Journal of Cellular Biochemistry 75:414–423 (1999)

Abnormal Osteogenesis in Osteoporotic Patients Is Reflected by Altered Mesenchymal Stem Cells Dynamics

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Abstract Bone marrow contains a population of mesenchymal stem cells with the ability to differentiate into cells that form bone, cartilage, adipose, and other connective tissues. Stem cells can be isolated from bone marrow aspirates and expanded in vitro. Presently, most stem cells studies have been performed in cells obtained from "healthy" control subjects. The goal of this study was to compare the functional characteristics of mesenchymal stem cells derived from "healthy" control and osteoporotic postmenopausal women to better understand the mechanisms involved in the pathogenesis of this disease. Osteoporotic and control stem cells have similar morphology and size and express similar cell surface antigens as evidenced by their reactivity with cell specific monoclonal antibodies. Mesenchymal stem cells from osteoporotic women differ from controls in having a lower growth rate than control cells, being refractory to the mitogenic effect of IGF-1, and exhibiting a deficient ability to differentiate into the osteogenic linage as evidenced by the alkaline phosphatase activity and calcium phosphate deposition. We conclude that in osteoporosis stem cell growth, proliferative response and osteogenic differentiation are significantly affected. Also, the study of mesenchymal stem cells from osteoporotic postmenopausal women may provide a better understanding of the mechanisms involved in the pathogenesis of the osteoporosis. It may also serve to test in vitro in rapid manner novel new therapeutic strategies. J. Cell. Biochem. 75:414–423, 1999.

Key words: mesenchymal stem cells; osteoporosis; osteogenesis in vitro; osteoprogenitor cells; IGF-1; differentiation

Bone formation is a complex process involving proliferation of pluripotent progenitor cells, their differentiation into osteogenic progenitor cells, migration of these cells to bone surface, and their differentiation into osteoblasts. This results in the secretion of abundant bone matrix proteins and the eventual calcification of bone extracellular matrix [Long et al., 1995; Lecanda et al., 1997].

It is well established that bone marrow stroma contains cells, known as mesenchymal stem cells (MSCs) that differentiate into bone, cartilage, fat, and a connective tissue. Recently, techniques for the isolation and extensive subcultivation of human MSCs have been developed [Haynesworth et al., 1992a,b; Caplan, 1991]. Conditions for optimal differentiation into specific mesenchymal cell lineages have not been fully established for all species studied. Yet, cultured expanded human MSCs have been demonstrated to be capable of differentiating along the osteogenic, chondrogenic, adipogenic, and marrow stromal lineages [Bruder et al., 1997].

Stem cells derived from the epidermis, gastrointestinal epithelium, and the hematopoietic compartment of bone marrow of human adults have been studied extensively [Bruder et al., 1997]. However, only recently the human population of MSCs has been studied in sufficient detail to advance our knowledge of the mechanisms involved in bone remodeling and regulation of osteogenesis. However, only few studies relate the origin of specific bone diseases with alterations in the dynamic response of bone cells progenitors [Gimble et al., 1996; Nutall et al., 1998].

Osteoporosis is characterized by a reduction in skeletal mass caused by an imbalance between bone resorption and bone formation. Loss of gonadal function and aging are the two most

Grant sponsor: FONDECYT; Grant number: 8970028.

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Received 1 February 1999; Accepted 29 April 1999

important factors contributing to the development of this condition. [Manolagas and Jilka, 1995]. Deficiency of the female hormone 17β-estradiol, caused by either menopause or removal of the ovaries, results in accelerated bone loss [Horowitz, 1993], which is associated with an increase in the rates of bone resorption and bone formation, with the former exceeding the latter, and an increase in the number of osteoclasts in the trabecular bone. Estrogens may also regulate the circuitry of cytokine action that control bone remodeling [Manolagas and Jilka, 1995].

Presently, there are multiple efforts to elucidate the origin and causes of osteoporosis and their therapeutic solutions. However, most of these in vitro studies have been performed using mature bone cells, osteoclasts, and/or osteoblasts. There are very few studies that analyze whether changes in the functional characteristics of progenitors cells of osteoclasts and osteoblasts, -GM-CFU, and MSC-, respectively, may contribute to pathogenesis of osteoporosis. There is only indirect evidence suggesting that changes in the functional characteristics of MSCs or changes in the regulation of the differentiation pathway may have important implications in some osteogenic disorders [Gimble et al., 1996; Nutall et al., 1998]. Thus, it has been postulated that age-related defects in osteoblast number and function may be due to quantitative and qualitative stem cell defects [Bergman et al., 1996]. It is now well accepted that plasticity of the stromal cell lineage may have important consequences in terms of formation of fully functional differentiated cells; this may be critical in the progression of bone diseases. Excess adipogenesis in postmenopausal women may occur at the expense of osteogenesis and, therefore, be an important factor in the fragility of adult bone [Nuttall et al., 1998]. Whatever the role of adipocytes in bone marrow, there is little doubt that adipogenesis increases as bone volume decreases. This phenomenon coupled to the possibility that osteoblast and adipocyte differentiation pathways are regulated jointly, suggests that marrow adipogenesis has important implications in osteogenic disorders [Nuttall et al., 1998].

We postulate that alteration in osteogenesis, as thus occur in osteoporosis, may be explained in part by changes in the functional dynamic response of progenitor bone cells. The objective of this study was to compare the functional dynamic response of MSCs derived from bone marrow of "healthy" control and osteoporotic postmenopausal women to better understand the mechanisms involved in the pathogenesis of this disease.

Our related interest was to obtain evidence that the study of MSCs could serve to understand the pathogenesis of osteoporosis. For this, we took advantage of the fact that it is possible to isolate and expand human MSCs in vitro, to study some functional dynamic responses of MSCs from osteoporotic patients. For these purposes, we obtained samples of bone marrow from "healthy" control and osteoporotic volunteers postmenopausal women (ages 65 to 75 years); we isolated and expanded MSC in cultures. In these cultures, we analyzed the proliferation rate, the differentiation of these cells into the osteoblastic lineage, and the effect of selected osteogenic growth factors, like IGF-1, on proliferation and differentiation.

MATERIALS AND METHODS

Subjects

Control and osteoporotic donors were selected from patients from the Traumatology Section, Hospital Sótero del Río in Santiago, Chile. Postmenopausal women (age range from 65 to 75 years) constituted both groups of bone marrow donors. "Healthy" women, without bone diseases, were selected to constitute the control group. Bone marrow was obtained by iliac crest aspiration during surgical procedures from control (four) and osteoporotic (four) donors after informed consent.

The diagnosis of osteoporosis was made by measuring bone mineral density using dual-energy X-ray absorptiometry (DXA; Lunar DPX-L, Software 1,3z, Lunar Corp., Madison, WI). DXA measures bone mass in the spine, the hip, and total body by scanning and filtering X-rays from a stable source.

For our purposes, osteoporotic donors were those showing a bone mineral density < 2.5 standard deviations (SDs) below the mean for young adults, and hip fracture. The bone mineral density for control donors ranged between a > -1.0 SD and < 2.5 SD [Raisz, 1997].

Cell Preparation and Culture Methods

MSCs were isolated from bone marrow as previously described [Jaiswal et al., 1997].

Briefly, 10 ml of bone marrow aspirate was added to 20 ml of Dulbecco's minimal Essential medium (DMEM; Sigma, St. Louis, MO) containing 10% fetal bovine serum (FBS) (culture medium), centrifuged to pellet cells, and discarded the fat layer. Cells were suspended in culture medium and fractionated on a 70% Percoll (Sigma) density gradient. The MSCs-enriched low-density fraction was collected, rinsed with culture medium and plated at 1–2 $\times$ 10^7 nucleated cells/100 mm dish (Nunc, Naperville, IL). Cells were cultured at 37°C in a humidified atmosphere of 5% CO_2. After 4 days in culture, non-adherent cells were removed and fresh culture medium was added. Culture medium was replaced by fresh medium twice weakly. When culture dishes became near confluent, cells were detached by a mild treatment with trypsin (0.25%, 5 min, 37°C) and replated at a 1/3 density for continued passaging. Experiments described here were performed after the fourth passage.

Cell Proliferation Assays

For cell proliferation studies, both control and osteoporotic MSCs were plated on 35 mm dishes at a density of 2 $\times$ 10^4 cells/dish (Nunc), and cultured in 1 ml of culture medium. At selected times, cells were detached by a mild treatment with trypsin (0.25%, 5 min, 37°C), and released cells were counted in a hemocytometer. Cell proliferation was measured in triplicate cultures. Also, plating efficiency of both types of cells was measured.

For this, cells were plated at a density of 2 $\times$ 10^4 cells/35 mm dish (Nunc), and cultured in 1 ml of culture medium. After 6 h in culture, non-adherent cells were removed, and the cells adhered to the culture dish were released from the plate and counted. The results are expressed as the percentage of adherent cells respect to the plated cells.

Alkaline Phosphatase Assays

Alkaline phosphatase activity associated to the cell layer was measured in culture, in the presence or in the absence of growth factors, using p-nitrophenyl phophate (Sigma 104 substrate) as substrate. For these purposes, cell layer was rinsed with TBS (20 mM Trizma Base, 150 mM NaCl, pH 7.5) and fixed with 3.7% formaldehide-90% ethanol solution for 30 seg at room temperature. Fixed cells were incubated with 1 ml of alkaline phosphatase substrate (1 mg/ml), in 50 mM sodium bicarbonate buffer (pH 9.6) containing 1 mM $MgCl_2$, at 37°C. After 20 min, the reaction was stopped with 0.5 ml of 3 N NaOH. The amount of product (p-nitrophenol) was measured at 405 nm. Enzymatic activity was expressed as p-nitrophenol produced (μmol/min $\times$ 10^6 cells) [Martínez et al., 1996].

Flow Cytometry Analysis

After the fourth passage, these cells are identified as MSCs by their reactivity with SH-2, -3, and -4 antibodies, as described previously [Haysnesworth et al., 1992a; Jaiswal et al., 1997]. SH-2, SH-3, and SH-4 antibodies recognize antigens on the cell surface of marrow-derived mesenchymal cells, but fail to react with marrow-derived hemopoietic cells. Also, these antibodies fail to react with the cell surface of osteoblasts or osteocytes, suggesting that the antigens recognized are developmentally regulated and specific for progenitor cells of the osteogenic lineage [Haysnesworth et al., 1992a].

In addition, differences in the pattern and/or in the extent of the reactivity of MSCs with these antibodies should reveal differential characteristics of MSCs from control and osteoporotic donors, related with the expression of specific antigens associated with the cell membrane.

Size, granularity, and reactivity of MSCs derived from control and osteoporotic donors to monoclonal antibodies SH-2, SH-3, and SH-4 were analyzed by flow cytometry. For these purposes, MSCs were cultured under the conditions described above. When cultures became near confluent, cells were detached by a mild treatment with trypsin (0.25%, 5 min, 37°C). Cells were incubated (30 min, at 4°C) at saturating conditions with monoclonal antibodies diluted (1:10) with 2% FBS-PBS, and sequentially incubated (30 min at 4°C) with fluorescein isothyocianate-conjugated goat anti-mouse immunoglobulin secondary antibody (Sigma), diluted 1:50 in 2% FBS-PBS. Cells were fixed with PBS-0.5% formaldehyde solution, and data was collected with a flow cytometer (FACScan, Becton Dickinson), and the analysis was performed using the LYSIS II program [Rodríguez et al., 1995].

Monoclonal antibodies SH-2, SH-3, and SH-4 were kindly provided by Dr. A. I. Caplan (Case Western Reserve University, Cleveland, OH).

Effect of IGF-1 on Cell Proliferation and Differentiation

IGF-1 is considered among the most important skeletal growth factors not only because they appear to be the most abundant factors present in bone [Price et al., 1994], but also because they have important actions on bone function [Canalis and Agnusdei, 1996]. Also, IGF-1 have been implicated as mediators of at least part of the effects on bone turnover of several hormones, growth hormone, estrogen, progesterone, parathyroid hormone; and the effects, among others, of cytokines (interleukin-1α and β) and bone morphogenetic proteins (BMP) [Langdahl et al., 1998].

Cells were cultured during 4 days in culture medium supplemented with different concentrations of IGF-1 (0; 5; 10; 25; and 50 ng/ml). At the end of the incubation period, cells were released by a mild treatment with trypsin and counted in a hemocytometer. In parallel experiments, the activity of alkaline phosphatase was determined, as a biochemical marker of differentiation. The activity was measured as described above.

Binding of IGF-1

Control and osteoporotic MSCs were cultured under conditions described above. When the culture reaches confluence, cells were transferred to a serum-free culture medium. After 24 h, cells were washed two times with PBS, and incubated during 4 h at 4°C with binding buffer (DMEM-1% BSA) supplemented with increasing concentrations of ^{125}I-IGF-1 (NEN, Boston MA, 287 μCi/μg) with or without unlabeled IGF-1 (Austral Biologicals, San Ramón, CA). At the end of the incubation period, cell layer was rinsed three times with cold binding buffer to remove unbound ^{125}I-IGF-1. ^{125}I-IGF-1 bound to cell layer was solubilized by treatment with 0.5 M NaOH. The contribution of non-specific binding to the total can be determined by measuring binding of ^{125}I-IGF-1 in the presence of a 100-fold excess of unlabeled growth factor [Takigawa et al., 1997].

Osteogenesis In Vitro

The ability of MSCs to differentiate into the osteoblastic lineage in vitro was evaluated measuring the activity of alkaline phosphatase, a marker of differentiation; and by the extent of calcium phosphate deposition on the cell layer. For this, MSCs were maintained during 16 days in culture medium supplemented with 100 nM dexamethasone, 10 mM β-glycerophosphate, and 50 μg/ml ascorbic acid added daily (osteogenic medium). On days 4, 8, 12, and 16 of culture, culture media was removed and alkaline phosphatase activity was measured as describe above. The crystals of calcium phosphate deposited on the cell layer were solubilized using a 0.5 N HCl solution, and the amount of calcium solubilized was measured by atomic absorption spectroscopy (423 nm).

RESULTS

Identification of MSCs

The results obtained by flow cytometry reveal that all cells isolated from bone marrow derived from control and osteoporotic donors exhibit a positive reaction with SH-2, SH-3, and SH-4 antibodies after the fourth passage. This strongly suggests that these cells correspond to the MSCs. This was confirmed by the ability of these cells to differentiate in vitro into osteogenic lineage. After the fourth passage the cell population in culture was highly homogenous, as suggests the fact that 100% of control and osteoporotic cells react with SH antibodies. The analysis of MSCs by flow cytometry also provides data about the size and granularity of cells. These data show that control and osteoporotic MSCs, expanded in vitro and analyzed after the fourth passage, behave as a single cell population in term of size and granularity, supporting the data obtained with SH antibodies. It is difficult to perform similar experiments in earlier passages or in the original bone marrow sample, because preliminary results show the presence of eight to 10 MSCs/1 $\times$ 10^6 mononuclear cells in the original bone marrow sample, measured as CFU-F assay.

Although, all the SH antibodies tested reacted with MSCs, minor differences were observed in the extent of the reactivity. Control and osteoporotic cells show the highest reactivity with SH-3 antibody as compared with all SH antibodies tested. But for both cell sources, the reactivity with SH-3 antibody is similar, suggesting that no important differences exist in the amount of cell surface antigens recognized by SH-3 antibody expressed by MSCs derived from control and osteoporotic donors.

The reactivity with SH-2 and SH-4 antibodies was similar in both groups. However, slight differences were observed in the reactivity of SH-2 and SH-4 with control and osteoporotic cells. Although the difference observed in reactivity with SH-2 and SH-4 antibodies was small, always we observed that normal cells exhibit a lower reactivity with SH-2 antibodies than osteoporotic cells (15–20%), and an opposite pattern with SH-4 antibodies.

Cell Proliferation

Human MSCs isolated from bone marrow derived from osteoporotic donors can be expanded in vitro as described previously for MSCs obtained from control donors [Haynesworth et al., 1992b; Bruder et al., 1997]. Osteoporotic cells show important differences in the proliferation rate as compared with control cells. While, MSCs obtained from osteoporotic patients show a constant proliferation rate during the culture period tested, MSCs derived from control donors show bimodal proliferation curve with two marked different proliferation rates. Thus, until day 7 of culture both types of cells show a similar and low proliferation rate. However, after day 7, while osteoporotic cells maintain a proliferation rate similar to the former seven days of culture, control cells increase their proliferation rate, as evidenced by the change observed in the slope of the growth curve (Fig. 1). After day 7 of culture, we can estimate a doubling time for control cell population of 72 h that is lower (three times) than those calculated for control cell population. On tenth day of culture, control cells cultures are reaching confluency, and cell proliferation is inhibited by cell to cell contact. On the contrary, osteoporotic cells cultures are in the logaritmic phase of growth and do not have physical restrictions to growth. By this reason, it is difficult to compare proliferation rates between control and osteoporotic cells after day 10.

At present, we do not have an explanation for this difference. This difference can not be explained by a difference in the plating efficiency, because both types of cells exhibit a similar plating efficiency, being, even slightly higher in the MSCs derived from osteoporotic donors than those derived from control donors. Thus, we observed that after six hours in culture 75.6 ± 7.9% and 63.5 ± 9% of osteoporotic and control MSCs adhere to the culture plate, respectively.

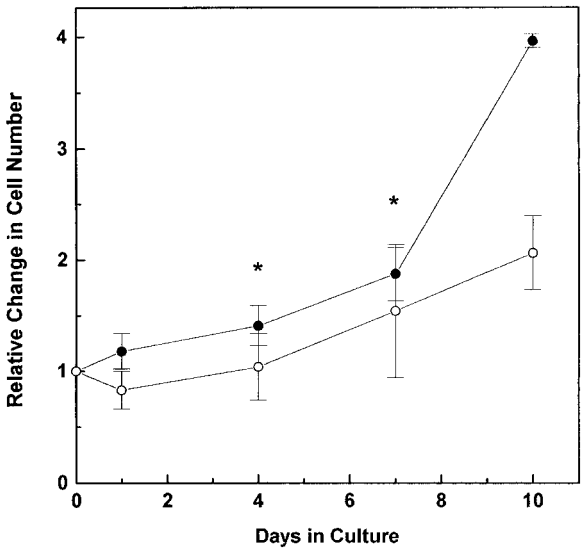

Fig. 1. Cell proliferation. MSCs are cultured as described in Materials and Methods. At selected times, culture medium is removed, cells are released by a mild treatment with trypsin, and counted in an homocytometer. Cell proliferation is expressed as the relative increase of cell number respect to the number of cells plated (2×10^4 cells/dish). The results are obtained from eight different donors: Four derived from control (closed circles) and four derived from osteoporotic donors (open circles). Each experiment is performed in triplicate. The results are expressed as mean ± SD ($P < 0.05$; $^*P > 0.05$).

Effect of IGF-1 on Cell Proliferation

MSCs derived from control and osteoporotic postmenopausal women were cultured during 4 days with increasing concentrations of IGF-1 (up to 50 ng/ml). At the end of this period, cells were released and counted in a hemocytometer. The results show a differential effect of IGF-1 on proliferation of cells derived from control and osteoporotic donors (Fig. 2). Thus, we observe that the presence of IGF-1 in the culture medium increases 2.5–3 times the number of MSCs derived from control donors. The number of cells derived from osteoporotic donors remains constant at the same concentrations of IGF-1 added to the culture medium. Figure 2 shows that IGF-1 exerts the mitogenic effect in a concentration dependent manner, but approximately 70–80% of the maximum proliferative effect is reached at relatively low concentrations of growth factor (below 10 ng/ml).

Effect of IGF-1 on Cell Differentiation: Activity of Alkaline Phosphatase

We observe that IGF-1 has a slight and nonsignificant effect into promoting the differentiation of MSCs to the osteogenic lineage in cul-

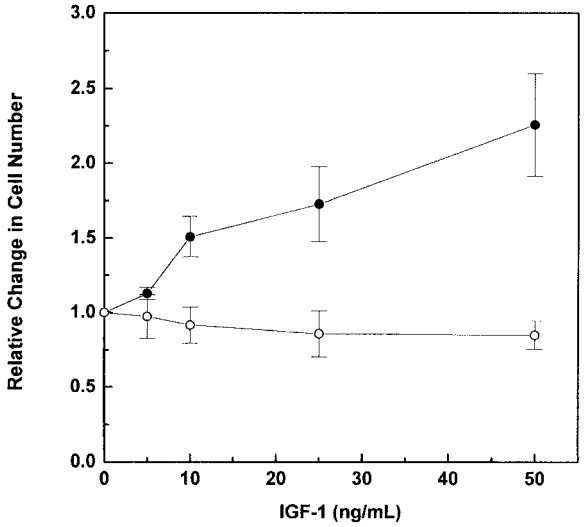

Fig. 2. Effect of IGF-1 on cell proliferation. Cells are incubated with different concentrations of IGF-1 (up to 50 ng/ml) during 4 days. After this, cells are released and counted in a hemocytometer. The results, at each concentration of IGF-1, are expressed as a relative cell number respect to cell number at 0 ng/ml of IGF-1. The results are obtained from eight different donors: Four derived from control (closed circles) and four derived from osteoporotic donors (open circles). Each experiment is performed in triplicate. The results are expressed as mean ± SD ($P < 0.05$).

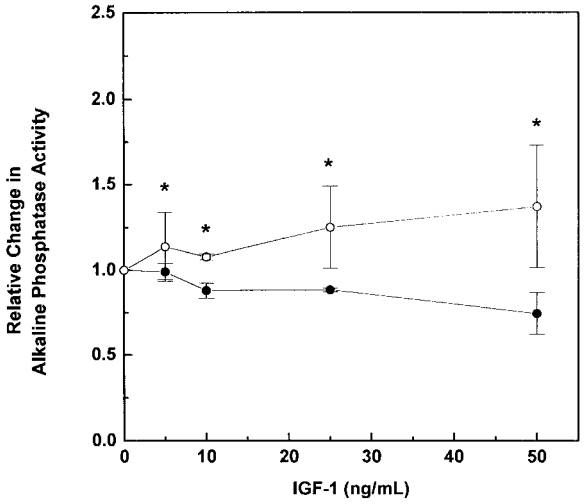

Fig. 3. Effect of IGF-1 on cell differentiation: Alkaline phosphatase activity. Cells are incubated with different concentrations of IGF-1 during 4 days. After this, the alkaline phosphatase activity is measured as a biochemical marker of differentiation. Enzymatic activity, at each concentration of IGF-1, is expressed in a relative manner respect to alkaline phosphatase activity at 0 ng/ml of IGF-1. The results are obtained from eight different donors: Four derived from control (closed circles) and four derived from osteoporotic donors (open circles). Each experiment is performed in triplicate. The results are expressed as mean ± SD ($P > 0.05$).

ture. This effect is in opposite direction that observed for the mitogenic effect. MSCs derived from control donors do not change the basal activity of alkaline phosphatase at the different concentrations of IGF-1 tested (up to 50 ng/ml), but MSCs obtained from osteoporotic donors increase 15–20% their alkaline phosphatase activity (Fig. 3). It is important to note that the effect of IGF-1 on the differentiation of MSCs is lesser than its proliferative effect. We also observed that the effect of IGF-1 on the differentiation of MSCs derived from osteoporotic donors is seen only at high concentrations of growth factor, reaching its maximum effect at 50 ng/ml. At low concentrations of IGF-1, below 10 ng/ml, where 70–80% of the mitogenic effect is expressed, no differentiation effect is detected. (Fig. 3).

Our results obtained in human MSCs are in accordance with other studies in human marrow stromal cells obtained from normal healthy donors [Langdahl et al., 1998], which also investigated the mitogenic and differentiation response of human osteoblasts-like cells and human marrow stromal cells to IGF-1. Human marrow stromal cells exhibited the same response to IGF-1 as those described by us. That is, IGF-1 exerts proliferative effects on human marrow stromal cells, but does not have a differentiation effect on these cells.

Binding of IGF-1

We studied the ability of MSCs to bind IGF-1 to evaluate whether the differential effects of IGF-1 on MSCs derived from control and osteoporotic postmenopausal women may be explained by changes in the amount of binding sites or by changes in their affinity to the growth factor. The results show that both, MSCs derived from control and osteoporotic women, bind IGF-1 with a similar affinity ($K_D = 5.7–6.5 \times 10^{-11}$ M), and both type of cells present a similar number of binding sites at the cell membrane (28,500–30,700 binding sites/cell). Thus, the differential effect de IGF-1 cannot be explained by differences neither in the number of binding sites for IGF-1 or in the affinity of these sites. This suggests the presence of specific factors that could modulate the activity of IGF-1 (i.e., IGFBP) or alteration in signal transduction may account for the differences observed.

Osteogenesis In Vitro

To analyze the ability of MSCs to commit to the osteoblastic lineage in vitro, progenitor cells were culture in osteogenic medium, as described in Materials and Methods. The presence of cells with alkaline phosphatase activity and with the ability to deposit calcium phosphate crystals on the cell layer strongly suggests that MSCs differentiate into the osteoblastic lineage. During the 16-days assay period, control MSCs sustain a significant increase in alkaline phosphatase activity, reaching the maximum value at day 12 in culture (2–2.5 times). After this, the enzymatic activity decreases. On the other hand, no effect was observed on the alkaline phosphatase activity of MSCs obtained from osteoporotic donors. Thus, during all the experimental period MSCs obtained from osteoporotic donors exhibit a constant and basal level of alkaline phosphatase activity. At different times tested, always MSCs from control donors exhibited five to 10 times more activity of alkaline phosphatase activity than MSCs from osteoporotic donors (Fig. 4).

We also detected differences in the ability of cultures of MSCs to induce the deposition of calcium phosphate crystals on the cell layer. Control MSCs cultured in osteogenic medium were able to induce a significant mineral deposition. This calcium deposition was more evident during the latter half of the culture period tested, increasing two to three times as compared with osteoporotic cells (Fig. 5). Cultures of osteoporotic cells do not show a meaningful of calcium phosphate calcium deposition.

These results strongly suggest that under the culture conditions used in these studies, only the MSCs isolated from control donors behave as cells from osteoblastic lineage, suggesting the differentiation of MSCs to osteoblastic lineage cells.

DISCUSSION

The results presented here show that MSCs derived from both control and osteoporotic postmenopausic women share some functional dynamic response but differ importantly in others. MSCs derived from osteoporotic donors can be isolated from bone marrow and expanded in culture as previously described for MSCs obtained from control human donors [Haynes-

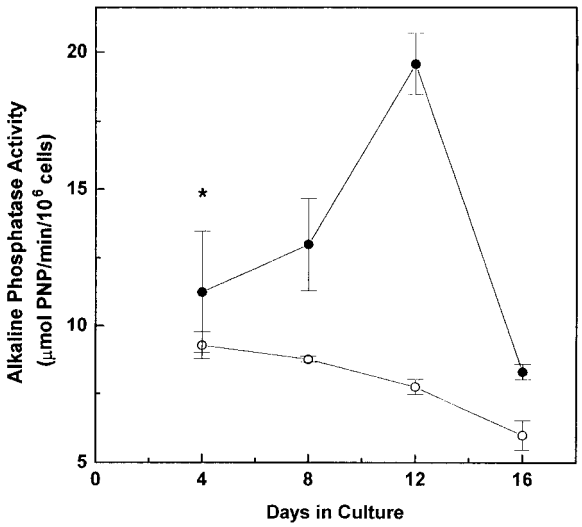

Fig. 4. Differentiation in vitro: Alkaline phosphatase activity. Cells are cultured in a culture medium supplemented with 100 nM dexamethasone, 10 mM β-glycerophosphate, and 50 μg/ml ascorbic acid. At days 4, 8, 12, and 16, the medium is removed, and alkaline phosphatase activity is measured as described in Materials and Methods. The results are obtained from eight different donors: Four derived from control (closed circles) and four derived from osteoporotic donors (open circles). Each experiment is performed in triplicate. The results are expressed as mean ± SD ($P < 0.05$; $^*P > 0.05$).

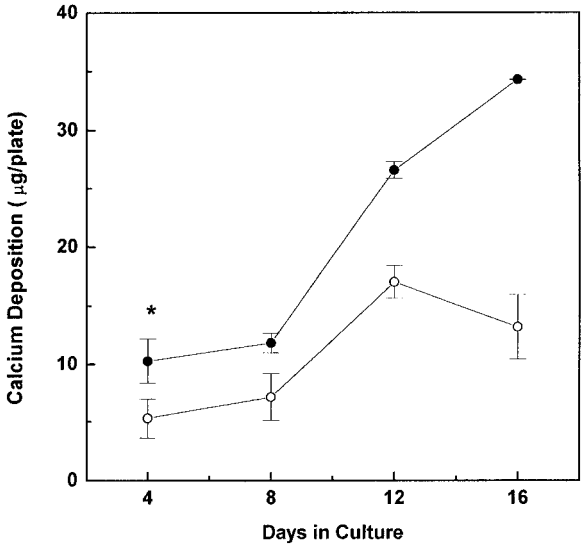

Fig. 5. Mineralization in vitro: Calcium phosphate deposition. Cells are cultured in a culture medium supplemented with 100 nM dexamethasone, 10 mM β-glycerophosphate and 50 μg/ml ascorbic acid. At days 4, 8, 12, and 16, the culture medium is removed, the crystals of calcium phosphate deposited on the cell layer solubilized, and the amount of calcium is measured by atomic absorption spectrometry. The results are obtained from eight different donors: Four derived from control (closed circles) and four derived from osteoporotic donors (open circles). Each experiment is performed in triplicate. The results are expressed as mean ± SD ($P < 0.05$; $^*P > 0.05$).

worth et al., 1992b]. MSCs isolated from osteoporotic donors express similar cell surface antigens, as evidenced by their reactivity with specific antibodies recognizing antigens present at the cell surface of progenitor cells [Haynesworth et al., 1992a].

However, these cells differ in important functional differences. Osteoporotic cells present a lower proliferation rate than control cells. Also, osteoporotic and control cells exhibit a differential response to IGF-1. While MSCs derived from control donors increase the cell number two to three times as a response to different concentrations of IGF-1, MSCs derived from osteoporotic donors do not change the cell number at all the concentration range of IGF-1 tested. The effect of IGF-1 on the differentiation process shows an opposite direction.

Another important functional difference observed in MSCs obtained from osteoporotic and control donors was their ability to respond to osteogenic stimulus differentiating into the osteogenic lineage. Thus, only MSCs derived from control donors cultured under osteogenic condition differentiate into osteogenic lineage as evidence the increased alkaline phosphatase activity and calcium phosphate deposition.

The results presented here strongly suggest that the MSCs isolated from the bone marrow of osteoporotic postmenopausal women behave differently from the MSCs derived from control postmenopausal donors. Some of the differences observed, like the differential mitogenic response to IGF-1 and the diminished ability of MSCs derived from osteoporotic donors to differentiate into the osteogenic lineage, suggest that these cells have a diminished ability to produce mature forming bone cells. This fact should be important to explain, in part, the decrease in bone mass observed in osteoporosis. In addition we think this methodology may serve to advance a molecular explanation for the differences observed in the functional characteristics of MSCs and may allow for the evaluation of specific therapeutic approaches to the treatment of osteoporosis.

At present, we do not have evidence to explain differences observed in proliferation rate. However, we know that the difference cannot be attributed to changes in the efficiency plating, because both types of cells adhere to the culture plate with similar efficiency.

The differential response of MSCs to insulin-like growth factor-1 appears important because insulin-like growth factors (IGF) are considered to be among the most important skeletal growth factors [Canalis and Agnusdei, 1996]. IGF should be considered important in the maintenance of bone mass, and alterations in the synthesis or activity of IGF may be relevant to the pathogenesis of osteoporosis and other metabolic bone disorders.

In this work we do not observe differences in the number and affinity of the binding sites for IGF-1 expressed by osteoporotic and control cells. Then, one must to look for alternative explanations in the presence of some molecules modifying the activity of IGF-1, i.e., IGF binding proteins (IGFBP), or in the modulation at the IGF-1 receptor-signaling pathway.

One could speculate that a decrease in IGF synthesis or activity may result in a decrease in bone mass. Then, we can assume that changes in selected IGFBPs may be involved in local regulation of IGF action in bone [Durham et al., 1994]. Thus, overexpression of some inhibitory IGFBPs, i.e., IGFBP-4, underexpression of some stimulatory IGFBPs, i.e., IGFBP-5, or the digestion of IGFBP-4 by specific serine protease could modify the rate of bone formation [Canalis and Agnusdei, 1996; Rees et al., 1998]. It is tempting to speculate that changes in the IGF/IGFBP axis in the skeletal cells or their precursors may be responsible for selected forms of osteoporosis.

The effect of IGF-1 also can be modulated at IGF-1 receptor signaling pathway. It is known that the phosphorilation of the IGF-1 receptor tyrosine kinase results in the stimulation of diverse intracellular pathways involving different signaling substrates. However, recent evidences show that the effect of IGF-1 can be inhibited modulating the IGF-1 receptor signaling, specifically by down-regulation of IGF-1-induced tyrosine phosphorilation of the IGF-1 receptor, or by an increase in membrane tyrosine phosphatase activity [Guvakova and Surmacz, 1997; Freiss et al., 1998]. Further experiments are necessary to elucidate the mechanism that may account of the differential effect of IGF-1.

Another important difference observed between osteoporotic and control cells is the distinct ability to differentiate to the osteogenic lineage in vitro. We can advance several different explanations to explain this difference.

Several evidences permit to conclude that marrow-derived mesenchymal stem cells are

the targets for endogenous osteoinductive molecules, such as bone morphogenetic proteins, BMPs [Kadiyala et al., 1997]. Although control mechanisms that orchestrate the proliferation, migration and differentiation of osteoprogenitor cells are still conjectural, evidence implicates BMPs as essential in this regard [Lecanda et al., 1997]. Recently it has been demonstrated that BMP-2 inhibits the proliferation but induce the differentiation of human osteoprogenitor cells [Lecanda et al., 1997; Fromigué et al., 1998]. These evidences suggest that changes in the expression or in the activity of molecules, like BMPs, should play an important role to explain the differential behavior of MSCs to proliferation and differentiation stimulus.

Because of the future potential use of BMPs in managing human bone diseases, there is a continued need to analyze the responses of control and osteoporotic osteoprogenitor cells to BMPs [Lecanda et al., 1997].

Currently, experiments are in progress in our laboratory to study the role that BMPs play in the differentiation of MSCs into the osteoblastic lineage, both in control and osteoporotic progenitor cells.

In order to understand the consequences that differ in the functional characteristics of MSCs may have in the pathogenesis of osteoporosis, also, it is important to consider the plasticity of MSCs to differentiate into several distinct cell lineages. Then, we can speculate that MSCs derived from osteoporotic donors may have a disminished ability to differentiate into the osteogenic lineage concomitant with an increased ability to differentiate to others cell phenotypes, like adipocytes. Thus, clinical and in vitro observations document an inverse relationship between adipocytes and osteoblasts. In osteoporotic patients, increased bone marrow adipose tissue correlates with decreased trabecular bone volume [Gimble et al., 1996]. Indeed, early histomorphometric observations suggested that a change in bone cell dynamics causing osteoporosis is the consequence of the adipose replacement of the marrow functional cell population [Meunier et al., 1971]. These findings suggest that a mechanism that could account for the decrease in bone volume, and hence mechanical strength, may result from opposing effects on differentiation of the two cell lines. The commitment to the adipocyte differentiation pathway occurs at the expense of osteoblast numbers and osteogenic function [Gimble et al., 1996; Nuttall et al., 1998]. In future studies, factors that control the balance between adipocytic and osteogenic differentiation need to be investigated [Bennett et al., 1991].

Specific inhibition of marrow adipogenesis and a concomitant enhancement of osteoblastogenesis of a common precursor cell may provide a novel therapeutic approach to the treatment of osteopenic disorders, like postmenopausal osteoporosis [Nuttall et al., 1998].

Results presented here show that MSCs derived from osteoporotic postmenopausal patients have important differences in functional dynamic responses when compared to control donors. This suggests that the characterization of progenitor bone cells may be a new approach to study the pathogenesis of osteoporosis. It may also allow for individual characterization of disease severity and response to treatment. It will be important to perform further studies to elucidate the molecular mechanisms that account for the differences observed in this study. The elucidation of these mechanisms could advance our understanding of the pathogenesis of this disease, and in the design of novel therapeutic strategies for the treatment of osteoporosis.

ACKNOWLEDGMENTS

The authors are grateful to Dr. R. Uauy for critical review of the manuscript and valuable comments. Also, the authors are grateful to Mrs. V. Simon, CEM-Universidad de Chile, for performing the flow cytometric analysis.

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