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Original Article

Age-Dependent Expression of Osteoblastic Phenotypic Markers in Normal Human Osteoblasts Cultured Long Term in the Presence of Dexamethasone

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Abstract. We have previously shown that osteoblasts derived from trabecular bone explants and cultured long term in 10 nM dexamethasone ((HOB+DEX) cells) exhibited properties consistent with a more differentiated phenotype compared with those grown in the absence of dexamethasone ((HOB−DEX) cells). To characterize these two cell models further, we measured the steady-state mRNA levels of the phenotypic markers alkaline phosphatase (ALP), collagen type I (COLL) and osteocalcin (OC), OC production, and the activities of ALP and parathyroid hormone (PTH)-stimulated adenylate cyclase. These findings were then correlated with the age and sex of the bone donors. Long-term culture in dexamethasone significantly increased ALP and OC mRNA levels and the activities of ALP and PTH-stimulated adenylate cyclase but not OC production, in (HOB+DEX) compared with (HOB−DEX) cells ($p < 0.05$). When the data were examined with respect to the age of the bone donor, age-dependent differences in the expression and responses to dexamethasone were apparent. ALP and PTH-stimulated adenylate cyclase activities decreased with increasing age of the bone donor in (HOB−DEX) and (HOB+DEX) cells ($p < 0.05$). There were no significant correlations between phenotypic marker mRNA levels and bone donor age in (HOB−DEX) and ((HOB+DEX) cells. All age-dependent decreases in ALP and PTH-stimulated cyclase activities were enhanced in the (HOB+DEX) cells. However, when the data were examined according to the sex of the bone donor, there were no differences in mRNA levels, OC production, or ALP and cyclase activities between cells from male and female donors. These results indicate an age dependence in the expression of osteoblastic markers in human bone cells at different stages of differentiation: thus osteoblastic cultures derived from older donors are likely to contain fewer osteoprogenitor cells, lower levels of glucocorticoid receptors or represent more differentiated osteblasts compared with those derived from younger donors.

Keywords: Adenylate cyclase; Age; Alkaline Phosphatase; Dexamethasone; Osteoblasts, mRNA

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Introduction

The use of osteoblast-like cells derived from adult human bone explants has recently gained popularity [1–19]. This technique has been applied to studies of cells derived from patients with metabolic bone diseases including pseudohypoparathyroidism [18] and osteoporosis [17,19]. However, these cultures contain heterogeneous populations of cells in different stages of differentiation. Indeed, Matsuyama et al. [5] and Manduca et al. [14] reported finding differential expression of alkaline phosphatase (ALP) activities in multiple subpopulations of osteoblasts from primary

and clonal cell cultures. Therefore, there is a need for well-characterized osteoblastic cell models of human origin.

In this respect, the development in our laboratory of two normal human osteoblastic cell models with distinct biochemical characteristics [6] should be useful. Thus, we showed that osteoblastic cells derived from iliac crest trabecular bone that had been explanted and cultured long term in media containing 10 nM dexamethasone ((HOB+DEX) cells) exhibited biochemical properties that were consistent with a more differentiated phenotype compared with cells grown in the absence of dexamethasone ((HOB−DEX) cells): the basal and 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) stimulated ALP activities and parathyroid hormone (PTH)-stimulated adenylate cyclase activities were significantly enhanced in (HOB+DEX) cells compared with those of (HOB−DEX) cells [6].

In the present study, we further characterized these two human osteoblastic cell models, the (HOB−DEX) and (HOB+DEX) cells, by comparing the steady-state mRNA levels of the osteoblastic phenotypic markers ALP, collagen type I (COLL) and osteocalcin (OC), the activities of ALP and PTH-stimulated adenylate cyclase, and OC production. We report here that the expression (steady-state mRNA levels and enzyme activities) of these osteoblastic phenotypic markers is higher in (HOB+DEX) cells and is age-dependent; also that the age-dependency is enhanced in the more differentiated cell model, the (HOB+DEX) cells.

Materials and Methods

Subjects

Trabecular bone from the iliac crest was obtained from 67 subjects (19 females and 48 males) aged 17–80 years (mean ± SD of 45 ± 17 years) undergoing iliac crest graft surgery for orthopedic procedures to correct mechanical back problems. The majority of the subjects had degenerative disc disease. None of the subjects had a history of metabolic bone disease or malignancy. The bone sampling protocol was approved by the Human Studies Review Committee of the University of Toronto. Informed consent was obtained from all subjects.

Cell Culture

The explant technique and subsequent cell culture were as previously described [1,6,18,19]. Briefly, bone fragments were cleaned of adherent tissue and blood, cut into 1–3 mm pieces, divided equally, and cultured separately, in 75 cm^2 flasks containing media (Ham's F-12 supplemented with 28 mM Hepes, 1.1 mM $CaCl_2$, 1% glutamine, 1% antibiotic–antimycotic solution (Gibco-BRL, Burlington, Ontario) and 10% fetal calf serum (Hyclone, Logan, UT)) with ((HOB+DEX) cells) or without ((HOB−DEX) cells) 10 nM dexamethasone (Sigma, St. Louis, MO). The medium was changed after 24 h and twice weekly thereafter. At confluency, the cells were harvested by trypsinization and plated at a density of 1×10^4 cells/cm^2 into 25 cm^2 flasks for RNA analyses and/or 12-well dishes for subsequent biochemical tests, depending on the cell number. Only cells from first passage were used in this study.

RNA Isolation

Cells were harvested at confluency after 7–10 days from 25 cm^2 flasks by trypsinization, and total RNA isolated by the method of Chomczynski & Sacchi [20]. Cells were lysed with a solution of 4 M guanidine thiocyanate (Sigma, St. Louis, MO), 25 mM sodium citrate, pH 7.0, 0.5% sarcosine and 0.1 M 2-mercaptoethanol, and the RNA extracted with phenol–chloroform. The RNA was further purified with repeated ethanolic precipitations and stored at −80 °C until required.

Northern and Slot Blot Analyses

RNA samples were analysed using slot blots due to the limited numbers of cells and, thus, the low RNA yield. Duplicate samples of 5 μg total RNA were applied onto nylon membranes using a Tyler slot blot manifold (BIO/CAN, Mississauga, Ontario). Northern analysis was used to determine the specificity of the cDNA probes. For Northern analyses, duplicate samples of 15 μg total RNA (as determined spectrophotometrically at 260 nm) were electrophoresed on 1.2% agarose gels and transferred onto Hybond nylon membranes [21] (Amersham, Oakville, Ontario). cDNA probes for ALP [22] (human, 2.5 kb), COLL (porcine α1 (I), 0.3 kb, 3′-untranslated region), OC (rat, 0.3 kb) and GAPD (glyceraldehyde-3-phosphate dehydrogenase, mouse, 1.0 kb) were labelled using [^{32}P]dCTP (3000 Ci/mmol, ICN Biomedicals, Mississauga, Ontario) and the Megaprime Labelling Kit (Amersham) to a specific activity of 1×10^9 dpm/μg. The labelled probes were then incubated with the blots at 65 °C for 2 h in Rapid Hybridization Buffer (Amersham) and washed according to the manufacturer's (Amersham) directions. The blots were exposed to X-ray film (Fuji NIF-RX). Slot blots were stripped after exposure and probed with each labelled cDNA in turn to allow for multiple analyses of marker mRNAs. Autoradiographs were scanned using a LECO 2001 Image Analysis System (LECO, Mississauga, Ontario). mRNA levels for ALP, COLL and OC were reported as ratios of hybridization signals for the markers divided by those obtained for GAPD.

There were no significant differences in the amount of RNA obtained from (HOB−DEX) and (HOB+DEX) cells. The amounts of RNA harvested per 25 cm^2 flask were (mean ± SEM) 18 ± 5 and 19 ± 7 μg, respectively.

Alkaline Phosphatase Activity

At confluency, cells grown in 12-well dishes were washed twice with 50 mM Tris HCl, pH 7.3, harvested by scraping, and ALP activity determined in cell sonicates as previously described [6], using a modified method of Lowry [23]. ALP activity was expressed as nanomoles *p*-nitrophenol released from *p*-nitrophenyl phosphate per milligram protein per minute of incubation time at 30 °C.

Adenylate Cyclase Activity

Adenylate cyclase assays were performed as described previously, using a modification of the method of Shimizu et al. [24]. Cells were preincubated with 1 μCi/ml [^{3}H]adenine for 2 h prior to a 5-min challenge with 10^{-6} M bovine PTH. [^{3}H]cAMP was isolated by the method of Salomon [25] and quantitated using a liquid scintillation counter. Data were corrected for cell number and recovery (mean ± SEM, 87 ± 5%).

Osteocalcin Production

OC levels were measured in culture media using a commercially available radioimmunoassay kit (Diagnostic Systems Labs, Burlington, Ontario) containing rabbit anti-bovine OC antisera and bovine OC standards.

Protein

Protein was determined using a commercially available Coomassie Blue dye reagent (Biorad, Mississauga, Ontario) of Bradford [26] and bovine serum albumin as the standard. There were no significant differences in the amount of protein obtained from (HOB−DEX) and (HOB+DEX) cells. The amounts of total protein per 25 cm^2 flask were (mean ± SEM) 870 ± 120 and 890 ± 110 μg, respectively.

Statistical Analyses

Data for each parameter were pooled from multiple determinations using cells derived from separate bone donors and examined according to the effect of the treatment (i.e. (HOB+DEX) v (HOB−DEX) cells) and the age and sex of the bone donors. Replicate samples were used to determine mean values but statistical analyses were based on single weight data from different donors. It should be noted that all statistical comparisons are unpaired since generally cells from different patients were used for each measurement.

Non-parametric Mann–Whitney tests were used to determine the statistical significance between two sample sets. Linear regression with ANOVA analysis (INPLOT, v.4.04, GraphPad Software, San Diego, CA) was used to determine the correlation between osteoblastic marker expression and age of bone donor. Since similar results were attained when raw data or transformed data (reciprocal, log values) were used for regression analyses, only the results for linear regression analyses of raw data are shown.

Results

Northern analyses using cDNA probes for the osteoblastic phenotypic markers showed that these cDNAs hybridized to mRNA species of approximately 2.5 kb for ALP, 5.4 and 6.4 kb for COLL and 0.6 kb for OC – values which agree with those published in the literature [22,27,28] (Fig. 1).

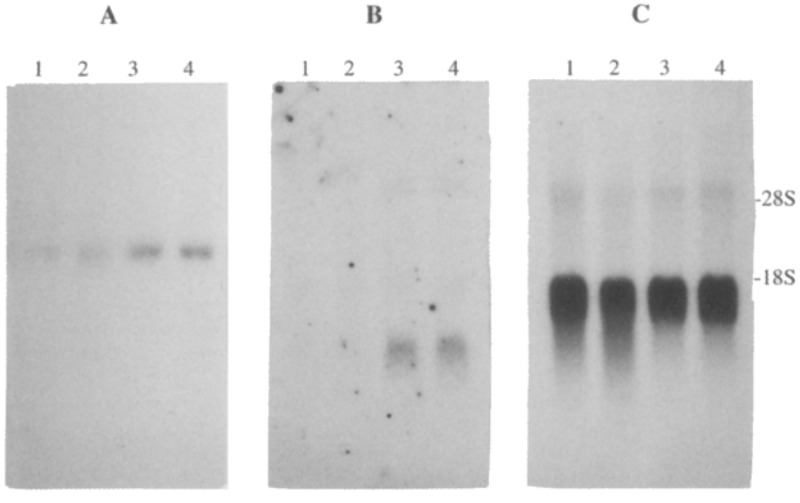

Fig. 1A–C. Northern analyses of mRNA levels for (**A**) ALP, (**B**) OC and (**C**) COLL and GAPD in RNA (15 μg) samples from (HOB−DEX) cells (lanes 1, 2) and (HOB+DEX) cells (lanes 3, 4). RNA was isolated from 10-day-old cultures after first passage and was processed as described under Materials and Methods. Autoradiographic exposure times were as follows: for ALP (2.5 kb), GAPD (1.0 kb) and COLL (5.4 and 6.4 kb), 24 h; for OC (0.6 kb), 48 h. OC mRNA was visible in (HOB−DEX) cells upon longer exposure (data not shown).

Effect of Long-Term Culture in Dexamethasone

The results of slot blot analyses of RNA from (HOB+DEX) and (HOB−DEX) cells are summarized in Table 1. Long-term culture of cells (i.e. 6–8 weeks) in dexamethasone significantly increased the steady-state mRNA levels for some of the osteoblastic markers. Indeed, steady-state mRNA levels for ALP and OC were observed to be higher in (HOB+DEX) cells compared with (HOB−DEX) cells ($p < 0.05$ and $p < 0.025$, respectively). The 1.2-fold increase in ALP mRNA levels corresponded with an observed 6.4-fold increase in ALP activities. Increased OC mRNA levels (1.35-fold) did not result in any corresponding significant change in OC production although protein levels appeared to be higher in (HOB+DEX) compared with (HOB−DEX) cells (Table 1). The effects of $1,25(OH)_2D_3$ on ALP and OC mRNAs or protein levels were not examined in this study.

There were no significant differences in the basal levels of adenylate cyclase activity between (HOB+DEX) and (HOB−DEX) cells (Table 1). However, PTH-stimulated adenylate cyclase activity was increased from 39.04 ± 8.39 to 347.62 ± 48.36 cpm/10^6 cells per 5 min, an increase of 8.9-fold, upon long-term culture in 10 nM dexamethasone ($p < 0.01$).

The data were further analysed to determine whether the expression of osteoblastic markers and their response to dexamethasone were dependent upon the age and sex of the bone donor.

Effect of Age on Marker Expression in (HOB−DEX) Cells

The effect of age on ALP, COLL and OC mRNA levels is shown in Fig. 2. Linear regression analyses of the data of each mRNA showed that in (HOB−DEX) cells there was no significant correlation between each osteoblastic marker mRNA level and donor age ($p > 0.05$). Figure 3 illustrates the effects of donor age on ALP and PTH-stimulated adenylate cyclase activities. ALP (Fig. 3A) and PTH-stimulated adenylate activities (Fig. 3B) were significantly lower in cells from older compared with younger donors. Indeed, linear regression analyses showed that there were significant negative correlations between both enzyme activities and donor age (for ALP, $n = 25, r = -0.40, y = 69.41 - 0.69x, p < 0.05$; for adenylate cyclase, $n = 41, r = -0.34, y = 6.00 - 0.061x$, $p < 0.05$). We previously reported age-dependent decreases in PTH-stimulated adenylate cyclase activities [19].

Effect of Age on Response to Dexamethasone

When the effect of age on the response to dexamethasone was examined, (HOB+DEX) cells from older donors appeared to express lower mRNA levels for ALP, COLL and OC compared with cells from younger donors (Fig. 4). However, this correlation did not reach statistical significance (Fig. 4; $n = 16, r = -0.30, y = 1.34 - 0.0050x, p > 0.05$). Figure 5 illustrates the effects of donor age on the responses of ALP (Fig. 5A) and

Table 1. Comparison of osteoblastic phenotypic marker expressions in (HOB−DEX) and (HOB+DEX) cells: effect of long-term culture in dexamethasone

1. mRNA levels[a]			
Cells	ALP	COLL	OC
(HOB−DEX)	0.94±0.03	0.99±0.05	0.94±0.07
(HOB+DEX)	1.12±0.06#	1.00±0.11	1.27±0.11*

2. Activity/protein levels[b]				
Cells	ALP	Adenylate cyclase		OC
		Basal	PTH	
(HOB−DEX)	40.93±5.58	14.32±4.55	39.04±8.39	13.56±2.92
(HOB+DEX)	262.49±42.58**	16.18±2.47	347.62±48.36**	17.17±3.56

Total RNA was isolated from (HOB−DEX) and (HOB+DEX) cells for slot blot analyses. OC production and the activities of ALP and adenylate cyclase were determined as described under Materials and Methods.

[a] mRNA levels (mean±SEM of duplicate determinations from cells of 24 and 16 subjects for (HOB−DEX) and (HOB+DEX) cells, respectively).

[b] ALP activity, expressed as nmol/mg protein per minute for the mean±SEM from cells of 25 subjects, was assayed in triplicate. Basal and PTH-stimulated adenylate cyclase activities are expressed as mean±SEM (cpm/10^6 cells per 5 min) from triplicate determinations of cells from 41 and 19 donors for (HOB−DEX) and (HOB+DEX) cells, respectively. OC production, expressed as ng/ml from the mean±SEM of media from cells from 8 subjects, was assayed in duplicate.

# $p < 0.05$ compared with (HOB−DEX); * $p < 0.025$ compared with (HOB−DEX), ** $p < 0.01$ compared with (HOB−DEX).

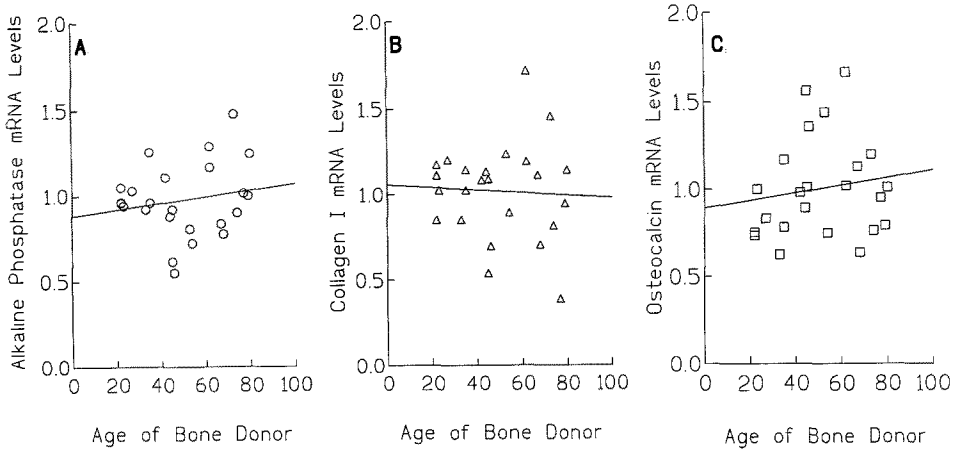

Fig. 2A–C. Linear regression analyses of ALP, COLL and OC mRNA levels in (HOB−DEX) cells v donor age. mRNA levels represent duplicate determinations of RNA isolated from 24 subjects. There was no significant correlation between marker mRNA levels and donor age.

Fig. 3A,B. Effect of donor age on ALP (**A**) and PTH-stimulated adenylate cylcase (**B**) activities in (HOB−DEX) cells. ALP and cyclase activities represent mean±SEM of triplicate determinations from cells from 25 and 41 subjects, respectively. There were 18 subjects in common. (**A**) ALP activity decreased with age ($n = 25$, $y = 69.41 - 0.69x$, $r = -0.40$, $p<0.05$). (**B**) Cyclase activity was expressed as PTH-stimulated/basal where basal = 14.29 ± 3.83 cpm/10^6 cells per 5 min. Cyclase activity decreased with donor age ($n = 41$, $y = 6.00 - 0.061x$, $r = -0.34$, $p<0.05$).

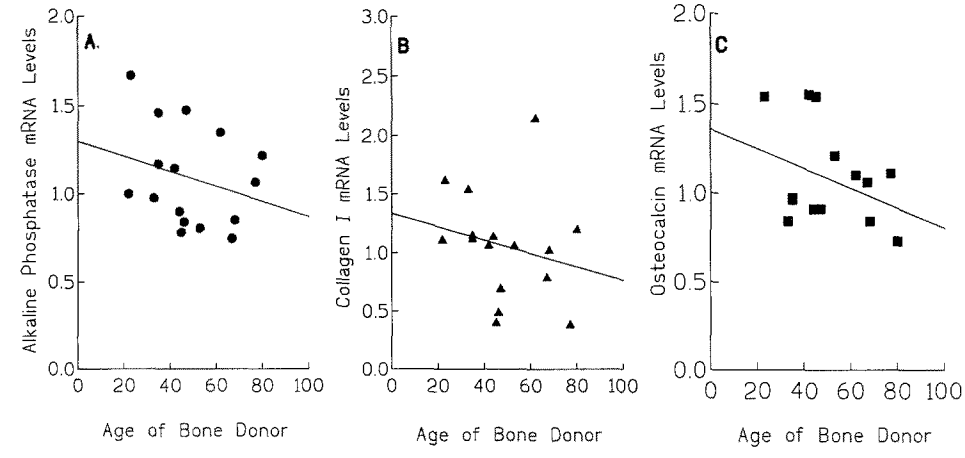

Fig. 4A–C. Effect of donor age on mRNA levels for ALP, COLL, and OC in (HOB+DEX) cells. mRNA levels represent mean ± SEM of duplicate determinations from cells from 16 of the 24 subjects mentioned in the legend to Fig. 2. There was no significant correlation between age and the expression of marker mRNAs in (HOB+DEX) cells ($n = 16$, $y = 1.32 - 0.0055x$, $r = -0.30$, $p>0.05$).

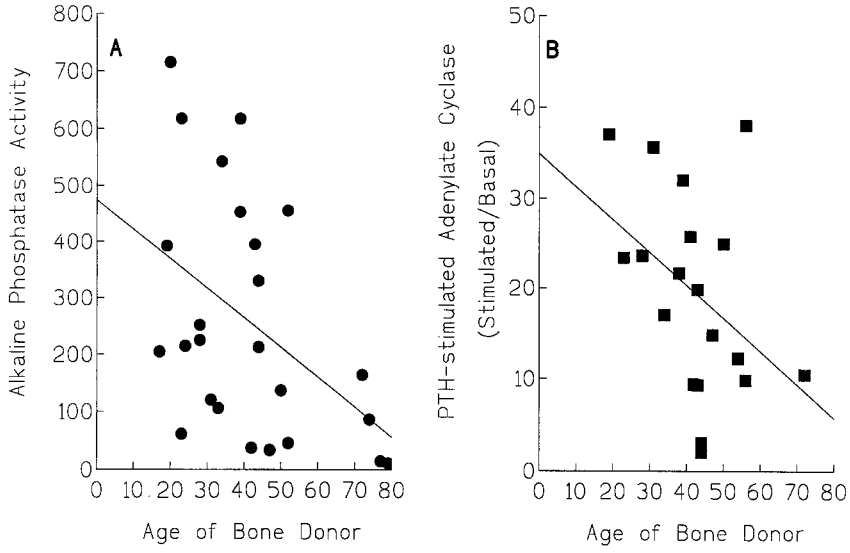

Fig. 5A,B. Effect of donor age on ALP (**A**) and PTH-stimulated adenylate (**B**) activities in (HOB+DEX) cells. ALP and cyclase activities represent mean ± SEM of triplicate determinations from cells from 25 and 19 subjects, respectively. Cells from these subjects were used for the data in Fig. 3. (**A**) ALP activity decreased with donor age ($n = 25$, $y = 474.02 - 5.22x$, $r = -0.46$, $p<0.025$). (**B**) Cyclase and basal activities were indicated in Fig. 3B. There was no significant correlation between donor age and adenylate cyclase activities in (HOB+DEX) cells ($n = 19$, $y = 34.94 - 0.36x$, $r = -0.41$, $p=0.065$).

PTH-stimulated adenylate cyclase (Fig. 5B) activities to long-term culture in dexamethasone. ALP activities were significantly lower in (HOB+DEX) cells derived from older donors compared with corresponding cells from younger donors. Indeed, linear regression analyses showed that ALP activity decreased sharply with age of the bone donor ($n = 25$, $r = -0.46$, $y = 474.02 - 5.22x$, $p < 0.025$). As can be seen by a comparison with Fig. 3A, this decrease was greater than that observed in (HOB−DEX) cells. However, when the data from older and younger donors were expressed as ratios of activities in (HOB+DEX) to those in (HOB−DEX) cells, no correlation was observed between these values and the age of the donors (data not shown), indicating that there were no significant differences in the magnitude of the ALP response to long-term culture in dexamethasone. Thus ALP activity was elevated approximately 6.4-fold by continuous culture in dexamethasone regardless of donor age.

Adenylate cyclase activities, expressed as ratios of PTH-stimulated to basal levels, were also lower in (HOB+DEX) cells from older donors compared with those measured in younger donors. Figure 5B illustrates that there appears to be an age-dependent decrease in PTH-stimulated adenylate cyclase activities in (HOB+DEX) cells, but this relationship was not statistically significant ($n = 19$, $r = -0.41$, $y = 34.94 - 0.36x$, $p=0.065$). This decrease appeared to be sharper than that found in (HOB−DEX) cells (comparison of the slopes of the regression lines: Fig. 3B and 5B).

When the data were examined with respect to the effect of the sex of the bone donors on the expression mRNA levels and enzyme activities) of the osteoblastic markers, no significant differences were observed in osteoblastic marker expression (data not shown).

Discussion

The results of our present study show that osteoblastic cells derived from iliac crest trabecular bone and cultured long term in dexamethasone ((HOB+DEX) cells) exhibited elevated PTH-stimulated adenylate cyclase and ALP activities compared with (HOB−DEX) cells. These findings confirmed our previous observation that (HOB+DEX) cells exhibit properties of a more differentiated phenotype compared with (HOB−DEX) cells [6]. Additional new findings in this study are that the steady-state mRNA levels of ALP and OC are higher in (HOB+DEX) cells compared with (HOB−DEX) cells, that the activities of PTH-stimulated adenylate cyclase and ALP in these cells are dependent upon the age of the bone donor, and that these age-dependent expressions are enhanced in cells cultured in dexamethasone. However, the contribution of cells other than osteoblasts in our cultures remains to be determined.

The effects of age on osteoblastic function reported in the literature are conflicting. Age-related decreases in the metabolic activity [2,29–32] and proliferative ability [15,29,31] of osteoblasts as well as in the number of osteoblastic progenitor [29,33] or mature osteoblastic [34] cells, have been documented. In cells derived from iliac crest and femoral head trabecular bone, cAMP responses to PTH challenge were reported to increase with the age of the bone donor [15], while the mitogenic response to growth factors [15] and the syntheses of proteoglycans [31] were reported to decrease with age. On the other hand, the effects of age on ALP activity and OC protein levels were controversial [2,15]. Others found no effect of age on osteoblastic metabolic activity [3,4,8]. Variations in culture conditions and osteoblastic

populations because of differences in the sources of bone (i.e. iliac crest v femoral [3,4,8,15] or vertebrae [2]) and bone donor populations (i.e. degenerative disc disease v traumatic bone fracture [2,3,4,8]) probably contribute to the discrepancies between our findings and those of others.

Our findings suggest that there are donor age-dependent changes in the metabolic function of osteoblasts in vitro. The extent of these changes appeared to be dependent upon the stage of osteoblast differentiation. Thus, greater age-dependent decreases were found in (HOB+DEX) as compared with (HOB−DEX) cells for all phenotypic markers. ALP activity levels in cells from older donors were significantly lower than those measured in cells from younger donors (Figs 3A, 5A). In (HOB+DEX) cells, this decrease in ALP activity appeared to correlate with the observed trend for an age-dependent decrease in ALP mRNA levels (Fig. 4), suggesting that in osteoblastic cells derived from older donors there may be a defect in the synthesis of ALP mRNA. Why a similar correlation does not appear in (HOB−DEX) cells is not known, but may be due to the greater heterogeneity of the culture population [2,5,6] in (HOB−DEX) as compared with (HOB+DEX) cells [6]. Linear regression analysis also indicated that there was an age-dependent decrease in the response of adenylate cyclase to PTH; this decrease may be due to age-dependent changes in the activity of the cyclase catalytic unit, in the levels of regulatory G proteins and PTH receptors or in the affinity of these receptors for the hormone. Studies using animal cells suggest that alteration in the levels of G proteins and not the catalytic unit are responsible for age-dependent decreases in adenylate cyclase activity [35–37]. That the number of PTH binding sites but not the affinity of these receptors for PTH also decreased in renal membranes from aging rats [38] indicates that the reduction in PTH receptor levels may also be another important factor to be considered. Further studies are required to determine which mechanism is responsible for the reduction in adenylate cyclase activity in human bone cells that we have found in this and previous studies [19].

In addition to the age-dependent decrease in enzyme activities, there was an age-dependent response of the osteoblastic cells to long-term culture in dexamethasone. We noted, however, that this was not observed with ALP. While osteoblastic cells derived from older donors exhibited decreased ALP activity, the magnitude of the dexamethasone-induced increase in ALP activity (6.4-fold) was similar in cells from both younger and older donors, indicating that the mechanism responsible for this effect was not altered by age. We have previously reported that the magnitude of the stimulation of ALP by $1,25(OH)_2D_3$ was also not affected by donor age [19]. The reason for the preservation of these responses with aging is not known, especially since age-dependent differences were observed with other parameters. mRNA levels of ALP, COLL and OC appeared to decrease with age in (HOB+DEX) cells (Fig. 4). We also observed that the magnitude of the dexamethasone-induced increase in PTH-stimulated adenylate cyclase activities was markedly lower in cells from older donors compared with younger donors (Fig. 5B). These findings suggest that osteoblastic cells from older donors were less responsive to glucocorticoid treatment. Possible explanations for this observation include the following: (1) the cells were at a stage of differentiation distinct from that found in cultures from younger donors, (2) there were lower numbers of the cell types responsive to dexamethasone, and/or (3) there is a decreased availability of osteoprogenitor [32,33] or matured [34] cells in bones from older donors. The effects of glucocorticoids on osteoblast phenotypic markers have been reported to be dependent upon a number of factors including the developmental stage of the osteoblast phenotype [12,39]. A fourth possibility is that cells from older donors responded less to dexamethasone because of decreased glucocorticoid receptor number or decreased receptor binding capacity. Although glucocorticoid receptors have been demonstrated in normal human osteoblast-like cells [12], the effect of age or dexamethasone treatment on receptor properties in these bone cells has not been studied. Receptor number has been reported to decrease with age in the liver [40,41] and testes [42] of the rat. However, others [43,44] found no evidence of age-related changes in glucocorticoid receptor numbers or receptor properties. Therefore, this hypothesis remains speculative but is supported by findings in other systems.

While many osteoblastic cell lines and models are available, the human trabecular bone explant technique is very popular [1–19], despite drawbacks which include its expense, labor-intensiveness and culture heterogeneity. It is currently the best system for obtaining primary human osteoblastic cells for the study of cell function in cells derived from bone specimens of subjects with and without metabolic bone diseases [6,17–19]. While there have been a number of studies reporting the effects of growth factors and hormones such as estrogen on human osteblastic function [7,9,10,11,16], the age of bone donors was not considered in these studies. Furthermore, cells from a number of patients of varying ages were often pooled to obtain results [9,10]. In the present study, we have shown that donor age is an important factor with respect to the metabolic function and the hormone responsiveness of osteoblasts in vitro. Furthermore, these age-dependent differences were enhanced in cells cultured long term in dexamethasone. Recently we reported that cells derived from osteoporotic subjects exhibited aberrant ALP responses to $1,25(OH)_2D_3$ [19] and PTH-stimulated adenylate cyclase response to dexamethasone [45] compared with age-matched control subjects. That these differences occurred in osteoblasts which already exhibit age-dependent decreases in metabolic function serves to emphasize the fact that abnormalities exist in osteoblasts derived from subjects with osteoporosis. In light of these observations and the age-related changes reported by others [2,15,29–34], the age of the bone

donor is clearly an important factor to be considered in all studies concerning osteoblastic function.

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