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The effects of recombinant human interleukin-1β on cellular proliferation and the production of prostaglandin E_2, plasminogen activator, osteocalcin and alkaline phosphatase by osteoblast-like cells derived from human bone

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There is mounting evidence implicating cytokines such as interleukin-1 in the local regulation of bone homeostasis. In this report we show that recombinant human interleukin-1β (rhIL-1β) influences several activities of osteoblast-like cells derived from human trabecular bone explants *in vitro*. rhIL-1β stimulated cellular proliferation and the synthesis of prostaglandin E_2 and plasminogen activator activity in the cultured human osteoblast-like cells in a dose-dependent manner. However, the induction of osteocalcin synthesis and alkaline phosphatase activity in response to $1,25(OH)_2D_3$, two characteristics of the osteoblast phenotype, were antagonized by rhIL-1β over a similar dose range. This study adds further support to the potential role of interleukin-1 in the physiological and pathological modulation of bone cell metabolism.

The potential contribution of resident immune cells and their products to the localized remodelling processes involved in bone metabolism is receiving increasing attention (1). Studies have demonstrated that one such cytokine, interleukin-1 (IL-1), can modulate several aspects of the activity of various bone cell types. IL-1 is a potent stimulator of bone resorption *in vitro* (2-6), an action apparently partially mediated via the stimulation of osteoclast cell formation (7). In addition, IL-1 also regulates several processes involved in bone formation (8,9) indicating the modulation of osteoblast cell activity by IL-1. Previous studies have demonstrated that partially purified IL-1 (10-12) and homogeneous IL-1β (13) regulate several aspects of the functional activity of human osteoblast-like cells *in vitro*. In this study we have examined whether these effects are exhibited by recombinant human IL-1β (rhIL-1β) on human osteoblast-like cells derived from human bone. In addition, the actions of recombinant human IL-1α (rhIL-1α) were compared with those of rhIL-1β.

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Abbreviations used:
rhIL-1β, recombinant human interleukin-1β; rhIL-1α, recombinant human interleukin-1α; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; PGE_2, prostaglandin E_2; EMEM, Eagle's minimal essential medium; PDGF, platelet-derived growth factor.

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MATERIALS AND METHODS

rhIL-1β and rhIL-1α

rhIL-1β was a kind gift of Glaxo Group Research Ltd., Greenford Road, Middlesex, UK. The specific activity of the rhIL-1β preparation was 10^8 U/mg. It was diluted in phosphate buffered saline and subsequently into experimental culture medium. rhIL-1α was a kind gift of Dr P.T. Lomedico, Dept. of Molecular Genetics, Hoffmann-La Roche Inc., Nutley, New Jersey, USA. The specific activity of the rhIL-1α preparation was 4×10^7 U/mg. It was diluted in phosphate buffered saline and subsequently into experimental culture medium.

Human bone cell culture

Explants of human trabecular bone were cultured as described previously in (14). The cells obtained by this method have been routinely characterized and shown to express an osteoblast-like phenotype in culture (14,15,16). All experimental incubations, with the exception of the determinations of plasminogen activator activity, were conducted in EMEM containing 5% (v/v) foetal calf serum previously depleted of vitamin D metabolites by charcoal extraction. The medium was additionally supplemented with L-ascorbate (50 μg/ml), vitamin K (10^{-8}M) and L-glutamine (2mM).

Extraction of $1,25(OH)_2D_3$ from FCS

Extraction of $1,25(OH)_2D_3$ was accomplished by adding dry Norit A charcoal (BDH Ltd., Warwickshire, UK.) (40 mg/ml) to the FCS and incubated, with rotation, at 4°C for 2h. The charcoal was removed by centrifugation (10,000g, 30 mins, 4°C). The vitamin D-depleted FCS was sterilized by filtration through sterile 0.22 μm pore size filters.

Cell proliferation

Cell proliferation was assessed by the incorporation of [^{14}C]-thymidine into materials precipitable by trichloroacetic acid. Cells were pulsed for the final 24h of a 48h incubation period.

Plasminogen activator assay

The plasminogen activator activity associated with the human osteoblast-like cells was measured using [^{125}I]-fibrin coated multiwells as described in (17). Results are expressed as % plasminogen-dependent fibrinolysis per 24h.

Osteocalcin assay

Osteocalcin released into the culture media, over a 72h incubation period was measured using a specific radioimmunoassay with an antibody raised in rabbits against purified bovine osteocalcin (14). Results are expressed as ng osteocalcin per μg cell protein.

Alkaline phosphatase and protein assay

Alkaline phosphatase activity in the solubilized cell layer was measured by monitoring the release of p-nitrophenol from disodium p-nitrophenyl phosphate. The assay buffer consisted of 0.1M diethanolamine, supplemented with 0.5M magnesium chloride, pH 10.5. Results are expressed as μmoles per μg cell protein per h. The protein content of the cell layer was measured by the method of Lowry (18), using bovine serum albumin as standard. Cells were incubated in the appropriate culture medium for 72h.

Prostaglandin assay

Prostaglandin E_2 (PGE_2) released into the culture medium over a 72h incubation period was measured by radioimmunoassay using an antiserum with specificity towards PGE_2 (Steranti Research, UK) as described in (19). Results are expressed as ng PGE_2 per μg cell protein.

Statistics

Statistical differences between treatments were determined using analysis of variance.

RESULTS

The proliferation of the human osteoblast-like cells was stimulated in a dose-dependent manner by rhIL-1β over the concentration range of 1-100 U/ml (Fig. 1). The stimulation of

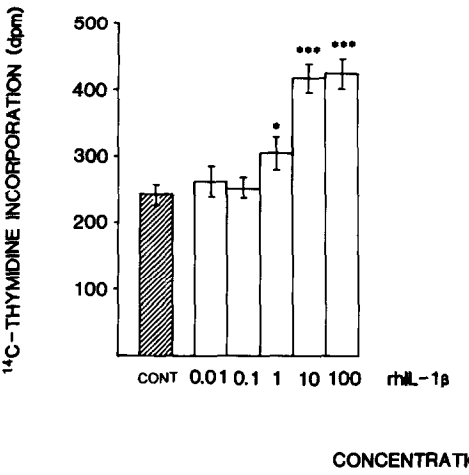

Fig. 1. Stimulation of cell proliferation of human osteoblast-like cells by rhIL-1β. Cell proliferation was assessed by [^{14}C]-thymidine incorporation as described in Materials and Methods. Values represent mean ± S.E.M. (n=4). Significant difference from control, * $p < 0.05$, *** $p < 0.001$.

cell proliferation was most pronounced at 100 U/ml, while concentrations below 1 U/ml had no detectable effect.

rhIL-1β stimulated the production of PGE_2 in a dose-dependent manner over the dose range of 1-100 U/ml with a maximal effect being observed at 100 U/ml (Table 1). Doses below 1 U/ml exhibited no detectable activity on the synthesis of PGE_2.

The plasminogen activator activity of the human osteoblast-like cells was stimulated by rhIL-1β in a dose-dependent manner (Fig. 2). Plasminogen activator activity was significantly stimulated by rhIL-1β over the dose range of 1-10 U/ml, with maximal stimulation being observed at 10 U/ml. Concentrations below 1 U/ml exerted no detectable effect on the basal plasminogen activator activity.

Table 1. The effect of rhIL-1β on the production of PGE_2 by human osteoblast-like cells

rhIL-1β (U/ml)	PGE_2 (pg/ug protein)
0	0
0.01	0
0.1	1.50 ± 0.10
1	3.25 ± 0.50
10	35.5 ± 3.30 ***
100	217 ± 25.0 ***

PGE_2 released into the culture media was measured as described in Materials and Methods. Values represent mean ± S.E.M. (n=4). Significant difference from control, *** $p < 0.001$.

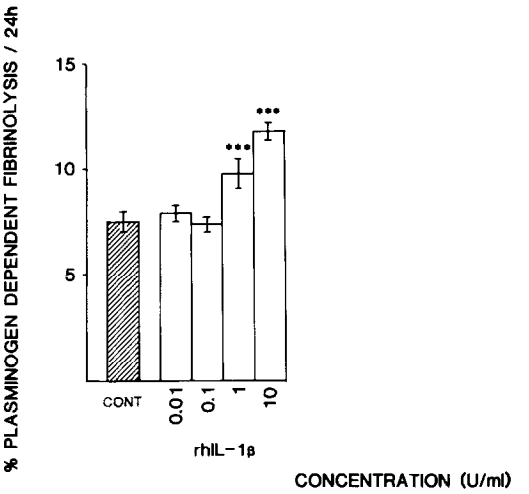

Fig. 2. The effect of rhIL-1β on the plasminogen activator activity of human osteoblast-like cells. Plasminogen activator activity was measured as described in Materials and Methods. The data shown represents the plasminogen dependent fibrinolysis of [^{125}I]-fibrin substrate. Values represent mean ± S.E.M. (n=4). Significant difference from control, *** $p < 0.001$.

The basal alkaline phosphatase activity of the human osteoblast-like cells was decreased by rhIL-1β over the dose range of 10-100 U/ml (Fig. 3). Concentrations below 10 U/ml exhibited no marked inhibitory action on basal enzyme levels. The induction of alkaline phosphatase activity by $1,25(OH)_2D_3$ was antagonized by rhIL-1β in a dose-dependent manner over a concentration range of 1-100 U/ml. Lower doses had no obvious effect on the alkaline

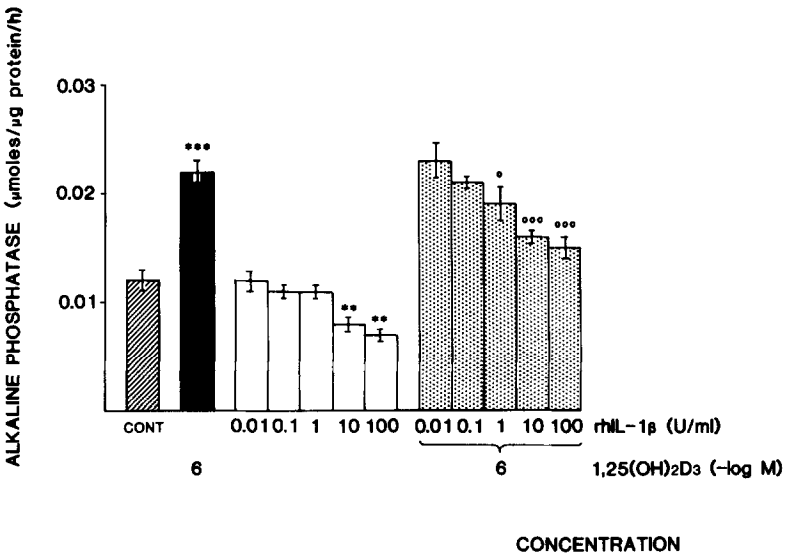

Fig. 3. The influence of rhIL-1β on the basal and $1,25(OH)_2D_3$-stimulated alkaline phosphatase activity of human osteoblast-like cells. The alkaline phosphatase activity of the solubilised cell layer was measured as described in Materials and Methods. Significant difference from control, ** $p < 0.01$. Significant difference from $1,25(OH)_2D_3$-treated cells, ° $p < 0.05$, °°° $p < 0.001$.

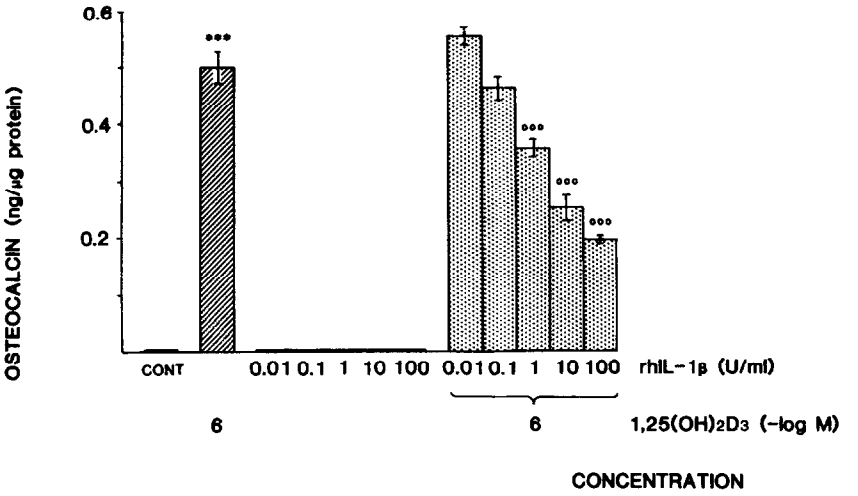

Fig. 4. Antagonism by rhIL-1β of the production of osteocalcin by human osteoblast-like cells induced in the presence of $1,25(OH)_2D_3$. The osteocalcin released into the culture medium was measured by specific radioimmunoassay as described in Materials and Methods. Significant difference from control, *** $p < 0.001$. Significant difference from $1,25(OH)_2D_3$-treated cells, °°° $p < 0.001$.

phosphatase activity (Fig. 3). The inhibitory actions on enzyme activity were most pronounced at the higher concentration of 100 U/ml of rhIL-1β.

rhIL-1β (0.01-100 U/ml) had no detectable effect on the production of osteocalcin by the human osteoblast-like cells when added alone, but antagonized the production of osteocalcin induced by $1,25(OH)_2D_3$ (Fig. 4). The production of osteocalcin stimulated by $1,25(OH)_2D_3$ was significantly antagonized by rhIL-1β over a dose range of 1-100 U/ml in a dose-dependent manner. Over the concentration range used, maximal inhibition of osteocalcin synthesis was observed at 100 U/ml.

Table 2. The effect of rhIL-1α on the cellular proliferation and the production of prostaglandin E_2, plasminogen activator, osteocalcin and alkaline phosphatase activity by human osteoblast-like cells

	Dose Range
Stimulation of cell proliferation	1-100 U/ml
Stimulation of prostaglandin E_2 synthesis	1-100 U/ml
Stimulation of plasminogen activator activity	1-10 U/ml
Inhibition of osteocalcin synthesis	1-100 U/ml
Inhibition of alkaline phosphatase activity	1-100 U/ml

Cell proliferation, prostaglandin E_2, plasminogen activator, osteocalcin and alkaline phosphatase assays were carried out as described in Materials and Methods. The dose range indicated represents the concentrations of rhIL-1α where a significant effect was observed. For the regulation of the synthesis of osteocalcin and alkaline phosphatase activity $1,25(OH)_2D_3$ (10^{-6} M) was added to the cultures to induce protein synthesis.

In a similar manner, rhIL-1α exhibited comparable activities to rhIL-1β (Table 2). Over the dose range of 1-100 U/ml, rhIL-1α stimulated cellular proliferation, the production of PGE_2 and plasminogen activator activity, whilst antagonizing the synthesis of osteocalcin and alkaline phosphatase activity. Lower concentrations of rhIL-1α (0.01-0.1 U/ml) exhibited no detectable effects on these measurements.

DISCUSSION

These studies indicate that rhIL-1β had several effects on human osteoblast-like cells *in vitro* and confirms previous observations describing modulatory actions of partially purified and natural homogeneous IL-1β on these cells (9-12).

rhIL-1β stimulated the plasminogen activator activity of the human osteoblast-like cells in a dose-dependent manner over a comparable concentration range to that observed with homogeneous IL-1β (12). The stimulation of plasminogen activator activity by IL-1 has been observed in several connective tissue cell types, including human chondrocytes (6). IL-1 is a potent stimulator of bone resorption both *in vitro* (2-6) and *in vivo* (20,21) through an action which may be mediated primarily via the osteoblast (22). The observation that rhIL-1β stimulates the plasminogen activator activity of the human osteoblast-like cells may indicate a potential mechanism for the osteoblast-mediation of bone resorption. Plasminogen activator has been associated in several processes governing connective tissue degradation (23). The existence of a plasmin-dependent proteolytic system in mineralized matrices has been described(24) and so may contribute to the breakdown of the constituents of the bone matrix (25). One mechanism whereby this may be acheived is the activation of latent collagenase via the action of plasmin, following its generation from plasminogen (26-28). Rodent osteoblasts produce latent collagenase in response to several bone resorbing agents (29), including IL-1 (30,31), but this has not been observed with the human osteoblast cells (32). Human osteoblast-like cells, however, produce high levels of a metalloproteinase inhibitor which may mask any collagenase activity (H.J. Andrews, personal communication).

In addition, the stimulation of production of PGE_2 by rhIL-1β may also contribute to bone breakdown since PGE_2 is a recognized stimulator of bone resorption *in vitro* (33) and also stimulates the plasminogen activator activity of osteoblast-like cells (34). The synthesis of PGE_2 in response to IL-1 can contribute to the biological actions of this cytokine in other tissues (35). Indeed, the stimulation of bone resorption by IL-1 can occur partially via PGE_2-dependent mechanisms (3,5) indicating that the synthesis of PGE_2 by osteoblast-like cells in response to IL-1 may contribute to this effect. Previous studies indicate that partially purified (11) and homogeneous natural IL-1β (13) stimulate the proliferation of the human osteoblast-like cells in the presence of indomethacin, a known inhibitor of PGE_2 synthesis (36). In contrast, the dose-dependent stimulation of the proliferation of the human osteoblast-like cells in response to rhIL-1β occurred in the absence of indomethacin. The significance of this observation is not known but may reflect subtle differences in the activity of different IL-1 preparations. However, stimulatory effects of IL-1 on the DNA synthesis of intact foetal rat calvaria in the absence of indomethacin have also been described (9).

Confirmation that rhIL-1β affects the osteoblast-like cells is obtained from the studies on the synthesis of osteocalcin and alkaline phosphatase, two widely accepted phenotypic markers of the osteoblast cell (37). The induction of osteocalcin and alkaline phosphatase in response to $1,25(OH)_2D_3$ was antagonized by rhIL-1β in a dose-dependent manner. In preliminary investigations the contribution of PGE_2 synthesis to the antagonistic action of rhIL-1β on the production of osteocalcin and alkaline phosphatase activity was assessed. The inhibition of synthesis of osteocalcin was partially abrogated in the presence of indomethacin, whilst the action on alkaline phosphatase activity was relatively unaffected (data not shown). This finding correlates with our previous observation of a differential regulatory mechanism in the synthesis of osteocalcin and alkaline phosphatase by human osteoblast-like cells (13). Thus, endogenous PGE_2 synthesis may partially contribute to some but not other activities of rhIL-1β on human osteoblast-like cells. The ability of rhIL-1β to suppress these markers of the mature osteoblast in association with the stimulation of cell proliferation is interesting. Several other cytokines and systemic hormones exhibit similar regulatory actions on the stimulation of proliferation with a concomittant inhibition of the expression of the mature phenotype (14,15,38,39) and visa versa (14,16). It would appear that the stimulation of the proliferation of the immature osteogenic cells parallels an inhibitory regulation of the mature osteoblast cell population. The significance of this observation may be relevant to the potential coupling phenomena whereby bone resorption links with bone formation in a co-ordinated manner (40). One way whereby an increase in new bone formation may occur is by an increase in the potential osteoblastic pool. Indeed, administration of IL-1 to the sub-cutaneous region of the calvaria of mice has recently shown that the initial induction of bone resorption by IL-1 is subsequently followed by a period of new bone formation (21).

There are two species of IL-1 namely IL-1α and IL-1β (41). Despite these two peptides being products of two different genes and exhibiting different isoelectric points, they exhibit similar biological activities (42). It is of interest therefore that both rhIL-1α and rhIL-1β exhibit a similar spectrum of activities on the human osteoblast-like cells. The mechanism whereby IL-1 induces the effects on the human osteoblast-like cells is not known at present. However, recently it has been reported that the mitogenic effect of IL-1 on fibroblasts and smooth muscle cells is mediated by the induction of platelet-derived growth factor (PDGF) (43). Indeed, PDGF exerts effects on osteoblast-like cells from rodents (44), but at present no similar reports exist describing the regulation of activity of human osteoblast-like cells by PDGF. Furthermore, osteosarcoma cells are known to produce PDGF (45) and so this raises the possibility that an induction of PDGF synthesis by IL-1 may contribute to a potential autocrine/paracrine regulation of osteoblast cell metabolism. This area is at present speculative and requires further clarification.

Elevated production of IL-1 has been implicated in the pathogenesis of osteoporosis (46) and with humoral hypercalcemia associated with squamous cell carcinomas (47,48). The present study therefore provides further support for the role of IL-1 in the processes governing bone metabolism. In addition, the demonstration of the production of IL-1-like factors by osteoblasts (49-52) may indicate a potential paracrine/autocrine regulatory mechanism affecting

both osteoblasts and osteoclasts and hence the cellular processes governing the regulation of bone metabolism.

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REFERENCES

(1) Baron, R., Vignery, A. and Horowitz, M. (1983) In Bone and Mineral Research Annual 2 (W.A. Peck, ed.) pp. 175-243. Elsevier, Amsterdam.
(2) Gowen, M, Wood, D.D., Ihrie, E.J., McGuire, M.K.B. and Russell, R.G.G. (1983) Nature 306, 378-380.
(3) Heath, J.K., Saklatvala, J., Meikle, M.C., Atkinson, S.J. and Reynolds, J.J. (1985) Calcif. Tissue Int. 37, 95-97.
(4) Gowen, M. and Mundy, G.R. (1986) J. Immunol. 136, 2478-2482.
(5) Stashenko, P., Dewhirst, F.E., Peros, W.J., Kent, R.L. and Ago, J.M. (1987) J. Immunol. 138, 1464-1468.
(6) Bunning, R.A.D., Van Damme, J., Richardson, H.J., Hughes, D.E., Opdenakker, G., Billiau, A. and Russell, R.G.G. (1986) Biochem. Biophys. Res. Commun. 139, 1150-1157.
(7) Pfeilschifter, J., Chenu, C., Bird, A., Mundy, G.R. and Roodman G.D. (1989) J. Bone Miner. Res. 4, 113-118.
(8) Stashenko, P. Dewhirst, F.E., Rooney, M.L. Desjardins, L.A. and Heeley J.D. (1987) J. Bone Miner. Res. 2, 559-565.
(9) Canalis, E.M. (1986) Endocrinology 118, 74-81.
(10) Beresford, J.N., Gallagher, J.A., Gowen, M., Couch, M., Poser, J.W., Wood, D.D. and Russell, R.G.G. (1984) Biochim. Biophys. Acta 801, 588-65.
(11) Gowen, M., Wood, D.D. and Russell R.G.G. (1985) J. Clin. Invest. 75, 1223-1228.
(12) Evans, D.B., Bunning, R.A.D., Dinarello, C.A. and Russell, R.G.G. (1985) Calcif. Tissue Int. 38, 58A.
(13) Evans, D.B., Bunning, R.A.D., Van Damme, J. and Russell, R.G.G. (1989) Biochem. Biophys. Res. Commun. 159, 1242-1248.
(14) Beresford, J.N., Gallagher, J.A., Poser, J.W. and Russell, R.G.G. (1984) Metab. Bone Dis. Rel. Res. 5, 229-234.
(15) MacDonald, B.R. Gallagher, J.A. and Russell, R.G.G. (1986) Endocrinology 118, 2445-2449.
(16) Beresford, J.N., Gallagher, J.A. and Russell, R.G.G. (1986) Endocrinology 119, 1776-1785.
(17) Meats, J.E., Elford, P.R., Bunning R.A.D. and Russell, R.G.G. (1985) Biochim. Biophys. Acta. 838, 161-169.
(18) Lowry, O.H., Rosebrough, R.J., Farr, A.L. and Randall, R.J. (1951) J. Biol.Chem. 193, 265-275.
(19) Richardson, H.J., Elford, P.R., Sharrard, R.M., Meats, J.E. and Russell, R.G.G. (1985) Cell Immunol. 90, 41-51.
(20) Konig, A., Muhlbauer, R.C. and Fleisch, H. (1988) J. Bone Miner. Res. 3, 621-627.
(21) Boyce, B.F., Aufdemorte, T.B., Garrett, I.R., Yates, A.J.P. and Mundy, G.R. (1989) Endocrinology 125, 1142-1150.
(22) Thomson, B.M., Saklatvala, J. and Chambers, T.J. (1986) J. Exp. Med. 164, 104-112.
(23) Dano, K., Andreasen, P.A., Grondahl-Hansen, J., Kristensen, P., Neilsen, S., and Skriver, L. (1985) Adv. Cancer Res. 44, 139-266.
(24) Robinson, R.M, Taylor, R.E. and Birkedal-Hansen. (1984) Calcif. Tissue Int. 36, 31-38.

(25) Thomson, B.M., Atkinson, S., Reynolds, J.J. and Meikle, M.C. (1987) Bone 8,265-266A.
(26) Werb, Z., Mainardi, C.L., Vater, C.A. and Harris, E.D. (1977) N. Engl. J. Med. 296,1017-1023.
(27) Liotta, L.A., Goldfarb, R.H., Brundage, R., Siegal, G.P., Tarranova, V. and Garbisa, S. (1981) Cancer Res. 41, 4629-4636.
(28) Eeckhout, Y. and Vaes, G. (1977) Biochem. J. 166, 21-31.
(29) Sakamoto, S. and Sakamoto, M. (1986) In Bone and Mineral Research Vol 4 (W.A. Peck, ed.) pp. 49-102. Elsevier, Amsterdam.
(30) Delaisse, J-M., Eeckhout, Y. and Vaes, G. (1988) Endocrinology 123, 264-276.
(31) Shen, V., Kohler, G., Jeffrey, J.J. and Peck, W.A. (1988) J. Bone Miner. Res. 3, 657-666.
(32) Gowen, M., Wood, D.D., Ihrie, E.J., Meats, J.E. and Russell, R.G.G. (1984) Biochem. Biophys. Acta 797, 186-193.
(33) Klein, D.C. and Raisz, L.G. (1970) Endocrinology 86,1436-1440.
(34) Hamilton, J.A., Lingelbach, S.R., Partridge, N.C. and Martin, T.J. (1984) Biochem. Biophys. Res. Commun. 122, 230-236.
(35) Dinarello, C.A. (1984) Rev. Infect. Dis. 5, 51-93.
(36) Levine, L., Hinkle, P.M., Voelkel, E.F. and Tashjian, A.H. (1972) Biochem. Biophys. Res. Commun. 47, 888-896.
(37) Rodan, G.A. and Rodan, S.B. (1984) In Bone and Mineral Research Annual 2 (W.A. Peck, ed.) pp. 244-285. Elseveir, Amsterdam.
(38) Evans, D.B., Bunning, R.A.D. and Russell, R.G.G. (1989) Biochem. Biophys. Res. Commun. 160, 588-595.
(39) Gowen, M., MacDonald, B.R. and Russell R.G.G. (1988) Arthritis Rheum. 31, 1500-1507.
(40) Parfitt, A.M. (1983) Calcif. Tissue Int. 36, S37-S45.
(41) Oppenheim, J.J., Kovacs, K.J., Matsushima, K. and Durum, S.K. (1986) Immunol. Today 7, 45-56.
(42) Dinarello, C.A. (1989) Adv. Immunol. 44, 153-205.
(43) Raines, E.W., Dower, S.K. and Ross, R. (1989) Science 243, 393-396.
(44) Centrella, M., McCarthy, T.L. and Canalis, E. (1989) Endocrinology 125, 13-19.
(45) Heldin, C-H., Johnson, A., Wennergren, S., Wernstedt, C., Betsholtz, C. and Westermark, B. (1986) Nature 319, 511-514.
(46) Pacifici, R., Rifas, L., Teitelbaum, S., Slatopolsky, E., McCracken, R., Bergfeld, M., Lee, W., Avioli, L.V. and Peck, W.A. (1987) Proc. Natl. Acad. Sci. USA 84, 4616-4620.
(47) Sato, K., Fujii, Y., Ono, M., Nomura, H. and Shizume, K. (1987) Cancer Res. 47, 6474-6480.
(48) Sato, K., Fujii, Y., Kasono, K., Tsushima, T. and Shizume, K. (1988) Endocrinology 67, 592-601.
(49) Hanazawa, S., Ohmori, Y., Amano, S., Miyoshi, T., Kumegawa, M. and Kitano, S. (1985) Biochem. Biophys. Res. Commun. 131, 774-779.
(50) Hanazawa, S., Amano, S., Nakada, K., Ohmori, Y., Miyoshi, T., Hirose, K. and Kitano, S. (1987) Calcif. Tissue Int. 41, 31-37.
(51) Amano, S., Hanazawa, S., Hirose, K., Ohmori, Y. and Kitano, S. (1988) Calcif. Tissue Int. 43, 88-91.
(52) Hughes, D.E., Gowen, M. and Russell, R.G.G. (1988) J. Bone Miner. Res. 3, 509A.