Three isolation techniques for primary culture of human osteoblast-like cells

A comparison

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The culture of osteoblast-like cells of human origin has become an important experimental model in bone biology. We report here a comparison and evaluation of three of the most widely used systems available today: bone marrow stroma cell cultures (BMSC), human osteoblast explant cultures (hOB) and osteoblast explant cultures from collagenase-treated bone (hOB^{col}). Cultures from 16 bone specimens obtained from various donors were established and their expression of the osteoblast phenotype were then compared in secondary cultures by use of biochemical markers. BMSC had the highest basal and 1,25-dihydroxyvitaminD$_3$ (1,25(OH)$_2$D$_3$)-induced alkaline phosphatase activities in all cell isolations, with levels approximately twice those in explant cultures. Basal osteocalcin secretion was low-to-undetectable in all cell cultures but was detected in 1,25(OH)$_2$D$_3$-stimulated cultures. BMSC produced half of the amount of osteocalcin synthesized in explant cultures. The BMSC cultures also synthesized the lowest amounts of type I collagen, whereas collagen type III synthesis did not differ significantly among the various cultures. When secondary cultures were treated with 100 nM dexamethasone in the presence of ascorbic acid (50 μg/mL) and ß-glycerophosphate (10 mM), cultures deposited calcium mineral into the cell layer within 2–4 weeks. PTH-induced cAMP formation was detected in only 5 of 15 isolations and no consistent isolation-dependent response pattern was seen. We conclude that BMSC cultures differ significantly from explant cultures obtained from the same bone specimen. However, all cultures represent cells which can differentiate further and induce mineralization of the extracellular matrix in response to osteoinductive drugs.

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During the past half century, the development of isolation and culturing techniques of cells has resulted in many bone cell culture models. Today, cells from both the osteoclastic and osteoblastic lineages can be maintained in culture and thus details of the regulatory processes involved in bone turnover can be studied at the cellular level. Osteoblast-like culture models are based on immortalized cell lines, often derived from sarcomas, or primary cultures obtained through explant culturing or enzymatic release of cells from bone (Majeska 1996). Mills et al. (1979) were the first to describe primary isolation of human-derived bone cells and since then several groups have reported methods for isolating cells from humans with a specific osteoblastic phenotype. Beresford et al. (1986) showed that cells grown from trabecular bone fragments displayed a marked osteoblastic phenotype. The expression of some of the osteoblastic markers in the bone-derived cells was compared to the expression in fibroblasts derived from skin and profound differences were found. Thus, cells derived from trabecular bone expressed high levels of alkaline phosphatase (ALP) activity and responded to calciotropic hormones. They also secreted osteocalcin into culture supernatants (Beresford et al. 1984). Robey and Termine (1985) used another method, where bone fragments were pretreated with collagenase to remove all cells, except those immediately adjacent to the bone surface before further culturing. This has been claimed to give a purer osteoblastic cell population (Robey 1995). Recently, it has become increasingly popular to utilize bone marrow cultures, the rationale being that the marrow stroma contains the precursors that differentiate into the mature osteoblast (Owen and Friedenstein 1988, Beresford 1989). These cultures are therefore thought to represent earlier stages of the osteoblast differentiation pathway and have been used to study factors that trigger the differentiation of these precursors into mature osteoblasts (Cheng et al. 1994a, Jaiswal et al. 1997). Moreover, bone marrow cultures are presumed to be more heterogeneous, containing precursors that may differentiate not only in the osteogenic direction, but also towards fat, cartilage and muscle (Owen and Friedenstein 1988, Beresford 1989).

Numerous articles describe the characteristics of each of these systems, but there is no report comparing the different isolation techniques. We wanted to define the differences at the cell population level between primary isolated osteoblast-like cells obtained by three of the commonest isolation techniques, and have therefore measured established markers of the osteoblastic phenotype in secondary cultures of these cells. The starting material in this study was cancellous bone, obtained from patients during orthopedic surgery. The three cell populations compared were all derived from the same specimens and therefore we here report differences in phenotypic expression based solely on the isolation technique.

Material and methods

Material

Alpha modification of Eagle's medium (α-MEM) was purchased from Bio Whitaker, Walkersville, MI, USA; penicillin and streptomycin (PEST), L-glutamine, phosphate-buffered saline (PBS) and trypsin-EDTA from SVA, Uppsala, Sweden; amphotericin-B from Life Technologies AB, Täby, Sweden and fetal calf serum (FCS), L-ascorbic acid, the ALP enzyme assay (kit no. 104-LL), the reagents for ALP histochemistry, ß-glycerophosphate, Histopaque 1077, isobutyl-methylxanthine (IBMX), human recombinant parathyroid hormone (1-34) (PTH), forskolin, dexamethasone and crude collagenase type IA from Sigma-Aldrich, Stockholm, Sweden. 1,25 dihydroxy vitamin D_3 ($1,25(OH)_2D_3$) was kindly provided by LEO, Ballerup, Denmark. The osteocalcin ELISA was purchased from Osteometer, Herlev, Denmark; the RIA for the procollagen type I carboxyterminal peptide (PICP), and the procollagen type III aminoterminal peptide (PIIINP) was from Farmos Diagnostica, Turku, Finland. The cyclic adenosine monophosphate (cAMP) RIA was obtained from NEN Life Science Products, Boston, MA, USA.

Patients

Cells from 16 patients (11 women, age range 18–82 years) were obtained by the procedure described below. The patients were treated for vertebral fractures (n 10), coxarthrosis (n 4) or ankle instability (n 1). The bone specimens were trabecular bone from the iliac crest (n 12) or the upper femur (n 4). The study was approved by the local ethics committee.

Cell isolation techniques

The specimens were briefly rinsed in PBS and then cut into small pieces (1–2 mm in diameter), which were rinsed thoroughly in PBS. The PBS, containing bone marrow cells, was collected and subsequently spun through a column of histopaque 1077 (250 × g for 30 min). The cells at the interface were pelleted, counted and seeded in 75 cm^2 culture flasks at a density of $8 \times 10^6/75\ cm^2$ flask. The bone fragments were divided into two equally large groups. One group was gently shaken with 2 mL crude collagenase at 37 °C for 2 hrs and then rinsed in PBS. The bone fragments and isolated cells were incubated in α-MEM supplemented with PEST (100 U/mL penicillin, 100 μg/mL streptomycin), amphotericin-B (0.5 μg/mL), L-glutamine (2 mM) and FCS (10%) and were left undisturbed for 4 days before the first medium change. The medium was thereafter changed once every week. When primary cultures were confluent, cells were detached with trypsin/EDTA and pelleted. The cells were reconstituted in 10 mL of α-MEM and carefully counted in a hemocytometer to ensure that an equal number of cells were seeded in the various experimental cultures. All the different secondary experimental cultures were then set up from the same cell solutions.

Total DNA assay

At the end of the experiments, cell supernatants were harvested and the cell layers were lysed with 1% SDS in EDTA (20 mM)/ Tris (10 mM) buffer.

Total DNA content in the lysates was analyzed by the method of Labarca and Paigen (1980). The fluorescence at 485 nm was measured when aliquots of the lysate were mixed with bisbenzamide in a high salt buffer. A standard curve was generated with salmon testis DNA.

Osteocalcin assay

Cells from each population were seeded in a 24-well culture plate at a density of 10^5 cells/well. The cells were allowed to settle for 48 hrs in a medium containing 10% FCS and then washed and incubated for 72 hrs with or without 100 nM $1,25(OH)_2D_3$ in a serum-free medium. After the experiment, the supernatants were collected and immediately frozen at –70 °C. Prior to analysis, the samples were lyophilized and dissolved in 120 μL assay buffer. 100 μL of each sample was used in the ELISA and the assay was performed according to the instructions of the manufacturer. The mean intraassay coefficient of variation (CV) was 16% and the interassay CV of the mean was 87%.

ALP activity assay

ALP activity in the cell layers of the various cell populations was measured by the specific conversion of p-nitrophenyl phosphate (p-NPP) into nitrophenol and quantified in a multititer spectrophotometer. Cells (5×10^4/well in 24-well plates) were stimulated with 100 nM $1,25(OH)_2D_3$ for 72 hrs in serum-free medium before the cell layers were washed with PBS and lysed in 1% Triton-X100 in PBS. The lysates were frozen and stored at –70° C, pending ALP activity determination. Prior to analysis, the lysate was freeze-thawed twice to release further the enzyme activity (Sabokbar et al. 1994). The enzyme reaction was set up by mixing 50 μL of lysate with 50 μL of substrate buffer containing 0.5 M 2-amino 2-methyl-1-propanol, 2 mM MgCl and 10 mM p-NPP at a pH of 10.3. The microtiter plate containing the enzyme reaction was incubated in a heated (37 °C) multititer spectrophotometer and absorbance at 405 nm was determined once a minute for one hour. The results were expressed as mOD/minute from the linear part of the enzyme reaction curve. Intraassay CV was 18% on average and the interassay CV of the mean was 115%.

Collagen propeptide assays

The PICP and PIIINP propeptides were measured in the supernatants obtained in the ALP-activity experiment according to instructions from the manufacturer. The supernatants were diluted 1:5 in water prior to analysis in the PICP assay. Intraassay CV was 15% on average and 25% for the PICP and PIIINP measurements, respectively. The interassay CV for the means in the 16 experiments were 40% and 76% for PICP and PIIINP, respectively.

cAMP assay

Cells were seeded in a 24-well culture plate at a density of 5×10^4 cells/well. The cells were allowed to adhere for 48 hrs in a medium containing 10% FCS, before they were rinsed and incubated for 30 min in serum-free α-MEM containing the phosphodiesterase inhibitor IBMX (0.2 mM). Test substances were added, and after 30 min the media were withdrawn. cAMP in the cell layers was extracted with 90% isopropanol at 4 °C for 24 hrs. The extracts were evaporated and reconstituted in assay buffer and cAMP was analyzed by RIA according to instructions in the kit. Intraassay CV was 24% on average and the interassay CV for the mean was 94%.

von Kossa mineralization assay

Cells were seeded in 6-well plates at a density of 10^5 cells/well, cultured in control medium (α-MEM supplemented with PEST, amphotericin-B, L-glutamine and 10% FCS) or osteoinductive (OI) medium (control medium containing ß-glycerophosphate (10 mM), dexamethasone (100 nM) and L-ascorbic acid (50 μg/mL). At various time intervals, the experiments were interrupted and the cell layers were washed with PBS, fixed in neutral formalin and then incubated in a 5% (w/v) silver nitrate solution for 60 min in UV light. The wells were then rinsed in water and neutralized with 5% sodium thiosulfate.

ALP histochemistry

Cells were grown on chamber-slides in the control or OI medium. The staining was performed according to the protocol in the kit. Cells were fixed for 30 sec in 60% citrate buffered acetone and then incubated with 0.25 mg/mL fast Blue RR salt in 0.1% naphthol

AS-MX phosphate at pH 8.6 for 30 min.

Statistics

Values are given as the mean (SEM). The statistical significance of differences between means was calculated by the t-test for paired samples. The mean marker level or activity for each culture was considered one observation.

Results

Culture characteristics

After the isolation procedure described above, the bone was distributed to 75 cm^2 culture flasks with, on average, 0.45 g bone tissue per flask. In a few cases, bone fragments obtained with or without the collagenase treatment were examined by histology, for comparison. In non-collagenase-treated bone, crypts and crevices were still cellular. This was also obvious macroscopically, since although extensively washed and cut into pieces 1–2 mm in diameter, patches of red bone marrow could still be seen in non-collagenase-treated bone. After collagenase treatment, the marrow was completely removed and the bone was macroscopically white. The marrow cell preparation yielded between 30 and 100 × 10^6 nucleated cells which were seeded at a density of 8 × 10^6 cells per flask. The cells were harvested when all cultures had reached confluence. The mean length of time for the primary cultures was 42 (2) (range 29–62) days and yielded on average 2.8, 3.6 and 3.0 × 10^6 of BMSC, hOB and hOB^{col} cells per flask, respectively. The difference between the hOB cell number and cells of the other cultures was statistically significant ($p < 0.05$ with the t-test for paired samples). We also repeatedly estimated the degree of confluence by inspection and found that hOBcol cells were slower than hOB cells to emerge from the bone chips and to reach a high degree of confluence. At 2 weeks of culture, the degree of confluence was estimated by the same observer to be on average 20%, 40% and 60% for the hOB^{col}, hOB and BMSC cultures, respectively. We also calculated the productivity of the cultures, by dividing the total number of cells obtained from each specimen by the culture time and the weight of the bone, and found that this measurement correlated with the age of the donor ($r = -0.70$; $p = 0.02$; Figure 1).

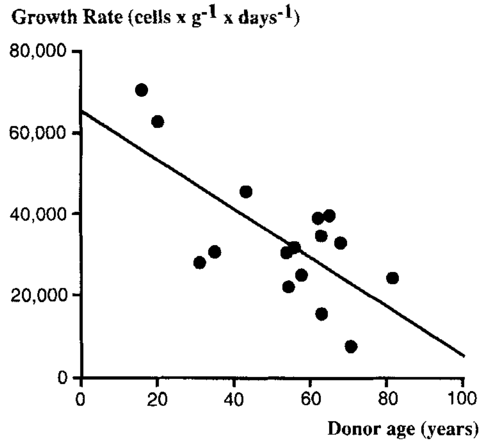

Figure 1. Correlation between donor age and culture growth. BMSC, hOB and hOB^{col} cells were isolated from 16 patients, as described in Methods. The cells were cultured until confluence. The total number of cells obtained from a bone specimen at the time of cell harvest was determined by counting in a hemocytometer. Cell numbers were divided by the size of the initial specimen and the culture time of the primary cultures and are shown here in relation to the age of the donor ($r = -0.70$, $p = 0.02$).

When the cells were passed to the experimental cultures, all possible care was taken to ensure equal cell numbers of the various cell types. However, since different plating efficiencies would result in differences in the number of cells in the experiments and introduce a major confounder, we measured the total DNA content in the cell layers after the experiments were harvested. The DNA contents were on average 73 (3), 81 (4) and 76 (5) µg/well for BMSC, hOB and hOB^{col}, respectively, representing no significant difference.

Osteocalcin synthesis

Basal synthesis of osteocalcin was low-to-undetectable. When it was detected, it was seen only in hOB and hOB^{col} cells (3 of 15 isolations). At 100 nM $1,25(OH)_2D_3$, osteocalcin was detected in all isolations and cell types. The levels were lowest in the BMSC cells in all isolations. BMSC cultures produced on average 43% of the amounts produced by explant cultures (Table). There was no statistically significant difference in osteocalcin secretion between hOB and hOB^{col} cells. However, hOB^{col} cells produced the highest amounts in 9 of 15 experiments.

Osteocalcin and collagen synthesis in primary isolated human bone cells

	BMSC	hOB	hOB^{col}
Osteocalcin (pg/1,000 cells)	147 (32)	336 (60) [a]	356 (66) [a]
PICP (ng/1,000 cells)	2.6 (0.3)	3.9 (0.4) [a]	4.3 (0.3) [a]
PIIINP (pg/1,000 cells)	37 (4)	28 (3)	30 (2)

BMSC, hOB and hOB^{col} cells were isolated from 16 patients, as described in Methods. The cells were cultured until confluence and then passed into 24-well culture plates at a density of 5×10^4 cells/well. In osteocalcin measurements, the cells were stimulated with 100 nM of $1,25(OH)_2D_3$ for 72 hrs. Osteocalcin in the supernatant was measured by ELISA. PICP and PIIINP levels in the supernatant were measured by RIA. The table shows mean levels (SEM) of cells from 16 different bone specimens.

[a] mean marker levels differ from the BMSC mean, $p < 0.01$ in the paired t-test.

Collagen synthesis

All cultures secreted high amounts of PICP into the culture medium. In all experiments, the mean level of PICP produced was lowest in the BMSC cultures. On average, the levels of PICP in BMSC cultures were 66% and 60% of levels produced in hOB and hOB^{col} cell cultures, respectively (Table). There was no statistically significant difference between the levels produced by hOB and hOB^{col} cells, although there was a trend towards higher levels in hOB^{col} cells. hOB^{col} cells secreted the highest amounts in 10 of 16 experiments. $1,25(OH)_2D_3$ had no significant effect on collagen synthesis in any cell type. The type III propeptide was detected in all cultures at a low level and there was no significant difference among the cell types (Table).

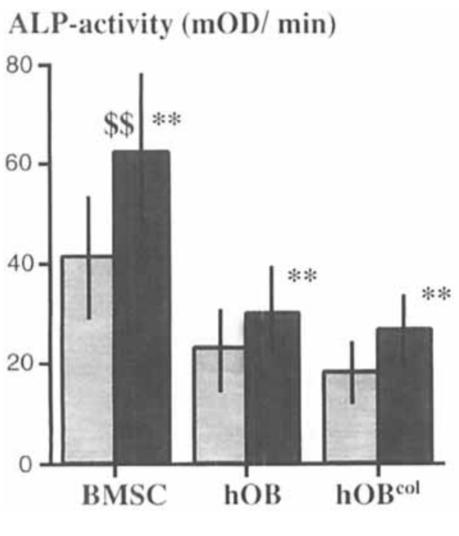

Figure 2. Alkaline phosphatase activity in primary isolated human bone cells. BMSC, hOB and hOB^{col} cells were isolated from 15 patients, as described in Methods. The cells were cultured until confluence and then passed into 24-well culture plates at a density of 5×10^4 cells/well. Half of the wells contained 100 nM of $1,25(OH)_2D_3$ and the cells were incubated for 72 hrs. ALP activity in the cell layer was measured spectrophotometrically, as described in Methods. Activity levels are expressed as mOD/min and the graph shows mean levels (SEM) of all isolations. ░ control culture, ■ 1,25(OH)2D3 (100 nM) culture. ** significantly different from control culture; $$ significantly different from hOB and hOB^{col} cultures, $p < 0.01$ in the paired t-test.

ALP activity

BMSC cells had the highest basal ALP activity (13 of 15 isolations) and $1,25(OH)_2D_3$-stimulated activity (14 of 15). The activity was on average 211% of the explant cultures as measured in the quantitative enzyme assay (Figure 2) and, as was seen in histochemical stainings, this was due to a higher percentage of ALP positive cells (data not shown). No significant difference was observed between the hOB and the hOB^{col} cells. The hOB^{col} cultures expressed significantly lower ALP activity than the hOB cultures in 7 of 15 isolations, whereas the reverse was true in 3 cases.

cAMP in response to PTH and forskolin

We detected a significant effect of PTH on cAMP accumulation in only 5 of 15 isolations and in those the effect was inconsistent the various cultures. However, there was a significant difference in the response to forskolin. BMSC cultures accumulated only 50% of cAMP levels in cells isolated from explants when stimulated with 10 μM of forskolin (Figure 3).

Mineral deposition

When first passage cells were cultured in the presence of ß-glycerophosphate (10 mM), ascorbate (50 μg/mL) and dexamethasone (100 nM) (OI-medium) for a prolonged period, calcium mineral was deposited in the cell layer of all cultures. This was usually observed after 4 weeks of culture but could, in some BMSC isolations, be detected after

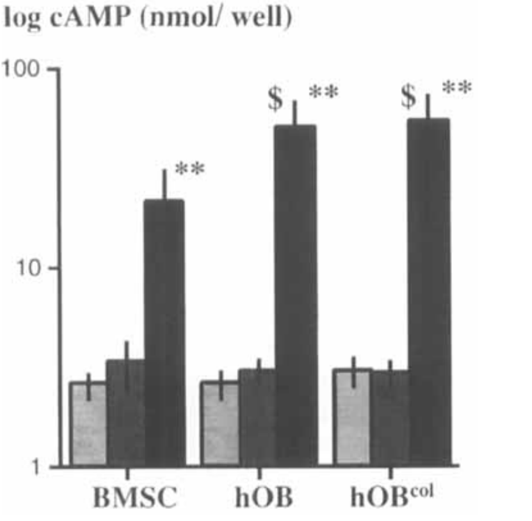

Figure 3. Accumulation of cAMP in the cell layer of PTH- and forskolin-stimulated cultures. BMSC, hOB and hOB^{col} cells were isolated from 15 patients, as described in Methods. The cells were cultured until confluence and then passed into 24-well culture plates at a density of 5×10^4 cells/well. When cells had adhered, they were preincubated with IBMX (0.2 mM) and then stimulated with ▒ control medium, ■ PTH (100 nM), ■ forskolin (10 µM). cAMP accumulation was measured by RIA, as described in Methods. ** significantly different from control culture, $p < 0.01$ in paired t-test.; $ significantly different from BMSC cultures, $p < 0.05$ in the paired t-test.

only 2 weeks (Figure 4). In half of the isolations, mineral deposits were detected in wells without dexamethasone after 4–6 weeks of culture. When this occurred it was most marked in the BMSC cells (Figure 4). No deposition occurred in cultures lacking both ß-glycerophosphate and dexamethasone. Parallel cultures were stained for ALP and it was clear that the difference in ALP activity detected at the beginning of secondary cultures was sustained in prolonged cultures and that OI medium strongly enhanced ALP activity (data not shown).

Discussion

The osteoblast is responsible for the production and mineralization of the organic matrix of bone. This cell has commonly been identified by histology and defined by morphological criteria, including cuboidal appearance, non-proliferative status and localization adjacent to newly formed bone (Holtorp 1990). However, these criteria are hardly applicable in systems of in vitro culture, since proliferation is a necessity and morphology is markedly changed. Moreover, no cell culture system described so far produces true bone tissue. Therefore the detection of the osteoblastic phenotype in cells propagated in culture must be based on other criteria. To this end, more or less specific biochemical markers have been established and shown to be expressed by osteoblasts in vivo (Majeska 1996). These include detection of the synthesis of bone matrix constituents, i.e., collagen type I and osteocalcin and activity of the bone-specific isoform of the enzyme alkaline phosphatase. The cells' ability to respond adequately to PTH and calcitriol are also strong indicators of the osteoblastic phenotype. In addition to detecting the osteoblastic phenotype in general, the markers may indicate the differentiation stage

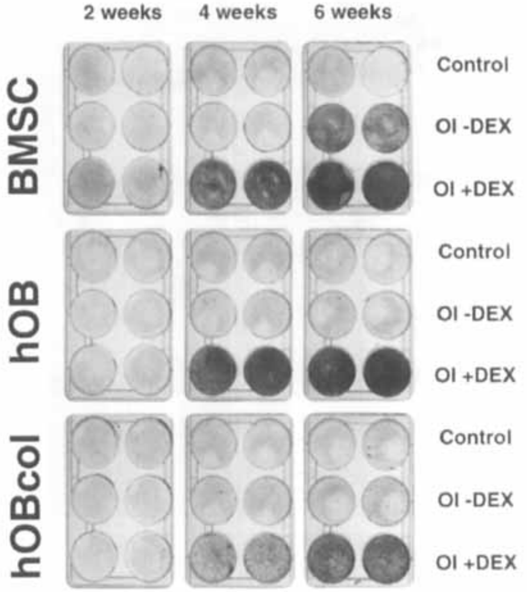

Figure 4. Deposition of mineral in of human bone cells cultured for a prolonged period. BMSC, hOB and hOB^{col} cells were isolated from 15 patients, as described in Methods. The cells were cultured until confluence and then passed into 6-well culture plates at a density of 10^5 cells/well, cultured for 7 days and then treated with OI-medium with or without dexamethasone or vehicle for the times indicated. Cells were fixed and stained for calcium deposits by the von Kossa method. The plates were then photographed and this figure shows representative results with cells from a 56-year-old male.

of the culture, since a temporal pattern of expression during differentiation has been described. Thus, in cultures of osteoblasts from fetal rat calvariae, the proliferation phase is coupled to collagen synthesis, followed by ALP activity and then osteocalcin secretion as the matrix mineralizes (Stein and Lian 1993). Using these markers, we have in this study, for the first time, shown that cells isolated from the adherent fraction of bone marrow differ phenotypically from cells grown from the trabecular bone containing the very same bone marrow. In a combined analysis of the markers, the patterns of these differences were the same in 16 consecutive isolations. The same pattern was observed regardless of patient gender, age or isolation site. Thus, the phenotype differences observed were consistent. This indicates that the isolation technique and therefore the localization of the precursors that produce the cell population is the main determinant of such differences. Although not unexpected, since the culture systems have been developed to study various aspects of the osteoblastic lineage, it remains an important observation since these two culture systems are used with increasing frequency to delineate expression patterns of gene products in relation to differentiation (Kassem et al. 1993, Cheng et al. 1994b, Kassem et al. 1994a, 1994b, Kimoto et al. 1994, Lecanda et al. 1997). On the basis of these data it may also be concluded that marrow stroma cells, remaining in the bone fragments despite extensive washing, rarely dominate the explant cultures because of a higher proliferation rate. If that had been the case, the explant and BMSC cultures would have been similar.

In these different polyclonal culture systems, the exact nature of the precursors of the confluent cells still remains unclear but must include different proportions of stem cells that are capable of differentiating into all mesenchymal lineages, partially committed precursors with various degrees of maturation, and mature non-proliferative cells that de-differentiate when placed in culture. With this in mind, we postulate that the isolation techniques used select for different sets of precursors. One hypothesis that could explain the differences between BMSC cultures and explant cultures is that the stromal cultures are mainly derived from partially differentiated osteoblastic precursors that acquire ALP positivity but also contain undifferentiated stem cells that produce low amounts of type I collagen, whereas the explant cultures are derived from mature osteoblasts or osteocytes that retain the capacity to secrete osteocalcin in response to $1{,}25(OH)_2D_3$. In this study, we found no differences between the hOB and hOB^{col} cells. However, trends in most markers point to a greater difference between the BMSC cultures and hOB^{col} cultures than between BMSC cultures and hOB cultures. It is therefore possible that the collagenase treatment increases the effectiveness of the washing step and removes marrow-derived precursors that to some extent influence the characteristics of the hOB cultures.

There was a large variation in the levels of markers between individual donors, as seen in the very high interassay CVs. In our material this variation could not be correlated to donor age, gender or isolation site and, apart from the variation that lies in the analytical methods, remains to be explained. However, we did detect a significant correlation between donor age and the number of cells produced by the cultures. Previous studies suggest that this is due to reduced responsiveness to growth factors and/or a reduced number of precursors available (Pfeilschifter et al. 1993, Fedarko et al. 1995, D'Avis et al. 1997).

One concern in our study is the effect of cell confluence. This factor has been known to influence differentiation of cells in general and, since we could not design our study to control for this, it may have influenced the results. Estimation of confluence by inspection is not an objective measurement, since it is a matter of personal opinion of the observer. However, collagenase treatment clearly slowed the cultures. It took longer for cells to emerge from the bone and the cultures lagged behind. The BMSC cultures were judged to reach confluence before hOB^{col} cultures. The cell number per unit area at passage into the secondary cultures is another measurement of confluence and hOB cultures contained significantly more cells per flask than did the other cultures. However, the main argument against this factor having influenced the results in any major way is that the difference in marker levels between hOB and hOB^{col} cells was small, and if present at all, was the opposite of what would have been expected if the cultures with the highest degree of confluence had been the most differentiated.

The one osteoblastic response that we found hard to detect was a clear cAMP response to PTH. The PTH receptors are known to be expressed on a subset of osteoblastic cells and our data therefore indicate that such cells are not enriched in these cultures (Turksen and Aubin 1991, Lee et al. 1994, McCauley et al. 1996). Others have generally found highly variable responses to PTH in human-derived cells and, compared to rat- and mouse-derived osteoblast-like cells, the response is usually weak (Peck et al. 1973, Auf'mkolk et al. 1985, Marie et al. 1989, Robey 1995, McCauley et al. 1996). Therefore the weak response to PTH seems a general feature in primary cultures of human osteoblast-like cells. This response may be increased by adjusting culture conditions and it is possible that the serum batch and lack of dexamethasone and ascorbic acid in the culture medium may be responsible for the failure to detect an overall significant effect in this study (Cheng et al. 1994a, Lennon et al. 1996, McCauley et al. 1996). Interestingly, the pattern of cAMP response to forskolin among the cultures was similar to type I collagen and osteocalcin patterns, indicating that the adenylate cyclase pathway may be quantitatively upregulated in differentiated osteoblasts.

We conclude that cells grown from explants have similar phenotypical characteristics regardless of collagenase treatment. These cells differ significantly from bone marrow stromal cells since they express lower levels of alkaline phosphatase, higher levels of type I collagen and vitamin D-stimulated osteocalcin secretion. All the cell cultures can mineralize matrix in vitro. We also demonstrate a clear influence of age on the rate of proliferation of these explants.

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