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Prostaglandin E_2, Interleukin 1α, and Tumor Necrosis Factor-α Increase Human Osteoclast Formation and Bone Resorption *in Vitro**

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ABSTRACT

Prostaglandin E_2 (PGE_2) and the cytokines interleukin (IL) 1α and tumor necrosis factor (TNF)α increase bone resorption *in vivo*, but the effect of these agents on osteoclastic bone resorption has never been studied in an *in vitro* human system. Our recently described human bone marrow culture system, in which osteoclasts are generated (vitronectin and calcitonin receptor-positive cells which resorb bone), was used to study the effects of these agents. Addition of indomethacin to macrophage colony-stimulating factor (M-CSF)-treated cultures nearly abolished osteoclast parameters, indicating that prostaglandins are virtually essential for human osteoclast formation. Additionally, PGE_2 dose responsively increased osteoclast numbers and bone resorption. The effects of M-CSF and PGE_2 are independent, as demonstrated by unaltered PGE_2 concentrations in culture supernatants in spite of the dose-responsive increase in osteoclast parameters in response to M-CSF. The generation of osteoclasts in the presence of PGE_2 occurred in favor of CD 14-positive macrophage formation.

IL 1α and TNFα increased osteoclast parameters in a dose-responsive manner. Maximum stimulation yielded culture supernatant levels of PGE_2 approximately the same as those concentrations of exogenous PGE_2 that dramatically induced osteoclast formation. This osteoclast-inducing effect was inhibited both by indomethacin and by the specific inhibitor of inducible prostaglandin G/H synthase, NS-398, and this was reversed by addition of exogenous PGE_2. These results demonstrate unequivocally that IL 1α and TNFα enhance human osteoclast formation and suggest that they mediate their effects through PGE_2. (*Endocrinology* **139:** 3157–3164, 1998)

PROSTAGLANDINS (PGs) are ubiquitous local hormones that have been previously reported to exert important effects on the skeleton (for review, see Ref. 1). In particular, *in vivo* studies in humans and mice provide evidence that PGs enhance bone resorption (for review, see Ref. 2). It has been shown that they increase bone resorption (3) and osteoclast formation (4, 5) in organ culture. It was subsequently found that PGs exert an anticatabolic effect by inhibiting bone resorption by mature isolated neonatal rat osteoclasts (6–8), implying that the PG-induced increase in bone resorption in organ culture was mediated through cells other than the mature osteoclast. The more recent finding that PGs stimulate bone resorption and calcitonin (CT) receptor-positive cell formation (9–11), as well as tartrate-resistant acid phosphatase-positive cell formation (12) in murine bone marrow (BM) cultures, suggests that the increase in bone resorption in organ culture may be the result of osteoclast formation.

It has been suggested that prostaglandin E_2 (PGE_2) exerts the opposite effect on human osteoclast formation, having been found to suppress the formation of osteoclast-like 23c6-positive cells in human BM cultures (13). 23c6 is a monoclonal antibody that identifies the vitronectin receptor and preferentially identifies osteoclasts (14, 15). However, bone resorption, which is unequivocal evidence that osteoclasts have developed, was not assessed in this study (13). We have previously reported, however, that PGE_2 enhances osteoclastic bone resorption in human BM cultures, suggesting that PGE_2 induces osteoclast formation in both human and murine species (16). This study was performed, however, before culture conditions had been optimized to enable reproducible formation of substantial numbers of human osteoclasts. Only a very small area of bone was resorbed in these cultures, and for this reason, osteoclast numbers were not assessed. The effect of PGE_2 on human osteoclast formation, therefore, remains contentious.

It is now possible to generate reproducibly large numbers of human osteoclasts, which resorb bone (17). This is achieved by adding macrophage colony-stimulating factor (M-CSF) to human BM cultures (17, 18). With the availability of a sound and reliable assay, we elected to test the effect of PGE_2 on human osteoclast formation, because it is obviously important to resolve the issue of whether this local hormone exerts similar or opposing effects on the generation of human and murine osteoclasts.

The proinflammatory cytokines, including interleukin (IL) 1α, tumor necrosis factor (TNF)α, and IL 6, are known to enhance bone resorption in murine cultures and in humans and mice *in vivo* (19). Evidence also exists, for the murine species, that many of the hormones and local factors that increase bone resorption also increase PG formation in bone cells (9, 20–25). There is evidence that this is regulated by the stimulation of an inducible form of the enzyme PG G/H synthase (PGHS-2) together with control of arachidonic acid release (25). However, we have been unable to demonstrate increased bone resorption in the presence of several of these

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cytokines in our human BM assay, in the absence of M-CSF (17); and although others have reported increased numbers of osteoclast-like cells in response to these agents, bone resorption was not assessed in these experiments (26). The failure of these factors to enhance human bone resorption *in vitro* might be caused by the regulation of human bone resorption differing from that in the murine species. We therefore elected to study the effect of these cytokines in our human osteoclast-forming culture system.

Materials and Methods

Human BM cultures

Osteoclasts were generated from human BM in a two-phase culture system, as previously described (17, 18). In brief, BM was aspirated, under 1% lignocaine local anesthesia, from the posterior iliac crest of healthy volunteers between the ages of 30 and 75 yr (approved by St Mary's Hospital medical ethics committee) into preservative-free heparin (CP Pharmaceuticals Ltd. Wrexham, UK). Marrow mononuclear cells were isolated by centrifugation over ficoll/hypaque density gradients (Sigma Chemical Co., Poole, Dorset, UK) and resuspended in phenol red-free RPMI medium (Sigma) supplemented with 10% heat-inactivated newborn calf serum (Harlan Sera Lab. Ltd., Sussex, UK), L-glutamine (2 mM), 100 IU benzylpenicillin per ml (Gibco Life Technologies, Paisley, Scotland, UK), 100 μg streptomycin per ml (Gibco), and 10^{-6} M hydrocortisone (Sigma Chemical Co.) and were incubated in tissue culture flasks in the presence of M-CSF (10 ng/ml; Genetics Institute, Boston, MA) at 37 C in a humidified atmosphere of 5% CO_2/95% air for 10–14 days (phase I). The BM cells were fed weekly by replacing half the medium. After this period, the cells were harvested from the flasks, using trypsin (Sigma), and 10^5 mononuclear cells per well were sedimented onto devitalized bone slices ($3 \times 3 \times 0.1$ mm) in a 96-well plate (Corning Glass Works) and were cultured in the presence of M-CSF at 25 ng/ml for 10 days (phase II). The effect of PGE$_2$ (Sigma; 10^{-9}–10^{-6} M), IL 1α, and TNFα (Genetics Institute; 0.01–10 ng/ml) were tested by adding them to phase II of the cultures in the presence and absence of indomethacin (10^{-6} M; Sigma) and PG endoperoxide synthase (PGHS-2) inhibitor, NS-398 (4×10^{-6} M; ICN Biomedicals Ltd, Thame, UK).

The cells on the bone slices were fed by replacing half the medium every 3–4 days. At the end of the experiment, the bone slices were fixed in acetone (Analar, Hayman Ltd., Essex, UK) and air dried. Osteoclasts were quantitated by counting the number of 23c6-positive cells, an antibody which preferentially stains osteoclasts (14, 15), kindly provided by Professor M. A. Horton, London. Macrophage numbers were assessed by labeling the cultures with an anti-CD 14 monoclonal antibody (Sigma). The entire surface of each bone slice was inspected, and the area of resorption was quantified by reflected light microscopy.

Labeling of cells with antibodies and iodinated (^{125}I) CT

Salmon CT (kindly provided by Rhone-Poulenc Rorer, Collegeville, PA) was iodinated using a modification of the chloramine T method (27). BM cultures were incubated in radioiodinated (^{125}I) CT (10 days after the cells were plated on bone slices, with or without excess unlabeled CT) and were washed, fixed, and stained with the antibody against the vitronectin receptor, 23c6, or against CD 14 (using conventional techniques) and were processed for autoradiography, as previously described (28). After development, the cultures were counterstained with toluidine blue.

Enzyme immunoassay (EIA) for PGE$_2$

PGE$_2$ concentration was determined using a commercially available EIA kit (Cayman Chemical, Ann Arbor, MI). Culture supernatants from all the cultures were collected onto ice at the end of the experiments. The supernatants from each treatment group were pooled, and the samples were subaliquoted before storage at $^{-}$70 C. Samples were assayed in duplicate and at two dilutions, according to the manufacturer's instructions. Briefly, the samples were added to the microtiter plates, which were supplied precoated with goat antimouse polyclonal antibody. Monoclonal anti-PGE$_2$ antibody and PGE$_2$ tracer were added to the wells, and the EIA plate was incubated at room temperature for 18 h, washed 5 times, and developed using Ellman's reagent for 60–90 min. The plate was read at 420 nm, and PGE$_2$ concentration was calculated by reference to a standard curve. Values shown are the mean of the four values obtained for each sample. The inter- and intraassay coefficients of variance were less than 4%.

The results were analyzed using ANOVA, where significance was accepted as $P < 0.05$. Results are displayed as mean $\pm$ SEM

Results

As previously reported, the addition of M-CSF to human BM cultures induced osteoclast formation and bone resorption, the former being identified as cells expressing the $a_v\beta_3$ subunit of the vitronectin receptor using monoclonal antibody 23c6 (Fig. 1). This has been found to be a specific marker for the osteoclast phenotype (17, 18). The 23c6-positive cells were further confirmed as osteoclasts by labeling some experiments with ^{125}IsCT (double-labeled cells, 87 $\pm$ 0.9%) (Fig. 1, *center panel*). Bone resorption was also assessed (Fig. 1). In this study, we found that addition of the cyclooxygenase inhibitor, indomethacin (at 10^{-6} M) to M-CSF-treated human BM cultures completely suppressed osteoclast formation, and this was associated with suppression of bone resorption (Fig. 2). Taken together with the finding that indomethacin (10^{-6} M) reduced the concentration of PGE$_2$ in the culture supernatants to minimal levels [less than 50 pg/ml (limit of assay detection, 7.8 pg/ml) mean percent reduction in M-CSF-treated cultures, 82.25 $\pm$ 10%] (Fig. 2), these data suggest that the indomethacin-induced inhibition of osteoclast parameters was the result of reduced levels of PGE$_2$, thereby demonstrating that PGE$_2$ is essential for human osteoclast formation.

PGE$_2$ reversed the inhibitory effect of indomethacin and enhanced M-CSF-induced osteoclast formation (Fig. 2). At 10^{-9} M, PGE$_2$ resulted in osteoclasts being formed in very small numbers, less than that seen in the control (M-CSF) cultures. At 10^{-8} M, there was a dramatic 3-fold increase in osteoclast numbers above that observed in M-CSF-treated cultures. The 23c6-positive cells in the cultures treated with M-CSF alone or with M-CSF with PGE$_2$ (10^{-8} M) contained the same number of nuclei, with a range from 1–6 (M-CSF, 1.45 $\pm$ 0.76; n = 97 cells *vs.* M-CSF + PGE$_2$, 1.48 $\pm$ 0.85; n = 128 cells). The increase in 23c6-positive cells was paralleled by a greater area of bone being resorbed in these cultures. Higher concentrations of PGE$_2$ (10^{-7} and 10^{-6} M) did not further induce osteoclast formation (Fig. 2).

Because M-CSF has previously been shown to be critical for osteoclast formation, we wished to determine whether this effect was mediated via PGE$_2$. To achieve this, we added increasing concentrations of M-CSF (0.5–50 ng/ml) to phase II of our cultures, and we measured the PGE$_2$ concentration by EIA in the culture supernatants. As expected, M-CSF stimulated osteoclast formation and bone resorption in a dose-responsive manner (17), but there was no increase in the level of PGE$_2$ in these cultures (Fig. 3), indicating that these two local hormones mediate their effect on osteoclast formation independently.

To test the hypothesis that PGE$_2$ results in the preferential formation of osteoclasts over macrophages, we assessed the numbers of osteoclasts and macrophages that were formed

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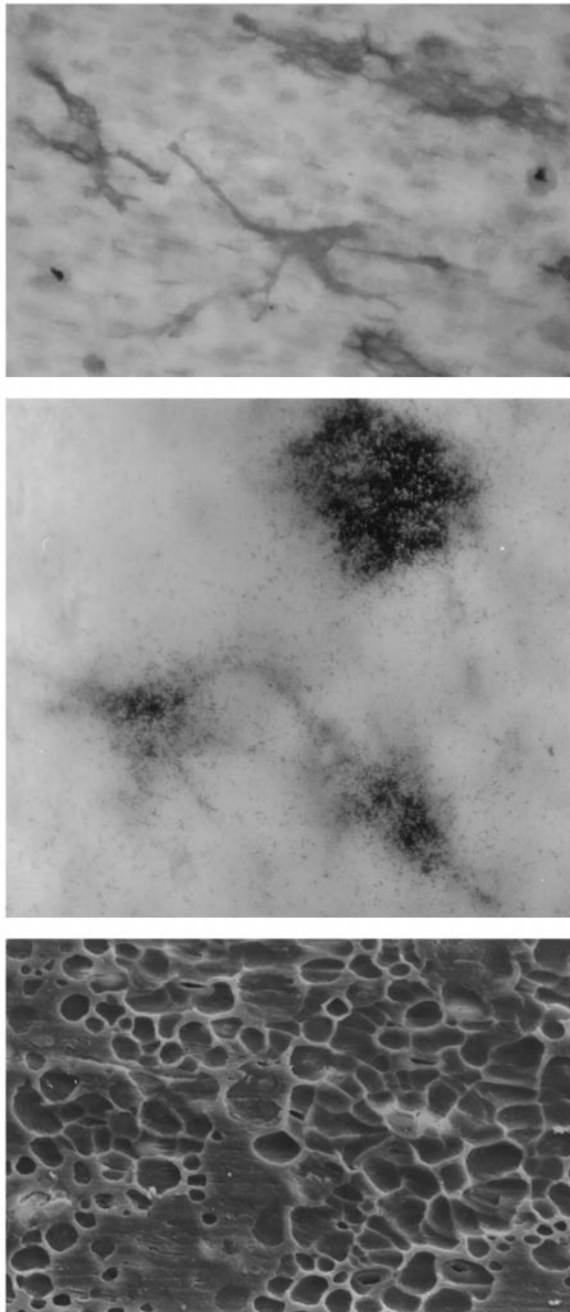

FIG. 1. Osteoclasts were defined by the following criteria: *upper panel*, 23c6- positive cells generated in human BM cultures containing 25 ng/ml M-CSF (magnification, ×200); *center panel*, 23c6-positive cells that coexpressed the CT receptor, as indicated by labeling with ^{125}IsCT (magnification, ×300); *lower panel*, scanning electron micrograph of osteoclastic bone resorption typical of that seen in our human BM cultures in the presence of M-CSF (magnification, ×500).

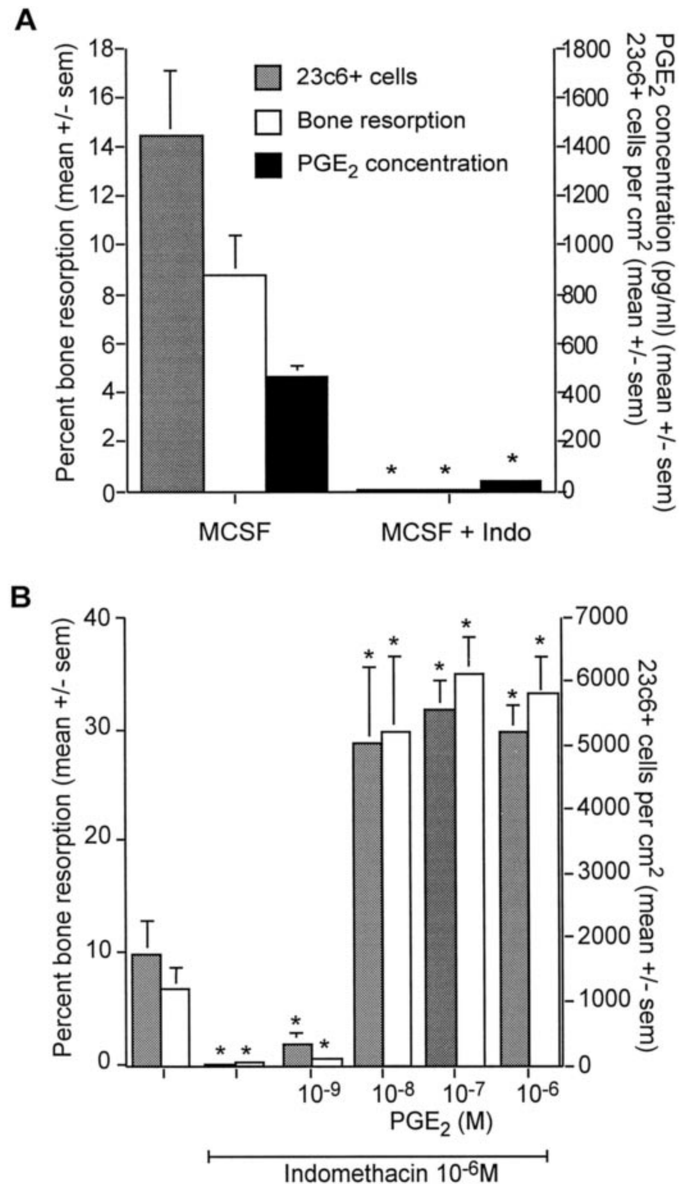

FIG. 2. A, Effect of indomethacin (10^{-6} M) on osteoclast parameters and PGE_2 concentration in culture supernatants in phase II human BM cultures containing M-CSF (25 ng/ml) [n = 4 experiments, with five or six bone slices per variable; $P < 0.0001$, compared with M-CSF control]. B, Effect of increasing PGE_2 concentrations in the presence of indomethacin (10^{-6} M) on osteoclast parameters in phase II human BM cultures containing M-CSF (25 ng/ml). The data derive from triplicate experiments, each containing five or six bone slices per variable per experiment; $P < 0.0001$, compared with M-CSF control.

in the presence of M-CSF with or without 10^{-6} M PGE_2 in the same human BM cultures. We found that M-CSF induced both osteoclast and macrophage formation, as well as bone resorption, above that seen in vehicle-treated cultures, whereas there was a significant reduction in the number of CD 14-labeled macrophages in the presence of indomethacin and PGE_2. In contrast, the addition of PGE_2 caused a significant increase in the number of 23c6-positive cells, which was associated with the expected increase in bone resorption (Fig. 4).

Both IL 1α and TNFα increased the number of 23c6-positive cells in a dose-responsive manner in the presence of M-CSF. This was associated with an increase in the area of bone resorption (Figs. 5 and 6). A maximal response occurred in response to both cytokines at 1 ng/ml, with IL 1α inducing a 5- to 10-fold increase in bone resorption and 23c6-positive cell numbers. TNFα induced a 3- to 7-fold increase in bone resorption and 23c6-positive cells. The maximum level of

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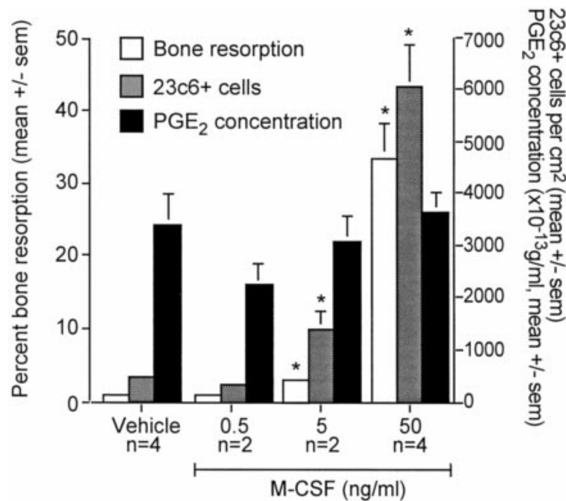

FIG. 3. Effect of increasing M-CSF concentrations in phase II human BM cultures on osteoclast parameters and PGE$_2$ concentration in culture supernatants. The data derive from two or four experiments, each containing six bone slices per variable per experiment; $P < 0.0001$, compared with vehicle.

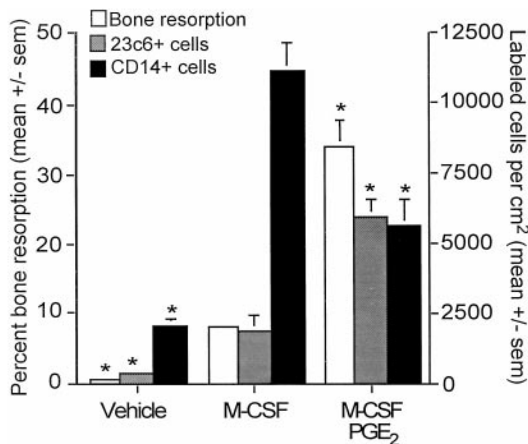

FIG. 4. Effect of M-CSF (25 ng/ml) ± indomethacin (10^{-6} M) with PGE$_2$ (10^{-6} M) on 23c6-positive and CD14-positive cell numbers and bone resorption in phase II human BM cultures. The data derive from triplicate experiments, each containing five bone slices per variable per experiment; $P < 0.005$, compared with M-CSF control.

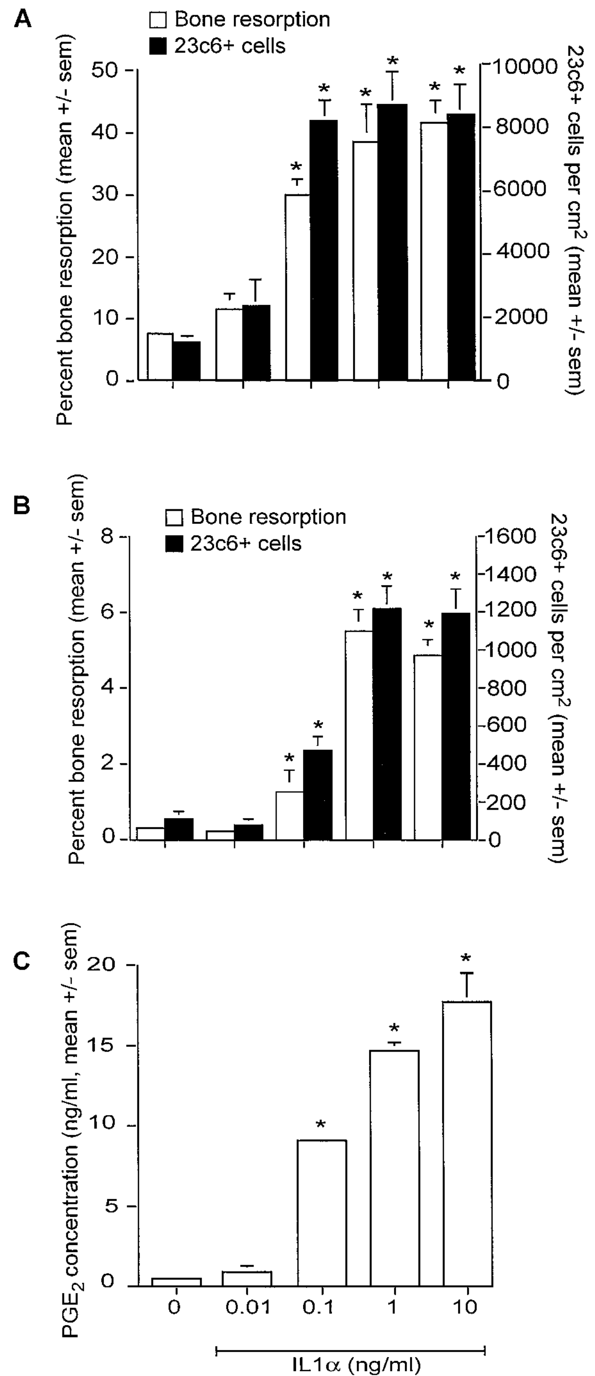

FIG. 5. Effect of increasing IL 1 α concentrations in the presence of M-CSF (25 ng/ml) on bone resorption and 23c6-positive cell numbers in Exp 1 (A) and Exp 2 (B). C, PGE$_2$ concentration in culture supernatants in phase II human BM cultures (n = 2). $P < 0.005$, compared with M-CSF control. Each experiment contains five or six bone slices per variable.

stimulation by the cytokines depended on the basal level of bone resorption in the experiment. BM experiments with high levels of basal osteoclast activity generally gave lower levels of stimulation, probably reflecting the variability in endogenous PGE$_2$ between BM donors. Above this concentration, the parameters plateaued in the presence of IL 1α and showed a reduction in the presence of TNFα. In the same experiments, we found that the increase in osteoclast parameters, in response to 0.1–10 ng/ml of IL 1α and 0.1 and 1 ng/ml of TNFα, was associated with an increase in the levels of PGE$_2$ in the culture supernatants at the end of the experiments (5, 6). The stimulation of bone resorption and PGE$_2$ concentration, in response to 1 ng/ml of both cytokines, was confirmed by further experiments (Figs. 7 and 8).

As further confirmation that PGE$_2$ is involved in the osteoclast-inductive effect of IL 1α and TNFα, we added indomethacin to the IL 1α- and TNFα-treated cultures. The increase in bone resorption in response to these cytokines (as originally demonstrated in Figs. 5 and 6) was virtually abrogated in the presence of indomethacin. The number of 23c6-positive cells followed the same pattern as bone resorption (data not shown). Addition of 10^{-7} M PGE$_2$ to the TNFα- or IL 1α indomethacin-treated cultures restored bone resorp-

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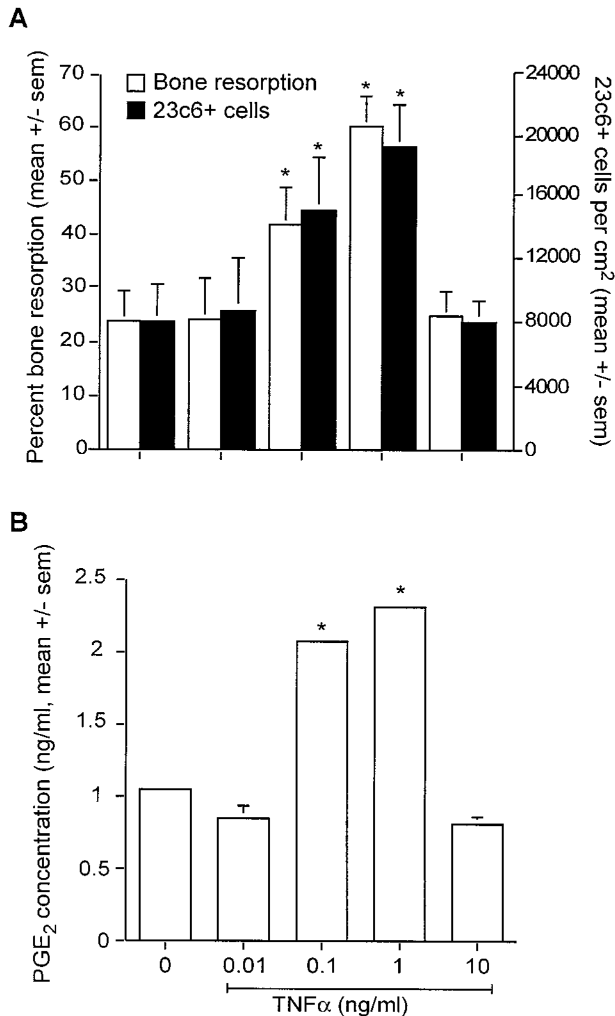

FIG. 6. Effect of increasing TNFα concentrations in the presence of M-CSF (25 ng/ml) on bone resorption and 23c6-positive cell numbers (A) and PGE$_2$ concentration in culture supernatants in phase II human BM cultures (B). n = 2 experiments, with five bone slices per variable per experiment. $P < 0.05$, compared with M-CSF control.

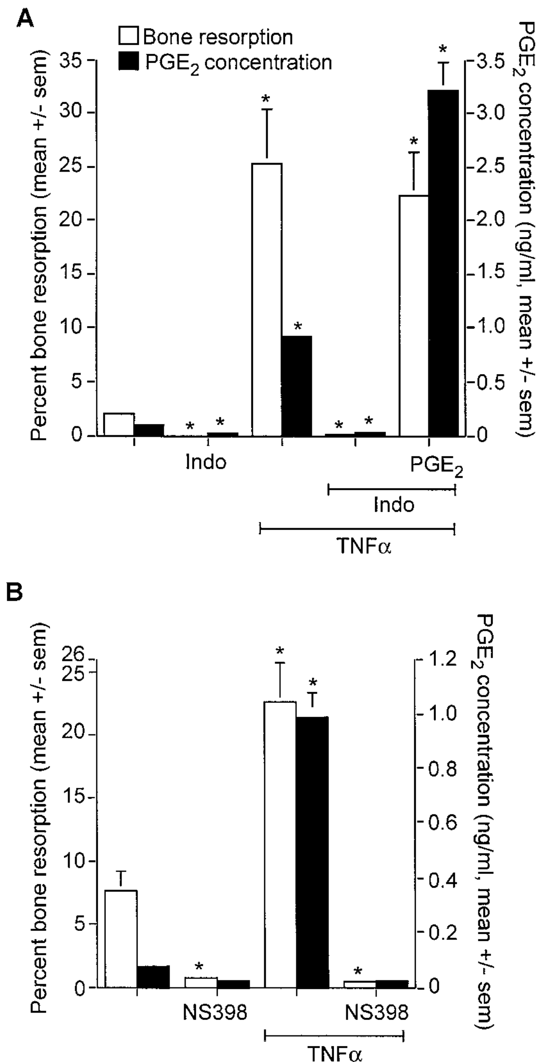

FIG. 7. A, Effect of indomethacin (10^{-6} M) ± TNFα (1 ng/ml) ± PGE$_2$ (10^{-7} M) on bone resorption (n = 3) and PGE$_2$ concentration in culture supernatants (n = 1) in phase II human BM cultures. $P < 0.0001$, compared with M-CSF control. B, Effect of NS-398 (4×10^{-6} M) ± TNFα (1 ng/ml) on bone resorption (n = 4) and PGE$_2$ concentration in culture supernatants (n = 1) in phase II human BM cultures. Each experiment contains five or six bone slices per variable. $P < 0.005$, compared with M-CSF control.

tion levels to those in TNFα- or IL 1α-treated cultures (Figs. 7 and 8).

Because indomethacin is a relatively nonspecific inhibitor of both the constitutive and the inducible forms of cyclooxygenase, PGHS-1 and -2, respectively (29), we added NS-398 as a specific inhibitor of the inducible form. We found that NS-398 exerted an effect similar to that of indomethacin (Fig. 7), except that the observed reduction in PGE$_2$ concentration was not as great, reducing PGE$_2$ levels by 66 ± 4%, as opposed to indomethacin reducing PGE$_2$ by 82.25 ± 10% in M-CSF-treated cultures (a difference of 16.25%, $P = 0.036$). In cytokine-treated cultures, the effects of indomethacin and NS-398 were to inhibit PGE$_2$ concentration by 99.45 ± 0.15% and 97.73 ± 0.14%, respectively (a difference of 1.72%, $P = 0.016$). Dose response experiments for indomethacin and NS-398 showed that they have almost identical inhibitory effects on bone resorption in our cultures (data not shown). The increase in the supernatant concentration of PGE$_2$ (in response to TNFα) and its suppression (in the presence of indomethacin and NS-398) provides additional evidence that increasing levels of cytokine are associated with elevated PGE$_2$ production. These results further indicate that PGE$_2$ is critical for osteoclast formation and bone resorption. Similar results were observed when IL 1α was substituted for TNFα (Fig. 8).

Discussion

We have found that PGE$_2$ enhances osteoclast formation and bone resorption in M-CSF-treated human BM cultures. This provides strong evidence that this local hormone exerts a similar effect on osteoclast formation in both the human and murine species. Our finding is accounted for by an increase in osteoclast numbers, which are identified as cells expressing the vitronectin receptor (using monoclonal anti-

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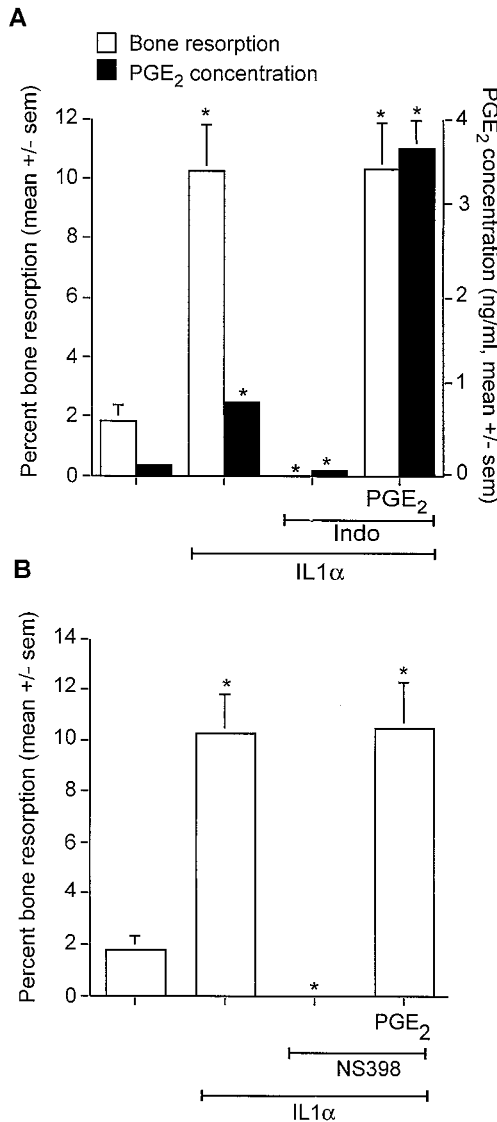

FIG. 8. A, Effect of IL 1α (1 ng/ml) $\pm$ indomethacin (10^{-6} M) $\pm$ PGE$_2$ (10^{-7} M) on bone resorption (n = 3) and PGE$_2$ concentration in culture supernatants (n = 1) in phase II human BM cultures. $P < 0.0001$, compared with M-CSF control. B, Effect of IL 1α (1 ng/ml) $\pm$ NS-398 (4×10^{-6} M) $\pm$ PGE$_2$ (10^{-7} M) on bone resorption in phase II human BM cultures (n = 3). Each experiment contains five or six bone slices per variable. $P < 0.0001$, compared with M-CSF control.

body 23c6) and CT receptor (using ^{125}IsCT). We have found previously that cells with this phenotype have a spatial and temporal relationship with the appearance of bone resorption (17). Our findings corroborate those of others, in that not only does PGE$_2$ increase osteoclast formation but, in its absence, osteoclast formation and bone resorption are virtually nonexistent (9, 11). Further studies, however, are required to assess whether PGE$_1$ or other prostanoids can be substituted for PGE$_2$ in human tissue, but data from murine cultures suggest that this probably is the case (10, 12). The additional finding that near-total abrogation of osteoclast parameters occurs in cultures containing exogenous M-CSF in the absence of PGE$_2$ implies that both these factors are required for osteoclast development and that they mediate their effects independently. Finally, the observation that increasing concentrations of M-CSF stimulate osteoclast formation in the absence of a rise in the levels of PGE$_2$ largely excludes the possibility, as suggested by others (11), that the defect in the op/op osteopetrotic mouse mutant is caused by a failure to produce sufficient PGE$_2$ from BM macrophages.

PGE$_2$ has previously been found to inhibit bone resorption by the mature neonatal rat osteoclast (6, 8). It may therefore have been predicted that there would be less bone resorption per 23c6-positive cell in the presence of PGE$_2$, compared with control cultures, as shown by Collins and Chambers (10) in murine osteoclast cultures. However, this was not the case in our experiments. It may be that PGs do not exert an inhibitory effect on human osteoclastic resorption activity. The alternative is that an effect was not detected because of the difficulty in assessing the quantity of bone resorbed per 23c6-positive cell in our cultures, because bone resorption represents the cumulative result of the activity of cells, which numbers vary during the culture period. To investigate this issue, it would be more appropriate to use the isolated mature human fetal osteoclast bone slice assay, just as we have done when we investigated the effect of M-CSF on mature human osteoclasts (30).

The osteoclastic response to PGE$_2$ was not gradual; instead there was a very dramatic increase in osteoclast parameters, which occurred at 10^{-8} M PGE$_2$. This suggests that PGE$_2$ concentration is a crucial means by which the effect of the hormone is controlled and, in particular, the means by which its action is contained locally. This provides insight into how PGE$_2$ could exert different effects on the same cells in different microenvironments [it inhibits mature rat osteoclasts from resorbing bone (6, 8) and stimulates bone formation (31, 32)].

It has previously been suggested that PGs inhibit human osteoclast-like cell formation *in vitro* (13). One possible explanation for this data being apparently contradictory to ours is that the osteoclast-like cells in that report may have been macrophage polykaryons and not bone resorptive osteoclasts. We propose this because we have demonstrated, for the first time, that PGE$_2$ reduces macrophage CD 14-positive cells in cultures where human osteoclast numbers and bone resorption are enhanced. Our finding implies that osteoclasts and macrophages share a common precursor, and this is consistent with reports showing that in cfos knock-out mice (33) and NF-kB knock-mice (34), osteoclasts are absent but macrophage numbers are increased.

This is the first report demonstrating that IL 1α and TNFα enhance human osteoclastic bone resorption *in vitro*. There are several findings to indicate that the osteoclast-inductive effect of IL 1α and TNFα was mediated by PGE$_2$, which itself induces osteoclast formation. First, the increase in osteoclast numbers and bone resorption that occurred in response to these cytokines correlated with increasing levels of PGE$_2$. Second, PGE$_2$ induced a dramatic increase in osteoclast parameters at 10^{-8} M, which was approximately the same PGE$_2$ concentration found in the cultures stimulated with 1 ng/ml of IL 1α or TNFα (10^{-8} M is approximately equal to 3.6 ng/ml; TNFα 1 ng/ml gives rise to a PGE$_2$ concentration of 2.3 ng/ml, or 7×10^{-9} M; IL 1α 1 ng/ml gives rise to a PGE$_2$ concentration of 14.6 ng/ml, or 4×10^{-8} M). Finally, the

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indomethacin and NS-398 inhibition of osteoclast numbers and bone resorption in cultures treated with TNFα or IL1 α was reversed by the addition of PGE_2.

The elevation in osteoclast parameters in response to PGE_2, whether this be in response to the addition of PGE_2 or the cytokines IL 1α or TNFα, occurred dramatically at a concentration of approximately 10^{-8} M PGE_2. In the presence of this concentration of PGE_2, osteoclast parameters were increased up to 10-fold. Because PGE_2 was only added at the beginning of phase II, and because we have previously shown that osteoclasts only start to develop on day 3 of this phase of the cultures, this suggests that PGE_2 exerts its effect on osteoclast formation by exerting an effect on a late precursor, possibly inducing differentiation rather than proliferation. This proposal is consistent with the report from Marshall *et al.* (35), who showed, using bromo-deoxyuridine, that osteoclast formation stimulated by PGE_2 was the result of differentiation and not proliferation.

PGE_2 is synthesized by the constitutive and inducible cyclooxygenase enzymes PGHS-1 and -2, respectively. The former is expressed in many mammalian cells, whereas the latter is generally undetectable under physiological conditions but is induced by inflammatory cytokines. Therefore, it is consistent that the addition of indomethacin, a relatively nonselective inhibitor of cyclooxygenase activity, should reduce PGE_2 concentration by 82.25 $\pm$ 10% in M-CSF-treated cultures. In contrast, NS-398, a specific PGHS-2 inhibitor that is 1,000 times more active on PGHS-2 than PGHS-1 (36), reduces PGE_2 levels by only 66 $\pm$ 4% in our cultures, implying that PGHS-2 was predominantly responsible for the PGE_2 production in the M-CSF-treated cultures. The greater inhibition of PGE_2 in the presence of cytokines, compared with cultures treated with only M-CSF, is caused by the increase in absolute PGE_2 concentration by IL 1α and TNFα. The differential inhibition of PGE_2 concentration by NS-398 and indomethacin probably reflects the inhibition of only the inducible form of the enzyme by NS-398. Suppression of PGE_2 levels by NS-398 in cultures that have not been treated with proinflammatory cytokines is probably explained on the basis that the technique of culturing cells, and the addition of serum to the cultures, induce PGHS-2 (25).

It is interesting to speculate that the increased level of PGE_2 in the culture supernatant, in response to IL 1α and TNFα in our cultures, is largely the result of up-regulation of PGHS-2, just as it is in the neonatal mouse calvarial system (25). However, an alternative explanation is that the cytokines increase release of arachidonic acid that can subsequently be converted into PGE_2.

Our *in vitro* results are consistent with the observations made *in vivo* that PGs are involved in the development of increased bone resorption in disuse osteopenia (37), and in some animal models of hypercalcemia of malignancy (38), and are associated with a small proportion of solid malignant tumors (39). However, apart from the work of Seyberth *et al.* (39), there is no convincing evidence that PGs reach levels in humans that are capable of inducing hypercalcemia by inducing bone resorption. This suggests that there are PG-independent mechanisms by which bone resorption can be increased, and there is now evidence that tumors producing PTH-related peptides account for many cases of hypercalcemia of malignancy (40). Nevertheless, this does not exclude the importance of PGs in local osteoclast formation and bone resorption at tumor sites.

This is the first report to establish unequivocally that PGE_2, IL 1α, and TNFα increase bone resorption in human BM cultures, and it provides a sound platform on which to base further experiments. Continued exploitation of this assay, including analysis of the expression of PGHS-1 and-2 mRNA and protein in human BM before culture and the effect of proinflammatory cytokines on their expression *in vitro*, will allow elucidation of the mechanisms by which local hormones interact to regulate human osteoclastic bone resorption in health and disease.

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