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Proliferation and Differentiation of Osteoblasts and Adipocytes in Rat Bone Marrow Stromal Cell Cultures: Effects of Dexamethasone and Calcitriol

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Abstract During bone loss, osteoblast population can be replaced by adipose tissue. This apparent reciprocal relationship between decreased bone density and increased fat formation can be explained by an imbalance in the production of bone-forming and fat-forming cells in the marrow cavity. Thus, osteoblast and adipocyte pathways seem more closely and inversely related. In the present study, we investigated the effects of dexamethasone (dex) and calcitriol [1,25$(OH)_2D_3$] on proliferation and differentiation of osteoblasts and adipocytes in rat bone marrow stromal cell cultures. Stromal cells were grown in primoculture in presence of dex and subcultivated in presence of dex and/or 1,25$(OH)_2D_3$. Total cell proliferation, osteoblast and adipocyte-cells number, and -mRNA specific markers were used to study the effects of hormonal treatment on stromal cells. Total cell proliferation was stimulated by dex and inhibited by 1,25$(OH)_2D_3$. Dex increased osteoblast and adipocyte cell population whereas calcitriol decreased bone-forming cell number and increased fat cell population. The presence of both hormones led to a strong decrease in osteoblastic cells and to a strong increase in adipocytic cell number. Dex induced mRNA osteoblastic markers expression like bone sialoprotein (BSP) and osteocalcin (OC) and an adipocyte marker expression, the fatty acid binding protein aP2. Calcitriol decreased the dex-induced BSP expression but stimulated slightly OC and aP2 mRNA. The effects of both hormones was to increase strongly OC and aP2 mRNA. These results support that, in rat bone marrow, adipocyte proliferation and differentiation are stimulated by glucocorticoids and calcitriol which act synergically, whereas osteoblastic cell proliferation and differentiation are increased by dex and inhibited by 1,25$(OH)_2D_3$. J. Cell. Biochem. 89: 364–372, 2003. © 2003 Wiley-Liss, Inc.

Key words: dexamethasone; calcitriol; bone marrow stromal cells; osteoblast; adipocyte; differentiation; proliferation.

Bone loss occurring in several cases, such as osteoporosis, age-related osteopenia, ovariectomy, immobilization, or glucocorticoid treatment, may be accompanied by an increase in marrow adipose tissue [Justesen et al., 2001; Verma et al., 2002]. The role of adipocyte in marrow is not yet understood but an excess of marrow adipose tissue is considered to be a significant negative risk factor for the long-term ability to maintain the mechanical strength of the skeleton [Nuttall and Gimble, 2000].

Bone histomorphometric observations suggested that a change in bone marrow stromal cell dynamics causes osteoporosis by replacing the functional bone-forming cell population with adipose tissue [Verma et al., 2002]. This apparent reciprocal relationship between decreased bone density and increased fat formation can be explained by an imbalance in the production of bone-forming and fat forming cells in the marrow cavity. So, an increase in the number of adipose cells could occur by replacing osteoblast population in osteopenic disorders.

Multipotential mesenchymal stem cells, present in bone marrow stroma, are able to differentiate into multiple cell lineages including myocytes, chondrocytes, osteoblasts, and adipocytes [Prockop, 1997]. Thus, osteoblast and

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adipocyte pathways seem more closely related. Several studies using a variety of stromal cells have demonstrated that the differentiation of adipocytes, chondrocytes, and osteoblasts can be influenced by hormones [Herbertson and Aubin, 1995; Locklin et al., 1995; Kelly and gimble, 1998; Thomas et al., 1999], cytokines [Gimble et al., 1994], and local growth factors [Gori et al., 1999; Chen et al., 2001; Ahdjoudj et al., 2002]. Factors that promote osteoblast differentiation are generally considered inhibitory for adipocyte differentiation and conversely [Beresford et al., 1992; Gori et al., 1999; Johnson et al., 1999].

Bone marrow stromal cells are known to be target cells for steroid hormones like calcitriol and glucocorticoids [Beresford et al., 1992; Herbertson and Aubin, 1995; Aubin, 1999; Atmani et al., 2002]. These hormones regulate osteoblastic cell proliferation, differentiation, and apoptosis [Cooper et al., 1999]. Glucocorticoids are known to be stimulatory for osteoblast and adipocyte differentiation [Beresford et al., 1992; Herbertson and Aubin, 1995]. Observations about effects of calcitriol on osteoblastic and adipocytic cell differentiation are more conflicting. It was reported that calcitriol stimulates [Liu et al., 1999] or inhibits [Lian and Stein, 1993; Atmani et al., 2002] osteoblastic differentiation. This hormone was also shown, in various cell culture models, to stimulate [Vu et al., 1996; Bellows and Heersche, 2001] or inhibit [Beresford et al., 1992; Kelly and Gimble, 1998] adipocyte differentiation. Osteoblasts can be identified by several markers like alkaline phosphatase enzyme (ALP), and the two matrix proteins, bone sialoprotein (BSP) and osteocalcin (OC). ALP and BSP are implicated in the mineralizing process of bone tissue [Ganss et al., 1999] and OC, the most specific marker of osteoblast, is involved in normal bone remodeling [Ducy et al., 2000]. The adipocyte fatty acid binding protein, aP2 (also called adipocyte lipid binding protein, ALBP) is involved in fatty acid storage, trafficking, and solubilization [Reese-Wagoner et al., 1999]. Its expression is associated with late stage of adipocyte differentiation [Ailhaud et al., 1992].

In this present study, we used cultures of marrow stromal cells isolated from rat long bones (femurs) to examine the effects of calcitriol and/or dexamethasone (dex) on proliferation and differentiation of adipocytic and osteoblastic cells.

MATERIALS AND METHODS

Reagents

Dulbecco's modified Eagle medium (DMEM), antibiotics (penicillin, streptomycin, amphotericin B, gentamycin), trypsin/ethylenediamine tetraacetic acid (EDTA), fetal calf serum (FCS), and cell culture disposables (6- and 24-wells plates, T75 flasks) were purchased from Life Technologies-GIBCO (Cergy Pontoise, France). Type IV collagenase, L-ascorbic acid 2-P, β-glcerophosphate, dex, and $1,25(OH)_2$ vitamin D_3 (calcitriol) were obtained from Sigma (Saint Quentin Fallavier, France), and [methyl-3H] thymidine was from Pharmacia (Orsay, France). Glutaraldehyde was obtained from Merck (Fontenay sous Bois, France) and hexamethyldizilasane from Acros Organics (Noisy le Grand, France).

For histochemical staining, naphthol AS-BI, fast blue BB salt, and osmium tetraoxide were purchased from Sigma. For RNA preparation and Northern blots, guanidine thiocyanate, formamide, formaldehyde, and salmon testes DNA were obtained from Sigma, nylon membranes from Appligene (Illkirch, France), agarose from Eurogentec (Herstal, Belgium). The DNA labeling kit was from Pharmacia and $[^{32}P]$-dCTP from NEN (Boston, MA). Rat OC (rOC) and mouse BSP (mBSP) cDNA probes were kindly provided by J. Aubin (Toronto, Ont., Canada), and the mouse aP2 (maP2) cDNA probe by Prof. Paul A. Grimaldi, Unité Inserm U470 (Nice, France).

Cell Culture

Isolation of rat marrow stromal cells. Rat bone marrow was prepared and cultured as previously described [Atmani et al., 2002]. Briefly, young adult males Wistar rats (3–4 weeks) were killed by cervical dislocation. Femurs were removed under sterile conditions and immersed in DMEM with antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin, 300 ng/ml fungizone). After removal of the bone heads, the marrow was collected by flushing repeatedly through the shafts with a syringe containing DMEM supplemented with antibiotics as above and 15% heat-inactivated FCS. Aggregates were removed from the cell suspension by sieving through a 70 μm cell strainer (Falcon, Grenoble, France). Cell number was determined using trypan blue staining with a Malassez cell.

Cell cultures. Cells were plated into 75-cm^2 tissue flasks at 10^6 cells/cm^2 density in DMEM medium containing 100 U/ml penicillin, 100 μg/ml streptomycin, 100 μM ascorbic acid 2-P, 10^{-8} M dex, and 15% FCS. DMEM supplemented by 100 μM of stable L-ascorbic acid 2-P was preferred to α-MEM that contains 50 μM of ascorbic acid which is unstable in solution at 37°C and neutral pH [Hata and Senoo, 1989]. Two days after plating, non-adherent cells were removed by two vigorous washings with phosphate buffer saline (PBS), pH 7.4. Thereafter, the medium was changed twice weekly until cell harvesting. After 7 days, primocultures were extensively rinsed with serum-free DMEM, and then incubated at 37°C for 2 h in serum-free DMEM containing 25 U/ml type IV collagenase and 2 mM $CaCl_2$. Cultures were then softly washed with PBS and treated by 0.1% trypsin–EDTA in PBS. After rinsing in PBS, cells were counted and plated in subculture conditions. All experiments were performed in subcultures. Cells, seeded at 5×10^3 cells/cm^2, were cultivated for 14 days in basic medium (DMEM supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 100 μM L-ascorbic acid 2-P, and 15% FCS) with different conditions: (i) 10^{-8} M dex, (ii) 10^{-8} M 1,25-$(OH)_2$ vitamin D_3, (iii) 10^{-8} M dex, and 10^{-8} M 1,25-$(OH)_2$ vitamin D_3, and (iv) hormone-free medium, but with hormone vehicle (0.1% ethanol) for control condition.

Cell Proliferation Assay

Proliferation was measured using [^{3}H] thymidine incorporation. Cells were cultivated in 6-well culture plates with a 0.5 ml medium volume per well. Six hours before harvesting, 1 μCi [methyl-3H] thymidine was added to each well. Then, medium was removed and cells were softly washed. Incorporated isotope was measured after trichloroacetic acid precipitation in a liquid scintillation analyser (Tri-carb 2300 TK, Packard, Meridien, CT). Measurements were done in triplicate for each condition at day 14 and results were expressed as percentage of variation versus control.

Histochemical Staining

After 14 days of subculture in six-wells plates, marrow stromal cells were rinsed three times with PBS and fixed with 3.7% formaldehyde in PBS for 10 min at 37°C. After extensive washing, cultures were processed for adipocyte or osteoblast characterization. Osteoblasts were identified by alkaline phosphatase histoenzymatic detection and adipocyte by osmium tetraoxide (OsO_4) histochemical staining of cytoplasmic lipid droplets. Although cell histoenzymatic detection of the alkaline phosphatase is not a specific marker of osteoblast, this staining is usually considered as representative for the osteoblastic population [Walsh et al., 2001]. For alkaline phosphatase staining, cells were incubated for 30 min at 37°C in Tris-buffer (pH 8.5) supplemented with 0.5 mg/ml of naphthol AS-BI phosphate and 1 mg/ml of fast blue BB salt. Presence of alkaline phosphatase was evidenced by a blue staining. For lipid droplets staining, cells were incubated for 30 min at room temperature in the presence of 0.25% (M/V) OsO_4 diluted in bidistilled water. Presence of lipid droplets was evidenced by brown round vesicles. All cell cultures were counterstained with 1% (M/V) solid nuclear red in bidistilled water. Osteoblasts were ALP-positive cells (ALP^+) and adipocytic cells were OsO_4-positive cells (OsO_4^+). Cells were counted in triplicate (3 well cultures, 20 running fields per well, 0.16 mm^2 per field) under light microscope (magnification × 400). For each condition, measurements were done on at least 300 cells per well. The percentage of ALP^+ and OsO_4^+ cells was determined and results were expressed as percentage of control.

SEM Study

Cells were fixed with 2.5% glutaraldehyde in 0.1-M cacodylate buffer (pH 7.4) for 30 min at room temperature, post-fixed in 1% OsO_4 in distilled water, for 30 min, dehydrated through a graded ethanol series, desiccated overnight in hexamethyldizilasane, and then carbon-coated by sputtering (Bal-Tec). Observations were made with a Jeol JSM 6301F scanning electron microscope.

RNA Preparation and Northern Blots

Total RNA extracts were prepared from cells plated at the initial density of 5×10^3/cm^2 into 75-cm^2 tissue flasks and cultivated in similar conditions as described above. For assay, cells were washed with PBS, pH 7.4, lysed with 6.8-mM guanidine thiocyanate, and RNAs were precipitated with isopropanol (v/v) and 2-M LiCl. Aliquots of RNAs (20 μg) were fractionated on 1.2% agarose formaldehyde gels. RNAs were transferred during 16 h by capillarity to 0.2 μm

nylon membranes and immobilized by baking at 80°C for 2 h. The membranes were prehybridized and hybridized at 42°C in 5 × Denhardt's solution, 5 × SSPE (solution of sodium phosphate EDTA), 50% formamide, 250 μg/ml salmon DNA testes with [^{32}P]-labeled cDNA probes for mouse aP2 (maP2), rat OC (rOC), mouse BSP (mBSP), and rat GAPDH (rGAPDH). The probes were ^{32}P labeled using a DNA labeling kit. The membranes were washed twice in 2 × SSC (solution of sodium citrate) and 0.1% SDS (sodium dodecyl sulfate) at room temperature, and twice in 0.1 × SSC and 0.1% SDS at 50°C. X-Omat AR radiographic films (Kodak, Rochester, NY) were exposed to the membranes, using two intensifying screens at −70°C.

Statistical Analysis

For assays in triplicate (proliferation, alkaline phosphatase bioactivity) and cell counts, means and standard deviations (SD) were performed using Systat software release 8.0 (SPSS, Inc., Chicago, IL). Significance was determined using the Student's *t*-test.

RESULTS

Osteoblast and Adipocyte Cell Observation

Based on histoenzymatical detection, cells presenting alkaline phosphatase activity were considered as osteoblast-like cells. Similarly, the OsO_4 staining was used to identify adipocytic cells which had numerous intracellular vacuoles containing lipid droplets. Light microscopy indicated that these culture conditions led to osteoblastic and adipocytic cell development (Fig. 1). As expected, ALP^+ cells showed strong membranous bioactivity and different morphologies (Fig. 1A). In adipocytic cells, OsO_4 stained lipid droplets, which were similar to those in brown fat tissue (Fig. 1B). Observations by scanning electron microscopy showed the flattened and globular appearance of osteoblastic cells (Fig. 1C), and a lot of lipid droplets in adipocytic cells (Fig. 1D).

Proliferation

Proliferation was measured, using ^{3}H thymidine incorporation, at day 14 of culture. This time point was considered in regard of the RNA expression of BSP, OC, and aP2, markers associated with late stage of osteoblastic and adipocytic cell differentiation.

At day 14, 10^{-8} M dex significantly increased proliferation of rat bone marrow cells in subcultures (+290% vs. control, $P < 0.001$) as measured by thymidine incorporation (Fig. 2A). In contrast, calcitriol at 10^{-8} M decreased cell proliferation slightly (−25% vs. control, $P < 0.05$), whereas the presence of both hormones had no significant effect on proliferation.

Adipocytic cells and osteoblastic cells were counted separately in control cultures as well in dex and/or calcitriol treated cultures. The numbers of adipocytic and of osteoblastic cells were determined by counting under light microscopy at day 14. All hormonal conditions stimulated adipocytic cell number {+180% with calcitriol, +660% with dex, and +1,200% in presence of both hormones versus control ($P < 0.001$ for all conditions) (Fig. 2B)}. For ALP^+ cells, the number was increased by +76% by dex but decreased by −78% by calcitriol which stopped the dex up-regulation (−94% versus control, $P < 0.001$ for all conditions) (Fig. 2C).

Expression of mRNA

Expression of mRNAs for the late differentiation markers of osteoblastic cells (OC, BSP) and of adipocytic cells (aP2) was determined at day 14 (Fig. 3). All these markers were not detected in absence of hormone. Dex strongly increased expression of BSP mRNA and slightly increased OC mRNA levels. Calcitriol did not induce expression of BSP mRNA but increased OC transcript levels slightly. However, calcitriol increased the stimulating effect of dex on OC mRNA expression, but decreased the stimulating effect of dex on BSP mRNA. For adipocyte differentiation, dex and calcitriol weakly enhanced each aP2 messengers and when they were associated, they strongly stimulated the expression of aP2 (Fig. 3).

DISCUSSION

Rat bone marrow stromal cells grown in medium supplemented with dex and ascorbic acid in primoculture, develop osteoblast-like and adipocyte-like cell populations [Herbertson and Aubin, 1995]. In the present study, cells were subcultivated in the presence of dex and/or calcitriol and long-term cultures develop osteoblastic and adipocytic cell phenotypes characterized by ALP histoenzymatic detection (for osteoblastic cells), lipid vacuole histochemical

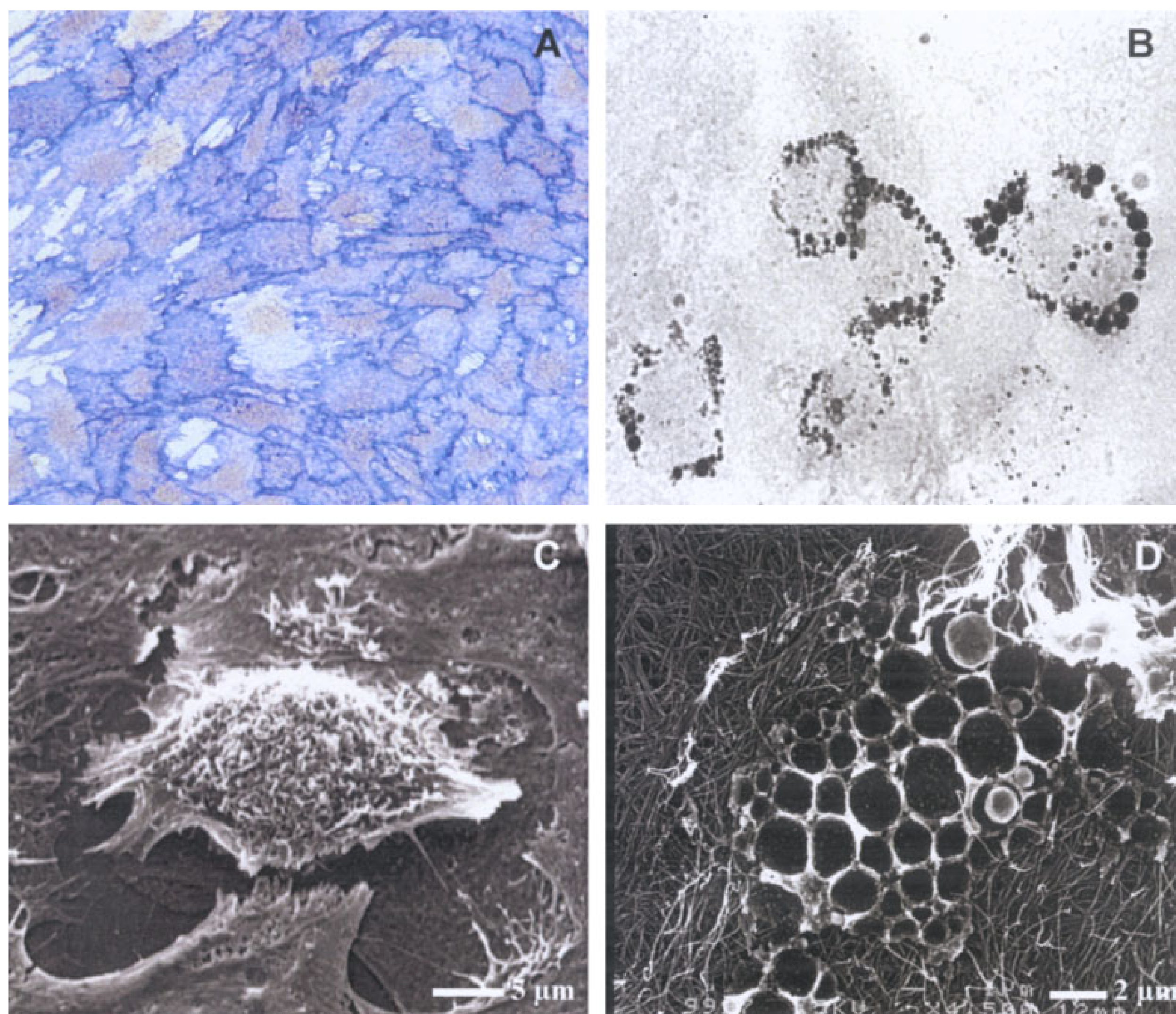

Fig. 1. Adipocyte and osteoblast. Rat bone marrow stromal cells were grown for 14 days in subcultures. Subcultures develop (**A**) alkaline phosphatase positive (ALP^+) cells and (**B**) adipocytic cells were characterized by their lipid vacuoles as shown under light microscopy. Observation in scanning electron microscopy showed different morphology between (**C**) osteoblastic cells and (**D**) adipocytic cells that contained several vacuoles with some resting droplets. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

staining (for adipocytic cells), and specific markers for mRNA expression.

Dex increased DNA synthesis significantly, as demonstrated by ^{3}H thymidine incorporation, whereas calcitriol decreased DNA synthesis and inhibited the stimulating effect of dex. Treatment with hormones, alone or in combination, increased the number of adipocytic cells and had a stimulatory effect on adipocytic differentiation, evidenced by aP2 mRNA expression. Similarly, the number of osteoblastic cells, characterized by their ALP activity, increased in presence of dex but decreased with $1,25(OH)_2D_3$ which blocked the stimulatory effect of dex. Moreover, dex, in contrast to calcitriol, stimulated osteoblastic differentiation, as shown by BSP and OC mRNA expression. These results indicated that dex increased the two populations of cells whereas calcitriol increased only the adipocytic population. Moreover, dex promoted both adipocyte and osteoblast cell differentiation whereas $1,25(OH)_2D_3$ stimulated only adipocytic cell differentiation.

It was demonstrated that glucocorticoids promoted differentiation of adipose precursors into mature cells in adipose tissue from several species, like human [Hauner et al., 1989], rat [Shinohara et al., 1991], mouse [Chapman et al.,

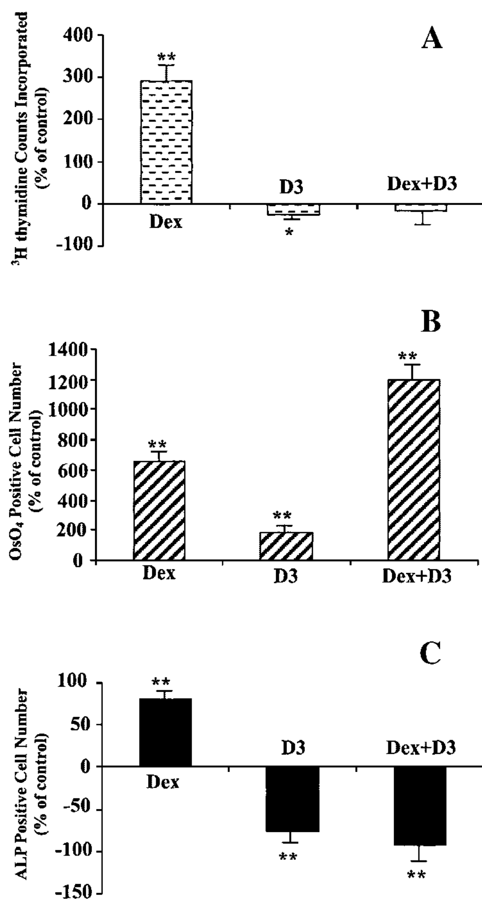

Fig. 2. Effects of dexamethasone (dex), calcitriol (D_3), or both on total marrow stromal cell proliferation, on OsO_4-positive (OsO_4^+) and ALP^+ cell numbers. Rat bone marrow stromal cells were grown in subcultures for 14 days. **A**: Total cell proliferation was estimated by ^{3}H-thymidine incorporation method. Counting of (**B**) adipocytic cells, identified by OsO_4 histochemical staining, and of (**C**) osteoblastic cells, identified by ALP histoenzymological detection was separately determined under light microscopy. Results were expressed as percentage versus control condition. $^*P < 0.05$ vs. control, $^{**}P < 0.001$ vs. control.

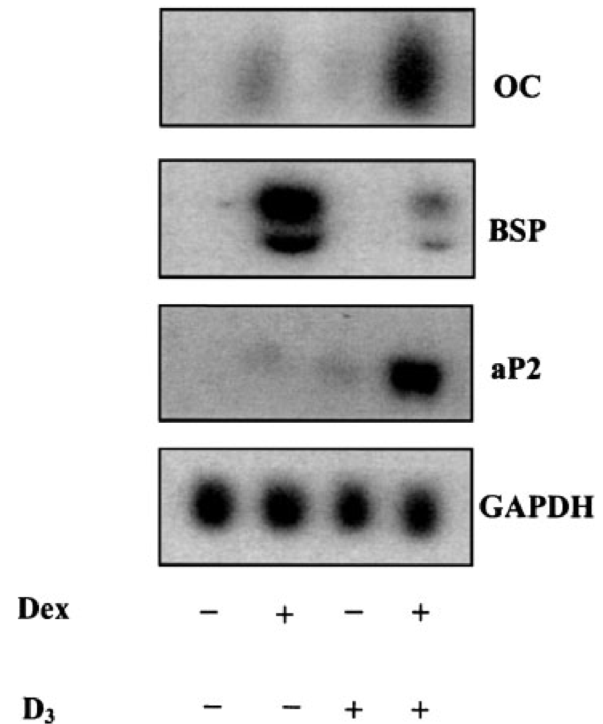

Fig. 3. Expression of osteoblast- and adipocyte-associated marker mRNAs. Rat bone marrow stromal cells were grown in primoculture for 7 days in the presence of 10^{-8} M dex and then subcultured for 14 days in presence of 10^{-8} M dex, or 10^{-8} M calcitriol (D_3) or both hormones. mRNAs were analyzed by Northern blot.

1985], rabbit [Nougues et al., 1993], and in human bone marrow stroma derived cell cultures [Locklin et al., 1999]. It is also known that glucocorticoids stimulate osteoprogenitor cell proliferation and differentiation in osteoblastic cell populations obtained from rat or human marrow cells [Aubin, 1999; Atmani et al., 2002]. The effects of $1,25(OH)_2D_3$ on osteoblastic differentiation show to be dependent on the stage of cell maturation. So, $1,25(OH)_2D_3$ inhibited osteoblastic differentiation of precusor cells [Owen et al., 1991] but had a stimulatory effect on mature cells. $1,25(OH)_2D_3$ increased expression of OC [Lian and Stein, 1993] and osteopontin [Chen et al., 1996] but decreased expression of BSP [Chen et al., 1996] and type I collagen [Rowe and Kream, 1982]. Reports of $1,25(OH)_2D_3$ on adipocyte differentiation are less clear. It has been described that calcitriol stimulates adipocyte differentiation in 3T3-L1 murine cells [Vu et al., 1996] and in fetal rat calvaria cells [Bellows et al., 1994; Bellows and Heersche, 2001]. Inversely, $1,25(OH)_2D_3$ has also been shown to inhibit adipocyte differentiation in cultures of 3T3-L1 murine cells lines [Kelly and Gimble, 1998], in bone marrow stromal cells isolated from mouse [Kelly and Gimble, 1998] and rat [Beresford et al., 1992]. These discrepant adipogenic effects of calcitriol could not be explained by differences in species or site of origin. Our results were obtained from male rat bone marrow. They are conflicting with reports using female mice [Kelly and Gimble, 1998] or female rat marrow stromal cells [Beresford et al., 1992], suggesting a possible role for animal gender.

The early step in the commitment of a mesenchymal progenitor cell to the adipocyte or to osteoblastic lineages depends on activation

and expression of the peroxisome proliferator-activated receptor $\gamma 2$ (PPAR-$\gamma 2$) and the Core Binding Protein α-1 (Cbfa1) also called Runx-2 [Wu et al., 1995; Ducy et al., 1997], respectively. Dex has been shown to stimulate expression of PPAR-$\gamma 2$ [Vidal-Puig et al., 1997] that is consistent with our results where dex increased adipocyte population and maturation. The increase in adipocytic cell population by calcitriol remains unclear. Some authors have shown that PPAR $\gamma 2$ is down regulated by $1{,}25(OH)_2D_3$ in 3T3-L1 cells [Hida et al., 1998] but previously others have described a stimulation of adipocyte phenotype in the same model, evidenced by calcitriol-induced lipoprotein lipase expression, a relatively early marker of adipocyte differentiation [Vu et al., 1996].

Dex has been shown to decrease Cbfa1/Runx-2 expression when added in proliferative phase [Viereck et al., 2002] but to increase osteoblast differentiation by stimulating osteogenic markers expression [Beresford et al., 1992; Lian and Stein, 1993; Herbertson and Aubin, 1995; Cooper et al., 1999; Atmani et al., 2002]. Calcitriol was shown to inhibit expression of Cbfa1 mRNA and protein in murine and rat cell lines [Drissi et al., 2002]. This is in agreement with our findings showing that calcitriol inhibits osteoblast differentiation from an early step. Moreover, other authors have reported that this hormone irreversibly inhibited osteoblast differentiation of rat fetal calvaria cells when added at the start of culture [Ishida et al., 1993].

Several reports have indicated a close relationship between adipocytic and osteoblastic lineages. Numerous factors were reported to inhibit osteoblast differentiation and to enhance adipocyte pathway and inversely [Beresford et al., 1992; Gori et al., 1999; Thomas et al., 1999; Lecka-Czernik et al., 2002; Okazaki et al., 2002]. Moreover, some studies reported that adipocyte cells can be induced to express osteogenic markers [Lecoeur and Ouhayoun, 1997] and osteogenic cells can express adipocytic phenotype [Nuttall et al., 1998]. These authors [Nuttall et al., 1998] suggested that these two lineages were derived from a common precursor. However, recently, Bellows and Heersche [2001] described, in fetal rat calvaria, that the large majority of osteoprogenitor are committed and restricted to the osteoblastic lineage and that the large majority of adipocyte progenitor are committed and restricted to the adipocyte lineage while the common osteoblast/adipocyte progenitor is present at low frequency. This common progenitor is suggested to be the source of the clonal cell lines that have been described to possess both adipogenic and osteogenic potential [Nuttall et al., 1998; Ahdjoudj et al., 2001]. However, Nuttall and Gimble [2000] favor the concept that plasticity can exist among cells of the stromal lineage and transdifferentiation of these late differentiated cells can still occur.

The role of adipocytes in bone marrow remains unclear. Multiple functions for these cells have been suggested (see review, Nuttall and Gimble, 2000), indicating that marrow adipocytes could sustain the function of other marrow cells and to serve as a source of energy, and of paracrine, or autocrine factors. Leptin, a protein secreted by adipose tissue, is implicated in the regulation of food intake and body weight by negative feedback at the hypothalamic nuclei. Recent studies have shown that human marrow stromal cells express leptin receptor and its activation enhances osteoblastic and inhibits adipocyte differentiation [Thomas et al., 1999]. Furthermore, it has been shown that dex increases leptin expression and secretion in human adipose tissue [Bradley and Cheatham, 1999] and in rat adipocytes [Williams et al., 2000]. Thus, stimulation of osteoblastic differentiation could be, in part, due to dex-stimulated leptin secretion. Inversely, it was reported that calcitriol inhibits leptin secretion by adipose tissue in adult humans [Menedez et al., 2001]. Thus, calcitriol-induced inhibition of leptin secretion could contribute to a decrease in osteoblastic differentiation.

Some authors showed recently, in a co-culture model, that mature adipocytes induce an inhibition of primary osteoblastic cell proliferation, probably mediated by factors secreted by adipocyte. They suggested polyunsaturated fatty acids as these factors [Maurin et al., 2002]. So, this inhibitory effect may contribute to the age-related bone loss. Furthermore, another coculture model showed that adipocytes, derived from murine bone marrow stroma, supported osteoclastic cell differentiation and function [Kelly et al., 1998]. In this way, adipose tissue seems to influence significantly the osteoblastic and osteoclastic cell differentiation and functions.

In conclusion, our studies showed that dex and calcitriol both enhance adipocyte differentiation and proliferation, whereas osteoblastic

cell proliferation and differentiation are enhanced only by dex in long-term rat bone marrow stromal cell cultures. The combining action of the two hormones strongly increases adipose population and differentiation but decreases the osteoblastic cell population.

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