation). In each assay system, IL-18 acted as a mitogen to the osteogenic and chondrogenic cells. Since IL-18 signal transduction may involve IFNγ or GM-CSF, we assessed their involvement in the IL-18 response. IL-18 did not induce IFNγ production by primary osteoblasts, but, of greater significance, IFNγ had the opposing action to IL-18 in that it inhibited the primary osteoblast cell proliferation. Although IL-18 rapidly induced GM-CSF production by primary osteoblasts, IL-18 was still mitogenic in osteoblast preparations established from GM-CSF-deficient mice. Combined, these studies indicate that IL-18 may have an autocrine/paracrine mitogen role for both osteogenic and chondrogenic cells, independent of the production of IFNγ or GM-CSF. (*Endocrinology* 144: 1194–1201, 2003)

BONE GROWTH is controlled by systemic hormones as well as by an increasing number of factors produced locally. Bone growth involves the complex coupling of the actions of osteoblasts and osteoclasts, which occurs by a contact-dependent mechanism and also involves paracrine and autocrine factors. It has recently been demonstrated that IL-18 suppresses the development of osteoclasts *in vitro* via granulocyte-macrophage colony-stimulating factor (GM-CSF; Ref. 1). The mechanism proposed for this action is that IL-18 binds to its receptor on T cells in the bone microenvironment to activate GM-CSF production, and the secreted GM-CSF, in turn, binds to its receptor on osteoclastic precursors to inhibit proliferation/differentiation (2).

IL-18 is a recently discovered cytokine originally named for its ability to induce interferon-γ (IFNγ) production by T cells (3). However, it has since been shown to be more than an inducer of IFNγ (4). IL-18 shares structural features with the IL-1 family of proteins and functional properties with IL-12 (5). IL-18 is a pleiotropic factor with many proinflammatory functions, such as inducing IFNγ, stimulating the proliferation of activated T cells and the differentiation of T helper type 1 cells (where IL-18 acts in synergy with IL-12), enhancing natural killer (NK) cell cytotoxicity (6), up-regulating intercellular adhesion molecules (7), and augmenting the production of GM-CSF (8). Both of the latter functions are independent of IFNγ production. The inhibitory effect of IL-18 on osteoclast generation is also independent of IFNγ (1).

Murine and human IL-18 gene sequences encode 192- and 193-amino-acid precursor proteins, respectively (9). These 24-kDa precursor molecules lack a signal peptide and have limited biological activity. The active mature 18.3-kDa molecule is cleaved from the precursor molecule by the action of IL-1β-converting enzyme, also known as caspase 1 (10, 11). Caspases belong to a protease family that plays a pivotal role in inflammation and apoptotic cell death (12). Yet another caspase, caspase 3, has a regulatory effect on IL-18, where it cleaves both precursor and mature forms of IL-18 into biologically inactive degraded products and may constitute a potential down-regulator of IL-18 (13).

The IL-18 receptor comprises two molecules, both of which belong to the IL-1 receptor family: IL-18R, a ligand-binding chain, and accessory protein-like (AcPL), an accessory chain (14, 15). IL-18R binds to IL-18 with low affinity, but the complex of the IL-18R and AcPL binds IL-18 with high affinity. IL-18 receptor genes are located in the region of chromosome 2, shared with other IL-1 receptor genes (16).

Antigen-presenting cells represent the major source of IL-18 production (13), but IL-18 expression has been demonstrated in a wide variety of cells, including osteoblasts and chondrocytes (1, 17). This study was designed to investigate further effects of IL-18 on skeletal cells, other than the cytokine's ability to inhibit osteoclastogenesis, and whether these effects were dependent on GM-CSF or IFNγ activity. We have used two culture systems that demonstrate the function of mature osteoclasts, namely, a bone organ culture

Abbreviations: AcPL, Accessory protein-like; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFNγ, interferon-γ; NK, natural killer; OPG, osteoprotegerin; TRAP, tartrate-resistant acid phosphatase.

and isolated mature osteoclast cultures, as well as various primary cultures of osteoblasts and chondrocytes to address these questions.

Materials and Methods

Animals

For the bone organ culture experiments ARC Swiss mice were used. Chondrocytes were isolated from cartilage removed from dogs purchased from the Dog Pound (Auckland, New Zealand). For osteoblast-like cell cultures, fetal Wistar rats were used, or cells were isolated from C57/BL6J mice (wild-type, purchased from Monash University Animal Services Center, Clayton, Australia) or GM-CSF-deficient mice (provided by Dr. Ashley Dunn, Ludwig Institute, Australia; Ref. 18). All experiments were conducted in accordance with the University of Auckland (New Zealand) and the National Health and Medical Research Council (Australia) guidelines and were approved by the institutional ethics committees.

Materials

IL-18 was provided by Dr. Kurimoto (Fujisaki Institute, Hayashibara Biochemical Laboratories, Inc., Okayama, Japan). EDTA and collagenase were obtained from Sigma-Aldrich (St. Louis, MO). Trypsin-EDTA, MEM, DMEM, medium 199, and fetal bovine serum (FBS) were obtained from Life Technologies, Inc. (Grand Island, NY). BSA was obtained from Immuno Chemical Products (Auckland, New Zealand). Pronase was purchased from Roche Molecular Biochemicals (Mannheim, Germany). [^{3}H]Thymidine and [^{3}H]phenylalanine were obtained from Amersham International (Little Chalfont, UK).

Mature isolated osteoclast culture

Rat osteoclasts were isolated from 1-d-old neonatal rats as previously described (19). Briefly, long bones were excised, chopped, and homogenized in acidified medium. The osteoclast-rich cell suspension (low numbers of stromal cells are also present) was collected and placed over bovine bone slices in 96-well plates and incubated for 35 min to allow the mature osteoclasts to settle. The bone slices were washed and placed in 6-well plates containing medium with cytokine or vehicle and were incubated for 20 h. Bone slices were fixed with 2.5% glutaraldehyde/PBS and stained for tartrate-resistant acid phosphatase (TRAP) using Sigma-Aldrich kit 387-A. The number of TRAP-positive multinucleated cells were quantified, and then the cells were removed by gentle scrubbing, stained with toluidine blue, and assessed for pits excavated by the osteoclasts using reflected light microscopy and metallurgic lenses.

Bone organ culture

Bone resorption studies were carried out in neonatal mouse calvariae as described previously (20). Two-day-old mice were injected s.c. with $^{45}CaCl_2$. Four days later the hemicalvariae were excised and cultured on small steel grids in 35-mm petri dishes containing medium 199 supplemented with 0.1% BSA. After a 24-h preculture period, fresh medium was added containing cytokine or vehicle. Calvariae were incubated for an additional 48 h. [^{3}H]Thymidine was added in the last 4 h of the incubation period. Bone resorption was measured by calculating the amount of ^{45}Ca released into the medium. DNA synthesis was assessed by measuring thymidine incorporation (21).

Primary osteoblast-like cell culture

Osteoblasts were isolated by sequential collagenase digestion of 20-d-old fetal rat calvariae as previously described (22). Digests 3–5 were pooled and grown in 75-cm^2 flasks in DMEM containing 10% FBS. Cells were grown to confluence, and then proliferation studies were performed. Cells were trypsinized and seeded into 24-well plates. Cells were serum-starved for 24 h in MEM/0.1% BSA, and then the medium was replaced, and cytokine or vehicle was added. The plates were incubated for an additional 24 h, with a pulse of [^{3}H]thymidine added 4 h before the end of the incubation period. Plates were assessed for thymidine incorporation or cell number.

The osteoblast-like character of these cells has been established by demonstration of high levels of alkaline phosphatase activity and osteocalcin production (23) and a sensitive adenylate cyclase response to PTH and prostaglandin E_2 (24). The osteoblast-like cells produced in this culture system are early in the osteoblast differentiation pathway and are often termed preosteoblastic.

Primary chondrocyte-like cell culture

Cartilage slices were removed from the articular surfaces of knee joints of adult dogs. The slices were finely chopped and incubated at 37 C first with pronase (0.8%, wt/vol) for 90 min, followed by collagenase (0.1%, wt/vol) for 18 h to complete the digestion. The isolated cells were washed and seeded into 75-cm^2 flasks containing DMEM supplemented with 10% FBS and 50 μg/ml ascorbic acid. Chondrocytes were grown

TABLE 1. Oligonucleotides to amplify and detect cytokine and receptor mRNAs

Oligonucleotide name	Orientation	Position (bp)	GenBank accession no.	Sequence (5′–3′)	Ref.[a]
GAPDH-1	Sense	686–705	NM_008084	GCTGTGGGCAAGGTCATCCC	25
GAPDH-2	Antisense	1004–1023	NM_008084	ATGAGGTCCACCACCCTGTT	1
GAPDH-4	Sense	352–371	NM_008084	CATGGAGAAGGCTGGGGCTC	25
GM-CSF-1	Sense	150–173	X02333	AGAAAGGCTAAGGTCCTGAGGAGG	26
GM-CSF-2	Antisense	504–528	X02333	CCGCATAGGTGGTAACTTGTGTTTC	26
GM-CSF-3	Antisense	339–359	X02333	GGAGAACTCGTTAGAGACGAC	26
GM-CSF-R-1	Sense	1099–1120	XM_109330.1	CGACTGGCTCTGTTTCCTTTGG	
GM-CSF-R-2	Antisense	1577–1599	XM_109330.1	TCCAAGTGTACTCTTCGCTCCAC	
GM-CSF-R-4	Antisense	1266–1285	XM_109330.1	CCTTGTTCCAGGTGCTTAAC	
IL-18-1	Sense	286–308	NM_008360.1	ACTGTACAACCGCAGTAATACGG	1
IL-18-2	Antisense	804–828	NM_008360.1	GGGTATTCTGTTATGGAAATACAGG	1
IL-18-3	Sense	641–660	NM_008360.1	TTGCCAAAAGGAAGATGATG	1
IL-18-R-4	Sense	480–499	NM_008365.1	GAACCTTTTTACCTGAAGCC	
IL-18-R-5	Antisense	1059–1076	NM_008365.1	CAACTCCACATCCTTTCC	
IL-18-R-6	Antisense	645–663	NM_008365.1	CCTCATCCTCCATCTCAAC	
IL-18AcPL-1	Sense	604–626	NM_010553.1	CTCCCATGCAAGTCAACTGTCAC	
IL-18AcPL-2	Antisense	990–1013	NM_010553.1	AAAGGCTTTCCAAGCTCTACGTC	
IL-18AcPL-3	Antisense	1217–1239	NM_010553.1	CCTTGTACCAGGTCATCTCTGGAC	
IFN-γ-1	Sense	130–150	M282621	TCTTGGCTTTGCAGCTCTTCC	26
IFN-γ-2	Antisense	560–582	M282621	CGAATCAGCAGCGACTCCTTTTC	26
IFN-γ-3	Sense	411–432	M282621	ACCTTCTTCAGCAACAGCAAGG	26

[a] Denotes citation to reference if oligonucleotide has been previously reported; no reference indicates that this is the primary publication for this oligonucleotide.

to confluence and seeded into 24-well plates, and proliferation assays were performed as detailed for the osteoblast-like cell culture.

RNA extraction, cDNA synthesis, and PCR

Total cellular RNA was extracted from newborn mouse calvarial cells using guanidine thiocyanate-phenol chloroform and used in RT-PCR essentially as previously described (25). Oligonucleotides (Table 1) were synthesized on an Oligo1000M DNA Synthesizer (Beckman, Fullerton, CA). The following oligonucleotides were used to amplify murine mRNA species: GM-CSF (GM-CSF-1 and GM-CSF-2), GM-CSF R (GM-CSF-R-1 and GM-CSF-R-2), IFNγ (IFNγ-1 and IFNγ-2), IL-18 (IL-18-1 and IL-18-2), IL-18R (IL-18-R-4 and IL-18-R-5), IL-18AcPL (IL-18AcPL-1 and IL-18AcPL-2), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; GAPDH-2 and GAPDH-4). The specificity of the products was confirmed by Southern blot detection using a ^{32}P-labeled internal oligonucleotide probe (GM-CSF-3, IFNγ-3, IL-18-3, IL-18-R-6, IL-18AcPL-2, and GAPDH-1) as previously described (26) or by nucleic acid sequencing of amplified products. Bound probe was detected by PhosphorImager analysis (Molecular Dynamics, Inc., Sunnyvale, CA) (26).

Statistical analysis

Data are presented as the mean ± SEM. The significance of differences between groups was determined by *t* test. The comparisons to be made in each experiment were specified *a priori*, so no adjustment for multiple comparisons was necessary. Where several experiments have been shown in one figure, the data are expressed as the ratio of results in treatment groups to those in the control group, and the *P* values shown were calculated using data from individual experiments before data were pooled.

Results

IL-18 does not inhibit mature osteoclast activity

IL-18 has been previously shown to inhibit osteoclast formation. We investigated whether IL-18 affected mature osteoclast activity using two different culture systems: 1) mature isolated osteoclasts, and 2) calvarial organ culture. IL-18 did not have any effect on mature osteoclast activity as measured by the number of bone-resorptive pits per TRAP-positive multinucleated cell (Fig. 1A). As a positive control, salmon calcitonin was analyzed in this culture system, and the peptide acted as a potent inhibitor of bone resorption (50% effective dose, 2.3×10^{-13} M; data not shown). We also demonstrated that IL-18 does not inhibit bone resorption in organ culture, as measured by calcium release from neonatal mouse calvaria (Fig. 1B). Bone resorption in this culture system predominantly reflects mature osteoclast activity rather than osteoclastogenesis due to the minimal amount of bone marrow in the calvarial tissue (27). However, IL-18 was found to significantly increase thymidine incorporation compared with that in controls in this organ culture (Fig. 1C). We have previously shown that increased thymidine incorporation in this model system reflects the proliferation of cells of the osteoblast lineage (28). As such, the effects of IL-18 on osteoblast and chondrocyte proliferation were assessed in primary cell cultures.

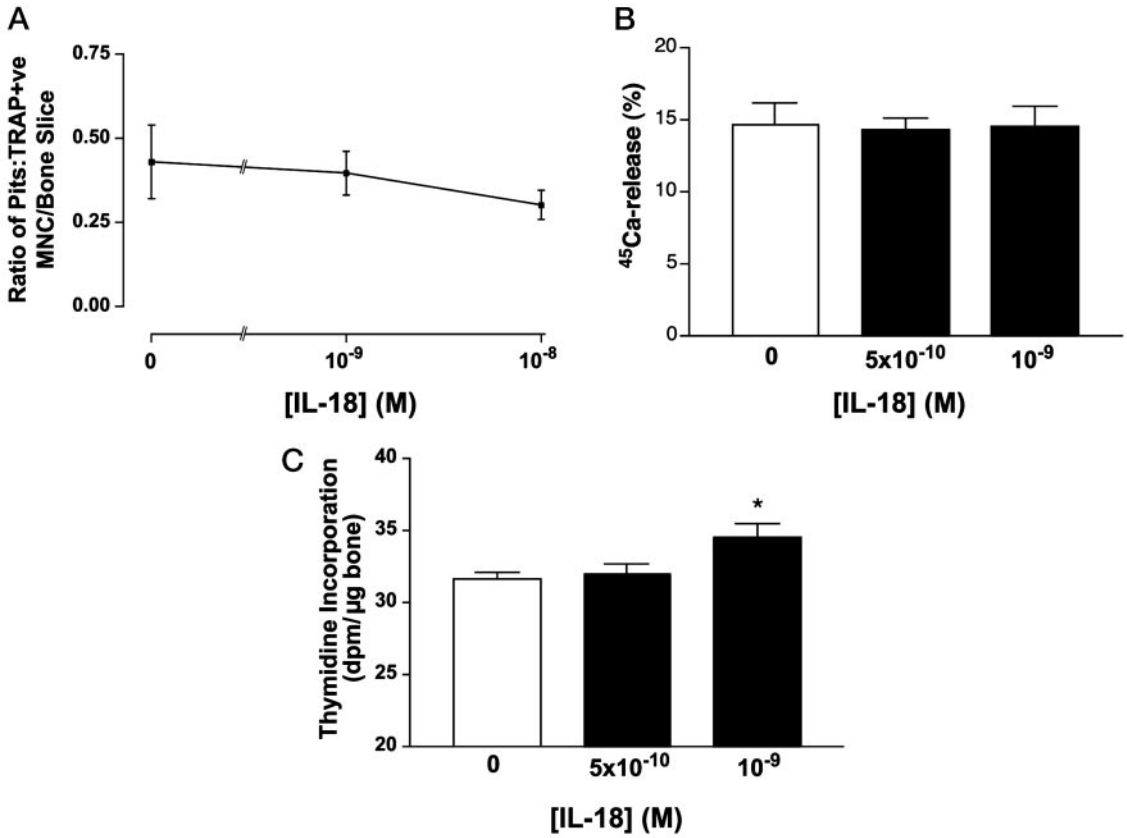

FIG. 1. Effects of IL-18 on neonatal rat mature osteoclast activity (A), bone resorption in neonatal murine calvarial organ culture (B), and DNA synthesis in organ culture (C). Data are expressed as the mean ± SEM number of bone resorptive pits per TRAP-positive multinucleated cell (A), percent calcium release (B), and [^{3}H]thymidine incorporation (C). *, Significantly different from control ($P < 0.03$).

IL-18 effects on chondrocyte proliferation

The proliferative effects of IL-18 on primary canine chondrocyte-like cells were assessed by the measurement of cell numbers and DNA synthesis. Treatment with IL-18 for 24 h produced a dose-dependent increase in cell numbers (Fig. 2A) and thymidine incorporation (Fig. 2B).

IL-18 effects on osteoblast proliferation

The effects of IL-18 on primary fetal rat osteoblast-like cells were assessed by measurements of cell numbers, DNA, and protein synthesis. Treatment with IL-18 for 24 h produced dose-dependent proliferative effects with increases in cell number (Fig. 3A) and DNA synthesis, as assessed by the measurement of [^{3}H]thymidine incorporation into cultured osteoblast-like cells (Fig. 3B). The magnitude of this response was comparable to that of classic osteoblast growth factors, such as TGFβ and epidermal growth factor (29). The effect of IL-18 on protein synthesis, as assessed by the measurement of [^{3}H]phenylalanine incorporation into cultured osteoblast-like cells, produced a dose-dependent increase (Fig. 3C). This increase in protein synthesis probably reflects the increased number of cells, because the percent increases in these two indexes were almost identical. Combined, these data demonstrate that IL-18 acts as a mitogen for osteogenic cells.

IL-18 mitogenic effect on primary osteoblasts is independent of both IFNγ and GM-CSF

IL-18 is known to signal through GM-CSF or IFNγ. To determine whether the enhanced osteoblast-like cell proliferation in response to IL-18 was a direct or an indirect action of IL-18, we examined the expression of GM-CSF, IFNγ, and receptor components in primary murine osteoblasts in response to IL-18 treatment. Primary murine osteoblasts were treated with IL-18 (10 ng/ml) over a 24-h time course (0.5, 1, 2, 4, 8, 12, and 24 h), RNA was extracted, and regulation of target genes was assessed by RT-PCR. The most striking regulation was observed with GM-CSF mRNA (Fig. 4). GM-CSF mRNA levels increased within 1 h of exposure to IL-18, and a 4-fold elevation in mRNA levels continued through to 24 h. A concomitant decrease in GM-CSF receptor mRNA was observed (Fig. 4). Although no detectable alteration in IL-18 R mRNA was observed, the mRNAs for IL-18 and IL-18AcPL gradually decreased over the 24-h time course (Fig. 4). IFNγ mRNA was not detectable (data not shown). The finding that IFNγ was not expressed by the primary osteoblasts, even in response to IL-18, indicated that it was unlikely that IFNγ was implicated in the osteoblast mitogenic response to IL-18. Since the primary osteoblast GM-CSF mRNA levels were elevated in response to IL-18, GM-CSF may be involved in the IL-18-induced mitogenic response. To test this possibility we studied the mitogenic response of IL-18 on osteoblast-like cells from GM-CSF-deficient mice. IL-18 significantly enhanced osteoblast proliferation whether the osteoblasts were obtained from GM-CSF-deficient (Fig. 5A) or wild-type (Fig. 5B) mice. This suggests that the IL-18 mitogenic effect on these osteoblast-like cells was GM-CSF independent.

In contrast to IL-18, IFNγ was not mitogenic to the primary osteoblasts, but, rather, IFNγ decreased cell numbers (Fig. 6A) and thymidine incorporation (Fig. 6B) of primary rat osteoblast cultures. This opposing action of IFNγ compared with that of IL-18 combined with the inability to detect IFNγ production by these osteoblasts would exclude IFNγ from this osteoblast mitogenic response to IL-18.

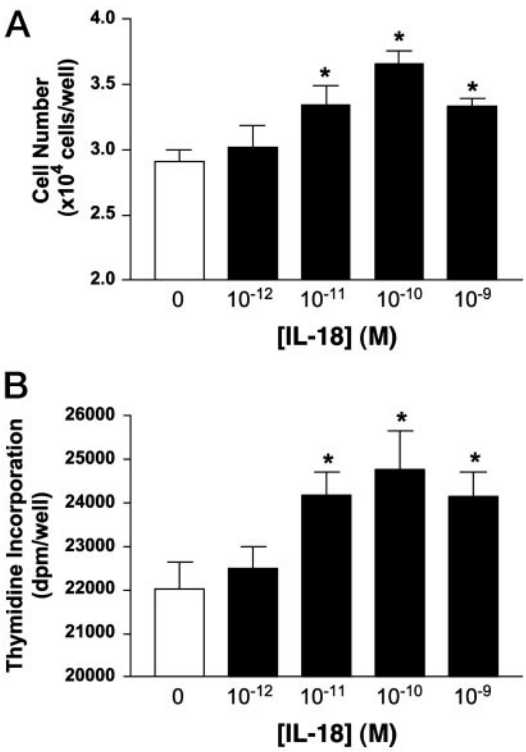

FIG. 2. Dose-dependent effect of IL-18 on primary canine chondrocyte cell number (A) and [^{3}H]thymidine incorporation (B). Data are the mean ± SEM. *, Significantly different from control ($P < 0.03$).

Discussion

In this study, we have demonstrated that IL-18 has direct effects on skeletal cells. IL-18 was a potent mitogen of primary osteoblast- and chondrocyte-like cells. This cytokine has been previously shown to be expressed by osteoblasts (1) and chondrocytes (17); thus, IL-18 is probably acting in a paracrine/autocrine manner in skeletal tissue. The mitogenic potential of IL-18 was independent of IFNγ or GM-CSF.

IL-18 was initially described as an IFNγ-inducing factor (30); however, it clearly has more functions than inducing IFNγ production alone, and many biological effects of IL-18 are now known to be independent of IFNγ's actions (4), including IL-18 inhibition of osteoclast formation (1). Our findings show that IFNγ is not expressed by primary osteoblast-like cells, and indeed, this cytokine has an inhibitory, rather than a proliferative, effect on primary osteoblasts, which confirms previous work performed in osteoblast-like cell lines (31). IL-18 acts to enhance not only IFNγ, but also GM-CSF, in a number of cell systems, such as peripheral blood monocytes (9). We have previously established that IL-18 inhibits osteoclast formation through stimulating the T

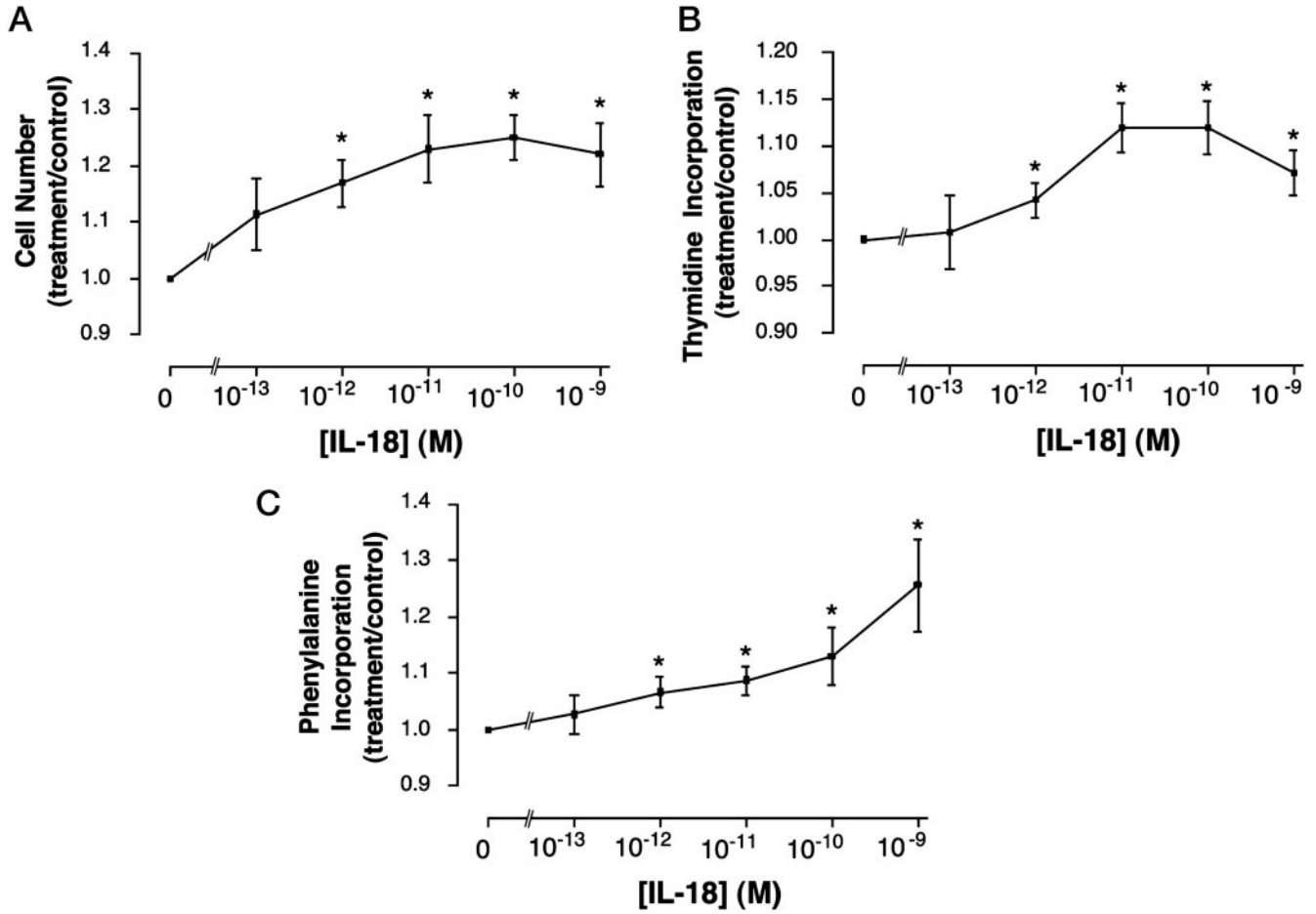

FIG. 3. Dose-dependent effect of IL-18 on primary fetal rat osteoblast cell number (A), [^{3}H]thymidine incorporation (B), and [^{3}H]phenylalanine incorporation (C). Data are the mean ± SEM. *, Significantly different from control ($P < 0.04$).

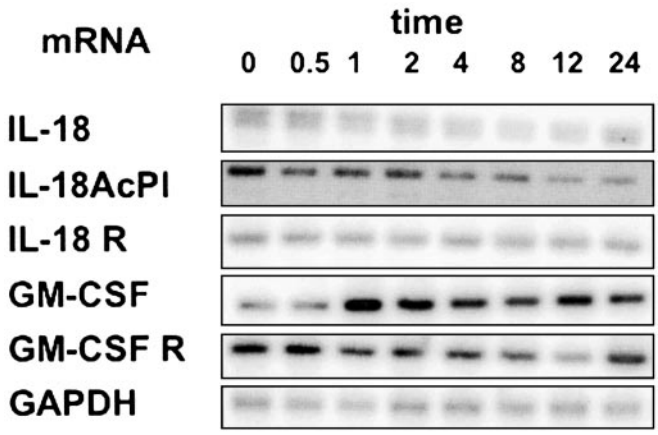

FIG. 4. Semiquantitative RT-PCR analysis of IL-18, IL-18AcPL, IL-18 R, GM-CSF, and GM-CSF R mRNA. Primary neonatal murine osteoblasts were treated with IL-18 (10 ng/ml) as indicated by the time course (0.5, 1, 2, 4, 8, 12, and 24 h), and total RNA was extracted. Total RNA samples were reverse transcribed with oligo(deoxythymidine) and subjected to PCR for IL-18 (30 cycles), IL-18AcPL (28 cycles), IL-18 R (34 cycles), GM-CSF (35 cycles), GM-CSF R (32 cycles), and GAPDH (20 cycles), which were in the log-linear range of amplification. Resultant PCR products were electrophoresed, transferred to nylon membrane, and hybridized with γ-^{32}P-labeled internal detection oligonucleotides specific to each of the cDNA products. RT-PCR analysis was repeated in triplicate.

cell production of GM-CSF (2). GM-CSF is a known autocrine mitogenic factor for osteoblasts (32), inducing a dose-dependent increase in human osteoblast proliferation (33). However, we were able to exclude GM-CSF in the IL-18 action by using osteoblasts isolated from GM-CSF-deficient mice. In such cultures IL-18 was still mitogenic. These findings imply that IL-18 is likely to be acting in an autocrine/paracrine manner for chondrogenic and osteogenic cells. The fact that the mitogenic action of IL-18 is independent of IFNγ or GM-CSF suggests that IL-18 is acting directly through the IL-18 receptor complex. Consistent with this proposition, both IL-18R and AcPL components were expressed by primary osteoblasts.

The finding that primary osteoblasts express both components of the IL-18 receptor, as also found in T and NK cells, implies that the osteoblasts are target cells responding to IL-18, and that IL-18 may have a physiological function on osteogenic cells. Further, the phosphorylation of MAPK in bone marrow stromal cells and osteoblasts in response to IL-18 supports this proposal (34). This observation is consistent with those in T and NK cells (35, 36), and it is possible that the phosphorylation of MAPK by IL-18 is involved in the proliferation of osteoblasts. However, as activation of nuclear factor-κB through the IL-1 receptor-associated kinase signaling pathway is considered a major pathway in the

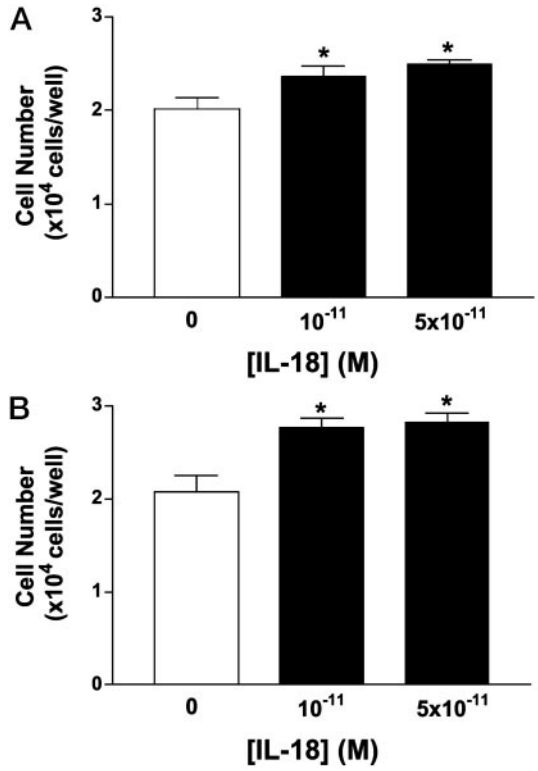

FIG. 5. Effect of IL-18 on neonatal murine osteoblast numbers from wild-type (A) and GM-CSF deficient (B) mice. Data are the mean ± SEM. *, Significantly different from control ($P < 0.03$).

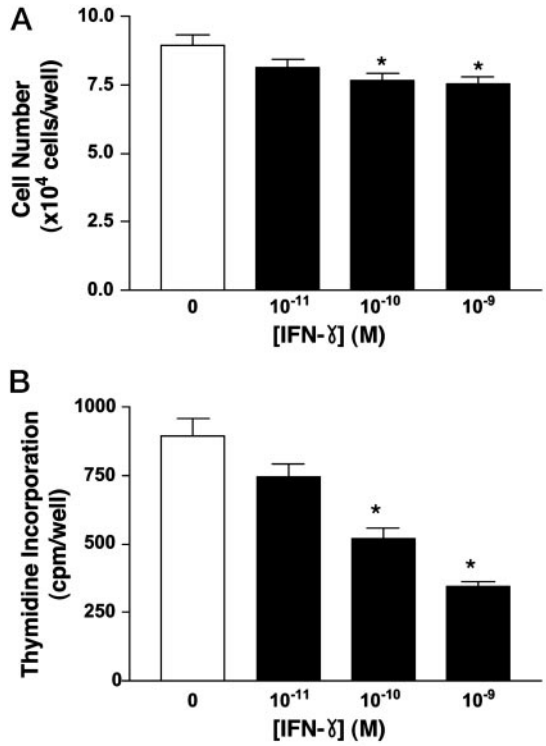

FIG. 6. Effect of IFNγ on fetal rat osteoblast cell number (A) and [^{3}H]thymidine incorporation (B). Data are the mean ± SEM. *, Significantly different from control ($P < 0.01$).

IL-18 signaling of T and NK cells (37), further studies are necessary to clarify how the proliferation of osteoblasts is regulated by IL-18.

Osteoprotegerin (OPG) has also been implicated as an IL-18 target gene in bone marrow stromal ST2 cells, osteoblastic MC3T3-E1 cells, and mouse calvarial osteoblasts (34). The expression of OPG mRNA was transiently, but modestly, increased, reaching a maximal level 3 h after IL-18 exposure. It is unlikely that the enhancement of osteoblastic OPG production would account for any activity to inhibit osteoclast formation, because the presence of T cells was obligatory for this IL-18 effect; IL-18 had no effect in cultures where osteoblasts were present, but T cells were absent (2).

The role of IL-18 on the osteoclast appears to be restricted to limiting osteoclast formation, as, in this current study, we found that IL-18 had no effect on the activity of mature isolated osteoclasts. In addition, we showed that IL-18 did not affect bone resorption, as measured by ^{45}Ca release from neonatal mouse calvarial organ cultures. In this organ culture system, bone resorption reflects predominantly mature osteoclast activity, although some osteoclasts may form from committed osteoclast precursors. Thus, it appears that IL-18 does not act directly upon hemopoietic cells or differentiated osteoclasts to affect osteoclast formation or activity. Further, IL-18 acts in synergy with IL-12 to inhibit osteoclast formation through the production of an as yet to be identified secreted factor from T cells (25). The ability of IL-18 to limit osteoclast formation *in vitro* lends support to the idea that IL-18 may be a useful therapy to limit cancer-induced bone loss. In proof of concept experiments, IL-18 inhibits osteolytic bone metastases by human lung cancer cells or human breast cancer cells in nude mouse models of cancer metastasis in bone (38, 39).

IL-18 has a key role in T cell development, activation, and IFNγ secretion by type 1 helper T cells and NK cells, and evidence is accruing that IL-18 has a proinflammatory role in rheumatoid arthritis and is implicated in diabetes (40–42). T cells are present in the bone microenvironment, and their impact on skeletal maintenance is only now being recognized. Their numbers and activities are modified in response to changes in estrogen levels, as a result of inflammatory conditions of bone such as the onset and development of rheumatoid arthritis and accompanying cancer metastasis in bone (43, 44). As a consequence of these conditions, T cells may produce activators of bone resorption such as receptor activator of nuclear factor-κB ligand (RANKL) or participate in inhibitory pathways of osteoclast formation such as that used by IL-18 (2, 45, 46). It is therefore not surprising that T cell-derived factors may not only regulate osteoclast formation, but may also influence osteoblast and chondrocyte activities, such as we describe here for IL-18.

In light of these stimulatory effects of IL-18 on osteogenic cells and considering that IL-18 has been previously demonstrated to have an angiogenic role (47) and to inhibit osteoclast development, we postulate that IL-18 may be yet another locally produced factor that can regulate bone growth. IL-18 may be important in bone development by

increasing bone and cartilage formation and inhibiting bone resorption. Preliminary work in one of our laboratories indicated that the IL-18 knockout mouse has reduced trabecular bone volume, which is consistent with the anabolic effect of this cytokine demonstrated in the present work (Sims, N. A., D. Mirosavljevic, M. J. Smyth, and M. T. Gillespie, in preparation). Consequently, IL-18 may be a physiological regulator of bone growth.

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