Journal of Cellular Biochemistry 62:476–483 (1996)

Dexamethasone Regulation of Marrow Stromal-Derived Osteoblastic Cells

A. Fried and D. Benayahu

Department of Cell Biology and Histology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 66978, Israel

Abstract The clonal subtypes of cells in the osteogenic family represented by fibroblastoid MBA-15.33, preosteoblast MBA-15.4, and mature osteoblastic MBA-15.6 cells were used to study the effects of glucocorticoid (dexamethasone). The role of dexamethasone was monitored on cell attachment when plated on various protein substrata (BSA, collagen I, and Matrigel). A 24 h exposure of the cells to 10^{-6} M or 10^{-7} M dexamethasone differential affects their attachment preference. MBA-15.33 and MBA-15.4 cells increased their attachment capability on collagen I, while MBA-15.6 cells' attachment was inhibited. Pretreatment with (10^{-6} M) dexamethasone caused an increase in attachment on Matrigel by MBA-15.33 cells and to less extent by MBA-15.4 cells. Additionally, measurements of two enzymatic activities were monitored; one is alkaline phosphatase (ALK-P), and the second is neutral endopeptidase (CD10/NEP). MBA-15.33, MBA-15.4, and MBA-15.6 cells were exposed to dexamethasone or to various growth factors (bone morphogenic protein (BMP-2 and BMP-3), TGFβ, and IGF-I). In some experiments, pretreatment of cells by dexamethasone was followed by exposure to the growth factors. The cells' challenged cellular responses were not uniform and revealed a differential pattern when their ALK-P and CD10/NEP enzymatic activities were measured. © 1996 Wiley-Liss, Inc.

Key words: stromal osteoblasts, dexamethasone, attachment, growth factors

The extracellular matrixes (ECM) promote cellular adherence that affects the growth, morphology, and phenotypic expression of osteoblastic cells. The matrix components mediate the interaction with cells through the existence of integrin or nonintegrin cell surface receptors. The expression of these receptors changed according to cell modulation with stimulators as hormones and growth factors as well as pharmacological components. Prolongation of treatment with glucocorticoids has been shown to decrease bone mass and induce osteoporosis in vivo [Dykma et al., 1985; Hahn et al., 1979]. The glucocorticoid affected the production of collagen synthesis [Dietrich et al., 1979; Canalis, 1983; Chyun et al., 1984] and its gene expression [Cockayne et al., 1986; Weiner et al., 1987]. Other proteins were also affected, such as osteocalcin [Beresford et al., 1984; Pockwinse et al., 1995], osteopontin [Yoom et al., 1987], and bone sialoprotein [Oldsberg et al., 1989]. Dexamethasone had a variable effect on cells at different stages of differentiation [Aubin et al., 1992; Breen et al., 1994; Fried et al., 1993]. We previously demonstrated the inhibition effect of dexamethasone on the growth rate of marrow stromal osteoblasts [Benayahu et al., 1994a] and their expression of mRNA for matrix protein as well as its effect on the mineralization of these cells. An acute effect of glucocorticoid may decrease bone mass by altering the osteoprogenitor and committed osteoblast function. A source of osteoprogenitor cells is the marrow stromal system [Benayahu et al., 1989; Owen and Friedenstein, 1988]. The systemic and local factors regulate the osteogenesis within the medullary cavity of bones. Such systemic factors as circulating hormones or a therapeutic drug such as the dexamethasone act on skeletal cells either directly or indirectly; this action modulates the synthesis, activation, and receptor binding of local growth factor. These changes may in turn stimulate or inhibit bone formation. The hormone may target a given growth factor to a specific cell [Canalis et al., 1991; Canalis, 1993], and this may further modify the hormone re-

Received December 1, 1995; accepted March 12, 1996.

Address reprint requests to Dafna Benayahu, Department of Cell Biology and Histology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel.

© 1996 Wiley-Liss, Inc.

sponse to the local growth factor that may change the replication or the differentiated of osteoblasts.

The role of glucocorticoid that provides tissue specificity for a given growth factor was our objective in this study. We used the marrow clonal-derived cell lines expressing osteoblastic-associated features. The clonal lines, derived from the MBA-15 line, were earlier described as presumably "arrested" at a particular stage along the osteogenic lineage [Fried et al., 1993]. They differ in their morphology, proliferation rate, synthesis of ECM proteins, and levels of adenylate cyclase. These cells' attachment preference to matrix proteins of collagenous and non-collagenous proteins was different [Benayahu et al., 1995a]. Enzymatic modulation of neutral endopeptidase (NEP) is identical to that of the CALLA antigen (CD10) and ALK-P activities previously shown to present on marrow stromal cell with higher expression by osteoblastic cells [Indig et al., 1990; Benayahu et al., 1994b, 1995b]. These enzymes are associated with the metabolism and regulation of a variety of peptides as well as of the mineralization process.

We now focus on the effects of dexamethasone modulation on differentiation in clonal MBA-15.33, MBA-15.4, and MBA-15.6 cells. The regulation of the cells demonstrated their ability to bind to various bone ECM components and the changes of their attachment following exposure to dexamethasone. The effect of dexamethasone also when priming the cultures to combined exposure to a series of growth factors on ALK-P and CD10/NEP activities was measured. This experimental system serves as a model to define the specific cell differentiation stages of the osteoblastic family and may shed light on the regulation in the marrow stroma compartments that occurs in situ.

MATERIALS AND METHODS

Cell Culture

The clonal MBA-15.33, MBA-15.4, and MBA-15.6 derived from mouse are marrow stromal cell lines, previously described [Fried et al., 1993]. Stock cultures maintained in the growth medium Dulbecco's modified Eagle's medium (DMEM) containing high glucose and supplemented with 10% fetal calf serum (FCS) (Beth Haemek, Israel). Cultures were passaged once weekly and medium changed every 3 days. All cultures incubated at 37°C in a humidified atmosphere of 10% CO_2 in air.

Hormone and Growth Factors

The glucocorticoid, dexamethasone was from IKAPHARM (Israel). rh-bone morphogenic protein 2 (BMP-2) (100 ng/ml) was a generous gift from the Genetic Institute (Cambridge, MA). Purified osteogenin (BMP-3) (10 ng/ml) [Luyten et al., 1989] was a kind gift from H. Reddi (Johns Hopkins University, Baltimore, MD). Transforming growth factor (TGF β) (1 ng/ml) and insulin-like growth factor (IGF-I) (10 ng/ml) were purchased from Collaborative Research (Bedford, MA).

Protein-Coated Dishes With Extracellular Matrix Proteins and Attachment Assay

The proteins included in this study were pepsin-solubilized collagen (Coll I) from bovine dermal collagen (Vitrogen 100; Collagen Corp., Palo Alto CA), solubilized basement membrane Matrigel™ (Collaborative Research, Inc., Bedford, MA), bovine serum albumin (BSA), fraction V (ICN Biochemical, Costa Mesa, CA). The proteins were used for coating non-tissue-culture, 96-well microtiter plates (Costar, Cambridge, MA), as described earlier [Benayahu et al., 1995a]. For attachment assays, the cells were incubated for 24 h with dexamethasone (10^{-6} M or 10^{-7} M) and then were washed out of culture medium and dispersed as a single cell suspension using trypsin-EDTA (Beth Haemek) and allowed to attach. The attached cells were quantitatively determined by the uptake of methylene blue and the dye released from the cells measured by its absorbency at a 550 nm wavelength using a microplate reader (vMaxMolecular Devices, Palo Alto, CA) [Benayahu et al., 1995a].

Enzymatic Determination

Experimental design. Cells were trypsinized and seeded at 1×10^4 cells/ml in 24-well plates after 24 h, the medium was removed, and the cells were supplemented with DMEM containing 2% charcoal FCS (chFCS) with or without dexamethasone (10^{-7} M). In priming experiments, cells were first exposed to the hormone for 24 h, after which the medium was changed and the cells supplemented with the growth factors for 48 h as described in Table I.

Biochemical determination. ALK-P activity was determined using 2 mM Paranitrophenyl phosphate at 37°C in 0.1 M 2-amino-2-methyl-1-propanol and 2 mM $MgCl_2$ [Majeska and Rodan 1982]. CD10/NEP activity was assayed by a two-stage assay at 37°C using the synthetic substrate Suc-Ala-Ala-Leu-NH-Np

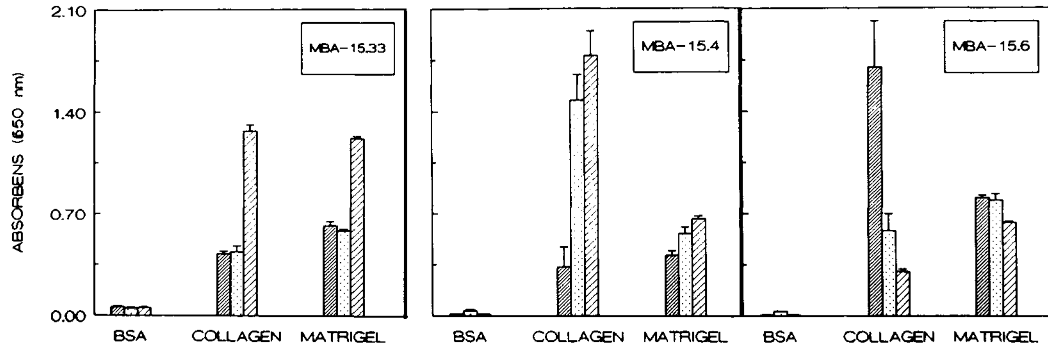

Fig. 1. The preferential attachment of various clonal cells MBA-15.33, MBA-15.4, and MBA-15.6 to matrix proteins such as BSA, collagen type I, and Matrigel was examined on untreated control (heavily hatched bars) or following preincubation with dexamethasone (10^{-6} M, lightly hatched striped bar; 10^{-7} M, dotted bar). Cells (40,000) were applied to each well for 3 h. The results are expressed by optical density read on a microplate reader at 550 nm. Assays were performed in triplicate on four to six separate wells. The values are of duplicate determination of three to four separate experiments as mean ± SEM ($P < 0.05$; Duncan's multiple range test).

TABLE I. Experimental Design

Treatment	Hours in culture			
	24	48	72	96
Control	10% chFCS	2% chFCS	2%chFCS	2% chFCS
GF	10% chFCS	2%chFCS	GF	GF
Hormone	10% chFCS	Hormone	2% chFCS	2% chFCS
Hormone + GF	10% chFCS	Hormone	GF	GF

supplemented with streptomyces griseus aminopeptidase I (SGAPI) [Indig et al., 1989]. Protein was measured using Lowry and colleagues' method [Lowry et al., 1951] with human serum albumin as a standard.

Statistical Analysis

The significance of differences between experimental and control groups was analyzed by Duncan's multiple range test.

RESULTS

Modulation of Attachment Capability According to Cellular Maturation Stage

In a previous study we were able to determine the differential in attachment capability on precoated wells with various matrix proteins by MBA-15 clonal cell lines [Benayahu et al., 1995a]. Herein we emphasize the effect of dexamethasone to modulate cell attachment. The clonal cell MBA-15.33 (fibroblastoid), MBA-15.4 (preosteoblast), and MBA-15.6 (mature osteoblasts) subpopulations were applied to precoated 96-well plates (Fig. 1). The cells were allowed to attach to different matrix proteins such as collagen type I and Matrigel that composed various basement membrane proteins, including collagens type IV and V. BSA was used for determination of nonspecific binding. The basal level of MBA-15.6 cell binding was higher than for MBA-15.33 and MBA-15.4, up to fivefold on collagen and up to twofold on matrigel. This pattern was changeable upon 24 h pretreatment of the cells with dexamethasone (10^{-6} M or 10^{-7} M). An increase in binding of pretreated MBA-15.33 cells with (10^{-6} M) dexamethasone and MBA-15.4 cells in both dexamethasone concentrations was observed. The effect of the drug on MBA-15.6 cells was the opposite: a decrease of 80% in cell attachment was monitored. The cells' binding capacity to Matrigel was increased when MBA-15.33 and MBA-15.4 were exposed to 10^{-6} M dexamethasone and was unchanged in MBA-15.6 cells.

Enzyme Activities

Response to growth factors and dexamethasone stimulation. The responses of the clonal cells that were challenged with BMP-2, BMP-3, TGFβ, and IGF-I were not uniform and revealed a differential pattern, as measured by enzymatic ALK-P and CD10/NEP activities. ALK-P activity increased up to 2.5 fold in MBA-

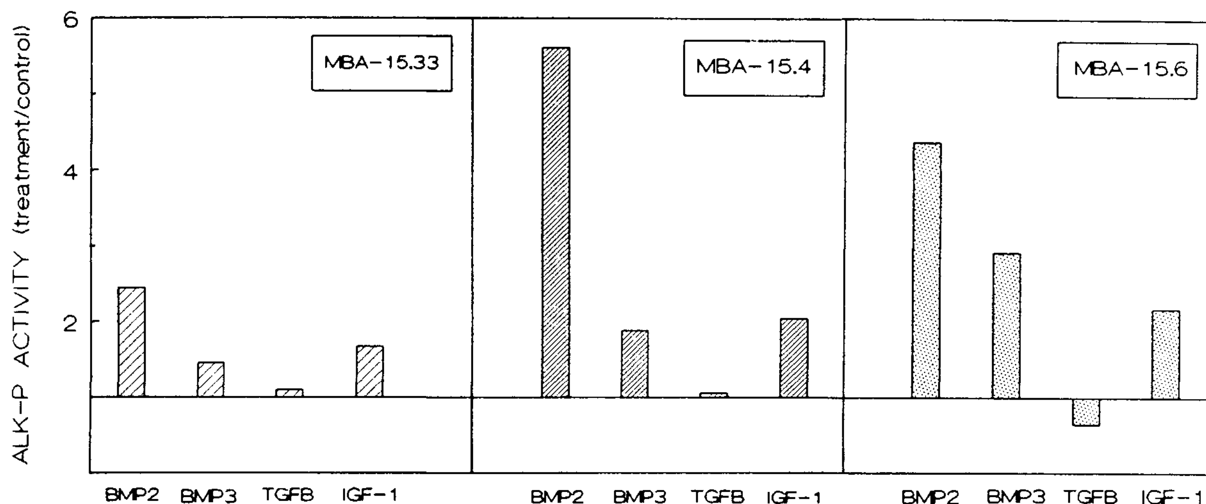

Fig. 2. MBA-15.33 (a fibroblastoid), MBA-15.4 (a preosteoblast), and MBA-15.6 (a mature cell) were exposed to various growth factors such as BMP-2, BMP-3, TGFβ, and IGF-I. ALK-P activity was determined in treated cultures and compared to an untreated control. Assays were performed in triplicate on four to six separate cultures. The results are expressed as ratio between treatment and control.

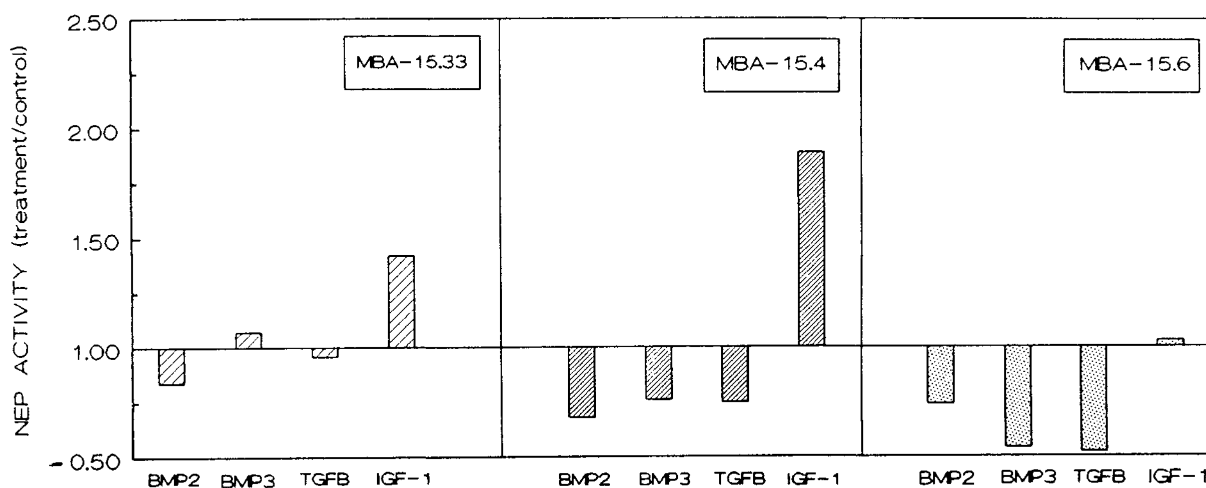

Fig. 3. MBA-15.33 (a fibroblastoid), MBA-15.4 (a preosteoblast), and MBA-15.6 (a mature cell) were exposed to various growth factors as detailed in the Fig. 2. NEP activity was determined in treated cultures and compared to an untreated control. Assays were performed in triplicate on four to six separate cultures, and the results are expressed as ratio between treatment and control.

15.33 up to sixfold in MBA-15.4, and up to fivefold in MBA-15.6 cells measured in the presence of BMP-2 (Fig. 2). BMP-3 stimulated ALK-P activity up to 1.5–2-fold in MBA-15.33 and MBA-15.4 cells and threefold in MBA-15.6 cells, whereas TGFβ inhibited the enzyme activity in MBA-15.6 cells but not in MBA-15.4 and MBA-15.33 cells. IGF-I had a stimulatory effect on ALK-P activity in all cells (Fig. 2). CD10/NEP activity exhibited a different pattern when these cells were introduced to the growth factors (Fig. 3). In MBA-15.6 and MBA-15.4 cells, the CD10/NEP activity was inhibited by BMP-2, BMP-3, and TGFβ but in MBA-15.33 cells activity was inhibited by BMP-2 or was unchanged by BMP-3 and TGFβ. IGF-I was a potent stimulator for the MBA-15.33 and MBA-15.4 cells, up to twofold, but did not alter the enzyme levels in MBA-15.6 cells (Fig. 3). Exposure of these cells to dexamethasone (Figs. 4, 5) did not significantly change the ALK-P activity over baseline levels in all cells. The drug effect on CD10 NEP activity was unchanged in MBA-15.4 and inhibited the enzyme activity in MBA-15.33 and MBA-15.6.

Effect of growth factors on cells primed by dexamethasone treatment. The pretreat-

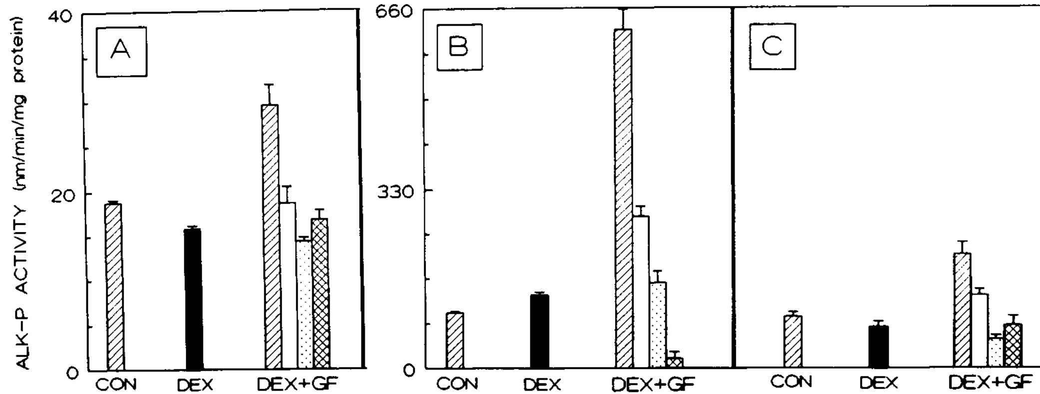

Fig. 4. ALK-P activity in clonal subpopulations. MBA-15.33 (**A**), MBA-15.4 (**B**), and MBA-15.6 (**C**) were exposed to dexamethasone (DEX) (10^{-7} M). Following 24 h of exposure, the cells were challenged with or without various growth factors (BMP-2, BMP-3, TGFβ, and IGF-I for an additional 48 h as described in the experimental design and compared to CON (basal levels). Assays were performed in triplicate on four to six separate cultures. The results are mean ± SEM ($P < 0.05$; Duncan's multiple range test).

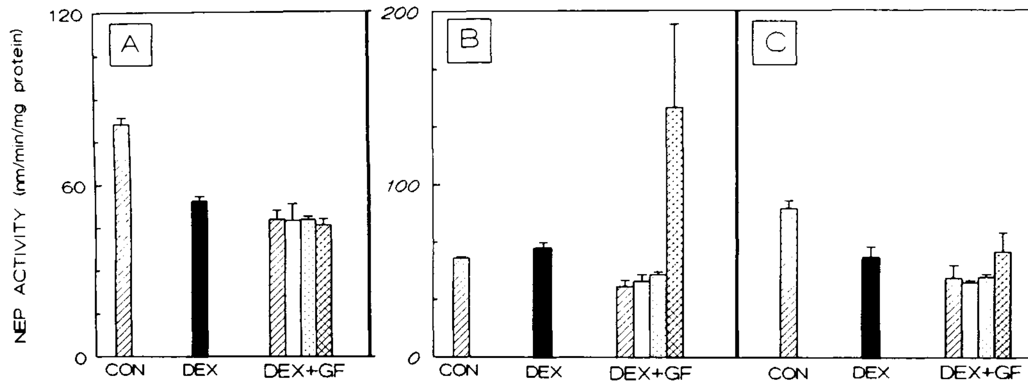

Fig. 5. NEP activity in clonal subpopulations. MBA-15.33 (**A**), MBA-15.4 (**B**), and MBA-15.6 (**C**) were exposed to dexamethasone (DEX) (10^{-7} M) as described in the legend for Fig. 4. Assays were performed in triplicate on four to six separate cultures. The results are mean ± SEM ($P < 0.05$; Duncan's multiple range test).

ment of MBA-15.4 cells with dexamethasone did not alter the ALK-P activity in the presence of BMP-2, BMP-3, and TGFβ from the response obtained when cells were challenged with the growth factors alone (Figs. 2, 4B). The MBA-15.33 and MBA-15.6 were lowered in their response to these growth factors by priming with dexamethasone (Fig. 4A,C). IGF-I stimulation of ALK-P activity was significantly inhibited following the priming by dexamethasone in MBA-15.33, MBA-15.4, and MBA-15.6 cells.

CD10/NEP activity was measured under the same culture conditions (Fig. 5). The basic inhibition effect of the growth factors BMP-2, BMP-3, and TGFβ was noted in MBA-15.4 and MBA-15.6. After priming MBA-15.4 cells with dexamethasone (Fig. 5B), these cells were unchanged from the basal growth factor level as presented in Figure 3. An exposure to IGF-I with or without priming by dexamethasone expresses an elevation in MBA-15.4 (Figs. 3, 5B). CD10/NEP activity in MBA-15.33 was unchanged when exposed to BMP-2, BMP-3, and TGFβ and elevated by IGF-I (Fig. 3). This pattern of enzyme activity was reduced when growth factors were added following exposure to the dexamethasone (Fig. 5A). The basic CD10/NEP activity in MBA-15.6 cells (Fig. 5C) in the presence of growth factors alone was not altered when introduced to cultures that were primed by dexamethasone challenge. Priming with dexamethasone did not alter the MBA-15.6 cells' response to BMP-2, BMP-3, or TGFβ.

DISCUSSION

The osteogenic potential is fully expressed only on bone surfaces where the cells interact with bone matrix that plays a role in the anchor-

age of cells and further modulates their differentiation. We used various clonal cells of the MBA-15 line that are at a different maturation stage in the osteoblastic lineage [Fried et al., 1993] to study their adhesion potential. The MBA-15.4 (preosteoblasts) adhesion was earlier quantitated in comparison to MBA-15.6 (a mature cell). The latter had the ability to adhere to the noncollagenous proteins (NCPs) more effectively [Benayahu et al., 1995a]. This suggested that not all cells in the osteoblastic lineage express the same expression of adhesion receptors, as was also pointed by others [Grzesik and Robey, 1994]. In this study we further emphasize the cells' attachment potential following an exposure to dexamethasone. This drug alters the cells' ability for attachment to the ECM constituents. MBA-15.33 and MBA-15.4 increased their attachment capability on collagen I, while attachment of MBA-15.6 cells was inhibited. This phenomenon possibly occurs due to changes in the family of integrins receptors [Gronowicz and McCarthy, 1995] identified on osteoblastic cells in vitro [Dedhar, 1989] and in vivo [Grzesik and Robey, 1994; Muschler and Horowitz 1991]. The osteoblastic cells' regulation was affected by dexamethasone. Thus, the prime importance in this study was to understand of specific cell's stage regulation that may clear the changes during bone remodeling by this drug therapy. A consequence of acute therapy by glucocorticoid is an induced osteopenia recognized in vivo [Dykma et al., 1985; Hahn et al., 1979]. Herein we emphasize the specific function of dexamethasone on osteoblastic cells. It is known that the hormonal modulation of cell function is mediated through local cytokines and growth factors. It is also possible that systemic factors play a concomitant role and alter the cellular response to the growth factors. The role assignment of the various growth factors at a particular point in the bone formation scheme is not a strict one, and certain factors may influence different cells or may affect these cells differentially. The effects of growth factors on marrow stromal osteoblasts was examined on a MBA-15 cell line in previous studies [Benayahu, 1991; Benayahu et al., 1994b, 1995b]. ALK-P activity increased following exposure to BMP-2 and BMP-3, while a decrease in enzyme activity was observed following TGFβ treatment [Benayahu, 1991; Benayahu et al., 1994b]. Neither IGF-I, platelet-derived growth factor [Benayahu, 1991], nor basic fibroblast growth-factor [Benayahu et al., 1994b] produced any changes in ALK-P activity and cell growth. In order to clarify the biological activities of local and systemic factors on the osteoblastic cell at different stages of differentiation, we used the clonal cell lines derived from the MBA-15 line. Priming of MBA-15.6 and MBA-15.4 cells with retinoic acid (RA) [Benayahu et al., 1994b], PTH, or $1,25(OH)_2D_3$ [Benayahu et al., 1995b] followed by exposure to BMP-2 and BMP-3 resulted in a synergistic effect on ALK-P activity and an antagonistic effect with TGFβ. However, dexamethasone did not alter the basic cell response to these growth factors. ALK-P activity monitored in the cell response to the IGF-I effect was unchanged following either $1,25(OH)_2D_3$ or PTH priming. Dexamethasone did not change the ALK-P activity in MBA-15.6 cells and inhibited ALK-P activity in MBA-15.4 and MBA-15.33 cells. CD10 also had NEP activity that highly correlated to the osteoblastic cells [Indig et al., 1990; Benayahu et al., 1994b; Howell et al., 1993; Ibbotson et al., 1992; Kee et al., 1992]. This enzyme was identified as a CALLA antigen and is an endopeptidase that is believed to act as a regulator by its proteolytic activity on local growth factors. MBA-15 and clonal subpopulations were demonstrated to have CD10/NEP activity and a diverse pattern upon modulation. A difference in ALK-P and CD10/NEP activity in response to $1,25(OH)_2D_3$ treatment was observed in clonal MBA-15.30 and MBA-15.33 cells. These cells' expressions for CD10/NEP activity increased in a dose-dependent manner with no parallel change in ALK-P activity [Indig et al., 1990]. PTH did not affect CD10/NEP activity, while $1,25(OH)_2D_3$ had a stimulatory effect only in the more mature MBA-15.6 osteoblastic cell; the inhibitory effect of BMP-2, BMP-3, and TGFβ is observed when PTH or $1,25(OH)_2D_3$ is used for pretreatment of cultures [Benayahu et al., 1995b]. When the cells were exposed to dexamethasone in the present study, CD10/NEP activity was inhibited in MBA-15.4 and MBA-15.6 cells when they were exposed to BMP-2, BMP-3, and TGFβ, but no changes were observed in MBA-15.33 cells. Under IGF-I treatment, MBA-15.33 and MBA-15.4 cells increased their CD10/NEP activity, which was unchanged following dexamethasone exposure in MBA-15.4 cells but inhibited in MBA-15.33 cells. These defined clonal osteoblastic cells strengthen the evidence that growth factors differentially affect cellular activities. As depicted by our studies,

combined exposure of osteoblast cells to systemic hormones (PTH, 1,25$(OH)_2D_3$, and RA) and BMPs enhanced their ALK-P activity. Pretreatment with dexamethasone did not alter the BMP's activity effects on ALK-P but differentially when combined exposure of dexamethasone to TGFβ and IGF-I. The combined exposure of the osteoblastic clonal cells to hormones and growth factors deepens our understanding of the series of events that occurs in bone formation. Both enzymes (ALK-P and CD10/NEP) are on stromal cells' surface, so their intense activity suggests their role as local regulators for various functions of bone cells in the local microenvironment. The varied responses in the different stages of cell differentiation demonstrated by the clonal subpopulations are described here.

ACKNOWLEDGMENTS

This study has been supported by grants from the Basic Research Fund of Tel Aviv University and The Fund from the Chief Scientist of the Israeli Ministry of Health to D.B.

REFERENCES

Aubin JE, Bellows CG, Turksen K, Liu F, Heersche JNM (1992): Analysis of the osteoblast lineage and regulation of differentiation. In Slavkin H, Price P (eds): "Chemistry and Biology of Mineralized Tissues." Amsterdam: Elsevier Science Publisher B.V., pp 267–276.

Benayahu D, Kletter Y, Zipori D, Wientroub S (1989): A bone marrow-derived stromal cell line expressing osteoblastic phenotype in vitro and osteogenic capacity in vivo. J Cell Physiol 140:1–7.

Benayahu D. (1991): "Mechanisms of Interactions Between Osteogenic and Non-Osteogenic Cells in the Marrow." Doctoral dissertation, Tel-Aviv University, Israel (In English).

Benayahu D, Kompier R, Shamay A, Kadouri A, Zipori D, Wientroub S (1994a): Mineralization of marrow stromal osteoblasts MBA-15 on three-dimensional carriers. Calcif Tissue Int 55:120–127.

Benayahu D, Fried A, Shamay A, Cunningham N, Blumberg S, Wientroub S (1994b): Differential effects of retinoic acid and growth factors on osteoblastic markers and CD10/NEP activity in stromal-derived osteoblasts. J Cell Biochem 56:62–73.

Benayahu D, Fried A, Efrati M, Gehron Robey P, Wientroub S (1995a): Bone-marrow interface: Preferential attachment of an osteoblastic marrow stromal cell line. J Cell Biochem 58:151–160.

Benayahu D, Fried A, Wientroub S (1995b): PTH and 1,25$(OH)_2$ vitamin D priming to growth factors differentially regulates the osteoblastic markers in MBA-15 clonal subpopulations. Biochem Biophys Res Commun 210:197–204.

Beresford JN, Gallagher JA, Poser JW, Russell RGG (1984): Production of osteocalcin by human bone cells in vitro. Effects of 1,25$(OH)_2D_3$, 24,25$(OH)_2D_3$, parathyroid hormone and glucocorticoid. Metab Bone Dis Rel Res 5:229–234.

Breen EC, Ignotz RA, McCable L, Stein JL, Stein GS, Lian JB (1994): TGF beta alters growth and differentiation related gene expression in proliferating osteoblasts in vitro, preventing development of the mature bone phenotype. J Cell Physiol 160:323–335.

Canalis E (1983): Effect of glucocorticoids on type I collagen synthesis, alkjaline phosphatase activity and deoxyribonucleic acid content in cultured rat calvaria. Endocrinology 112:931–939.

Canalis E, McCarthy TL, Centrella M (1991): Growth factors and cytokines in bone cell metabolism. Annu Rev Med 42:17–24.

Canalis E (1993): Regulation of bone remodeling. In Favus MJ (ed): "Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism," 2nd ed. New York: Raven Press, pp 33–37.

Chyun YS, Kream BE, Raisz LG (1984): Cortisol decreases bone formation by inhibiting periostal cell proliferation. Endocrinology 114:477–480.

Cockayne D, Sterling KM, Shull S, Mintz KP, Illeyne S, Cutroneo KR (1986): Glucocorticoids decreases the synthesis of type I procollagen mRNAs. Biochemistry 25:3202–3209.

Dedhar S (1989): Signal transduction via the beta 1 integrins is a required intermidiate in interleukin-1 beta induction of alkaline phosphatase activity in human osteosarcoma cells. Exp Cell Res 183:207–214.

Dietrich JW, Canalis EM, Maina DM, Raisz LG (1979): Effects of glucocorticoids on fetal rat bone collagen synthesis in vitro. Endocrinology 104:715–721.

Dykma TR, Gluck OS, Murphy WA, Hahn TJ, Hahn BH (1985): Evaluation of factors associated with glucocticoid-induced osteopenia in patients with rheumatic diseases. Arthritis Rheum 28:361–368.

Fried A, Benayahu D, Wientroub S (1993): Characterization of clonal subpopulations of marrow-derived osteogenic cells (MBA-15): Putative stages in osteoblast differentiation. J Cell Physiol 155:472–482.

Gronowicz G, and McCarthy MB (1995): Glucocorticoids inhibit the attachment of osteoblasts to bone extracellular matrix proteins and decrease beta 1-intergrin levels. Endocrinology 136:598–606.

Grzesik WJ, Gehron Robey P (1994): Bone matrix RGD glycoproteins: Immunolocalization and interaction with human primary osteoblastic bone cells in vitro. J Bone Miner Res 9:487–496.

Hahn TJ, Halstead LR, Teitelboum SL, Hahn BH (1979): Altered mineral metabolism in glucocorticoid-induced osteopenia, effect of 25-hydroxyvitamin D administration. J Clin Invest 64:655–665.

Howell S, Caswell AM, Kenny AJ, Turner AJ (1993): Membrane peptidases on human osteoblast-like cells in culture: Hydrolysis of calcitonin and hormonal regulation of endopeptidase-24.11. Biochem J 290:159–164.

Ibbotson KJ, D'Souza SM, Deschodt-Lanckman M, Appelboom TE (1992): Inhibition of bone resorption in vitro by human enkephalinase (EC 3.4.24.11), a neutral metalloendopeptidase. J Bone Miner Res 7:273–279.

Indig FE, Ben-Meir D, Spungin A, Blumberg S (1989): Investigation of neutral endopeptidase (EC 3.4.24.11) and

of netral proteinases (EC 3.4.24.4) using a new sensitive two-stage enzymatic reaction. FEBS Lett 255:237–240.

Indig FE, Benayahu D, Fried A, Wientroub S, Blumberg S (1990): Neutral endopeptidase (EC 3.4.24.11) is highly expressed on osteoblastic cells and other marrow stromal cell types. Biochem Biophys Res Commun 172:620–626.

Kee BL, Paige CJ, Letarte M (1992): Characterization of murine CD10, an endopeptidase expressed on bone marrow adherent cells. Int Immunol 4:1041–1047.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951): Protein measurement with folin-phenol reagent. J Biol Chem 193:265–275.

Luyten FP, Cunningham NS, Ma S, Muthukumara N, Hammonds RG, Nevis WB, Wood I, Reddi AH (1989): Purification and partial amino acid sequence of osteogenin, a protein initiating bone differentiation. J Biol Chem 264:13377–13380.

Majeska RL, Rodan GA (1982): Alkaline phosphatase inhibition by parathyroid hormone and isoproterenol in a clonal rat osteosarcoma cell line. Possible mediation by cyclic AMP. Calcif Tissue Int 34:59–66.

Muschler JL, Horwitz AF (1991): Dowe-regulation of the chicken alpha 5 beta 1 integrin fibronectin receptor during development. Development 113:327–337.

Oldsberg A, Jirskog-Hed B, Axelssen S, Heinegard D (1989): Regulation of bone sialoprotein (BSP) mRNA by steroid hormones. J Cell Biol 109:3183–3186.

Owen M, Friedenstein AJ (1988): Stromal stem cells: marrow-derived osteogenic precursors. In Evered D, Harnett S (eds): "Cell and Molecular Biology of Vertebrate Hard Tissue," Vol 136. New York: J. Wiley & Sons, pp 42–60.

Pockwinse SM, Stein JL, Lian JB, Stein GS (1995): Developmental stage-specific cellular responses to vitamin D and glucocorticoids during differentiation of the osteoblast phenotype: Interrelationship of morphology and gene expression by in situ hybridization. Exp Cell Res 216:244–260.

Weiner FR, Czaya MJ, Jefferson DM, Giambrone M-A, Tur-Kaspa R, Reid LM, Zern MA (1987): The effects of dexamethasone on in vitro collagen gene expression. J Biol Chem 262:6955–6958.

Yoom K, Bueraga R, Rodan GA (1987): *Tissue specific and* developmental expression of rat osteopontin. Biochem Biophys Res Commun 148:1129–1136.