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Mesenchymal Stem Cells from Osteoporotic Patients Produce a Type I Collagen-Deficient Extracellular Matrix Favoring Adipogenic Differentiation

J. Pablo Rodríguez,* Luis Montecinos, Susana Ríos, Patricio Reyes, and Jorge Martínez

Laboratorio de Biología Celular, INTA, Universidad de Chile, Santiago, Chile

Abstract Mesenchymal stem cells (MSCs), precursor cells resident in the bone marrow, have the capacity to differentiate into bone, cartilage, fat, and connective tissue. We have recently reported that MSCs from "healthy" donors differ from cells obtained from osteoporotic postmenopausal women in their proliferation rate, mitogenic response to osteogenic growth factors, and potential to mineralize. The purpose of this study was to examine the factors that explain the differential capacity of MSCs derived from "healthy" control and osteoporotic postmenopausal women to support mineralization. In addition, we examined the factors that regulate the differentiation of osteoporotic cells into adipocytes. For this purpose, we isolated MSCs from bone marrow of donors and analyzed the synthesis and deposition of type I collagen, the main component of bone extracellular matrix, the time course of gelatinolytic activity expression, the deposition of transforming growth factor β (TGF-β), and the ability of cells to differentiate into adipocytes. Our results indicate that cells derived from osteoporotic donors synthesized 50% less type I collagen than normal cells and maintained higher levels of gelatinolytic activity under differentiation conditions (70% versus 15% after 14 days in culture). MSCs derived from osteoporotic women produced 60–65% less TGF-β and expressed higher adipogenic capacity. We conclude that the capacity of MSCs derived from osteoporotic postmenopausal women to generate and maintain type I collagen-rich extracellular matrix is decreased, favoring their adipogenic differentiation. These observations may explain the decreased mineralization previously observed in these types of cells. J. Cell. Biochem. 79: 557–565, 2000.

Key words: adipogenesis; gelatinase; mesenchymal stem cells; metalloproteinases; osteoporosis; type I collagen

Bone remodeling is a process involving proliferation of pluripotent progenitor cells, differentiation along the osteogenic lineage, migration of these cells to the bone surface, and finally their differentiation into osteoblasts. Osteogenic cells secrete matrix proteins that are organized as an extracellular matrix (ECM), where calcium phosphate is deposited as hydroxyapatite crystals [Long et al., 1995; Lecanda et al., 1997]. Osteoporosis is an age-related disease characterized by a reduction in bone mass resulting from an imbalance between bone resorption and formation [Manolagas and Jilka, 1995]. Research efforts to elucidate the origin and cause of osteoporosis could help to develop new therapeutic approaches. Most studies have focused on enhanced osteoclast activity as a key factor to explain the decrease of bone mass [Horowitz, 1993; Manolagas and Jilka, 1995]. Altered bone formation as a pathogenic factor is also being explored [Rodríguez et al., 1999]. Suppression of bone formation may result from decreased synthesis and secretion of specific bone matrix constituents (type I collagen) and/or an increase in the activity of specific proteases. Matrix metalloproteinases are synthesized and secreted by osteoblasts and progenitor cells [Panagakos and Kumar, 1995].

Most of these in vitro studies have been performed in mature bone cells, osteoclasts, and osteoblasts. Very few studies have analyzed whether changes in the functional characteristics of the osteogenic progenitor cells may be responsible for disorders such as osteoporosis [Gimble et al., 1996; Nutall et al., 1998, Rodríguez et al., 1999]. The bone marrow stroma contains cells, known as mesenchymal stem cells (MSCs), which differentiate into bone,

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*Correspondence to: J. Pablo Rodríguez, Laboratorio de Biología Celular, INTA, Universidad de Chile, Casilla 138-11, Santiago, Chile. E-mail: jprodrig@uec.inta.uchile.cl

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cartilage, adipocytes, or connective tissue [Caplan, 1991; Haynesworth et al., 1992a, 1992b]. However, human MSCs only recently have been studied in sufficient detail to define the mechanisms involved in normal bone remodeling and regulation of osteogenesis under health and disease conditions. Altered dynamic responses of bone cells progenitors may be responsible for specific bone diseases [Gimble et al., 1996; Nutall et al., 1998, Rodríguez et al., 1999].

We have recently demonstrated [Rodríguez et al., 1999] that the proliferation rate of MSCs from osteoporotic postmenopausal women is decreased, that the mitogenic response to osteogenic growth factors, such as IGF-1, is also decreased, as is their ability to differentiate into the osteogenic lineage and to mineralize an ECM. These results are in agreement with previous assumptions that age-related defects in osteoblast number and function may be caused by quantitative and qualitative stem cell defects [Bergman et al., 1996; Oreffo et al., 1998]. The plasticity of the stromal cell lineage may be important in the differentiation of progenitor cells and may be critical for the progression of bone diseases [Park et al., 1999]. Excessive adipogenic differentiation has been observed in the bone marrow of osteoporotic postmenopausal women. This may occur at the expense of the osteogenic differentiation, suggesting that the regulation of the differentiation pathway may be important in defining the balance between bone formation and adipogenesis [Gimble et al., 1996; Nutall et al., 1998].

Bone mineralization depends on the formation of a dense collagenous framework where calcium deposition take place [Bellows et al., 1986]. In consequence, it is important to study the characteristics of the collagenous matrix, to better understand the origin and development of bone diseases that result in decreased mineral density (like osteoporosis). Type I collagen and mineral deposition are dynamic processes, both components being continuously removed and replaced during normal bone turnover [Bailey and Knott, 1999]. In spite of this, the exact role that ECM plays in adipogenesis is not known; it has been demonstrated that an increase in adipogenesis seems to be associated with a decrease in collagen synthesis [Bortell et al., 1994]

The enzymatic degradation of the collagen matrix in bone resorption is associated with production and activity of specific matrix metalloproteinases (MMPs) [Heath et al., 1984; Partridge et al., 1987]. Interestingly, although type I collagen is the main component of bone ECM, the specific enzyme responsible for its degradation is virtually absent in human osteoblasts [Johansen et al., 1992]. However, it has been demonstrated that the activity and steady-state mRNA level of MMP-2 and MMP-9 are enhanced considerably during formation and maturation of ECM [Panagakos and Kumar, 1995]

The purpose of this study was to examine the factors that explain the differential capacity of MSCs derived from "healthy" control and osteoporotic postmenopausal women to support mineralization. In addition, we sought to examine the factors that regulate osteoporotic cell differentiation into adipocytes. For this purpose, we isolated MSCs from bone marrow of both types of donors. Cells in cultures were used to study for their capability to synthesize and deposit type I collagen and transforming growth factor β (TGF-β), the time course of the gelatinolytic activity expression, and the ability of both types of cells to differentiate into adipocytes. Our results indicate that cells derived from osteoporotic donors synthesize lower amounts of type I collagen than cells from controls and that they also maintain high levels of gelatinolytic activity under basal or differentiation conditions. Furthermore, MSCs derived from osteoporotic women produce lesser amounts of TGF-β and express a higher adipogenic capacity than cells from control women. Results also suggest that MSCs derived from osteoporotic women produce an ECM deficient in type I collagen, and therefore are unable to sustain proper mineralization. Osteogenic differentiation blockage derived from the commitment of MSCs to the adipogenic phenotype in the bone marrow of osteoporotic patients may represent an important pathogenic mechanism for the progressive loss of bone mass.

MATERIALS AND METHODS

Subjects

Control and osteoporotic donors were selected from patients admitted to the Traumatology Section, Hospital Sótero del Río in Santiago, Chile. Both groups consisted of postmenopausal women similar in age (range, 65–75 years); none had endocrine diseases nor were receiving hormone replacement therapy.

As control group, we selected "healthy" women, which in addition to the conditions mentioned above, did not have evidence of bone diseases. Bone marrow was obtained from control and osteoporotic donors after informed consent by iliac crest aspiration during surgical procedures that these patients were undergoing as a part of their treatment.

The diagnosis of osteoporosis was made by measuring bone mineral density using dual-energy x-ray absorptiometry in the spine, hip, and total body. Osteoporotic donors were defined by a bone mineral density <2.5 SDs below the mean for young adults plus hip fracture [Raisz, 1997]. Bone mineral density for control donors ranged between <-1.0 SD and <2.5 SD of the standard.

Cell Preparation and Culture Methods of MSCs

MSCs were isolated from bone marrow as previously described [Jaiswal et al., 1997; Rodríguez et al., 1999]. Briefly, 10 ml of bone marrow aspirate was added to 20 ml of Dulbecco's Minimal Essential Medium (Sigma, St. Louis, MO) containing 10% fetal bovine serum (FBS) (culture medium), and centrifuged to form pellet; the fat layer was discarded. Cells were resuspended 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, nonadherent cells were removed and fresh culture medium was added. Culture medium was replaced by fresh medium twice weekly. When cultures became almost confluent, cells were detached by mild treatment with trypsin (0.25%, 5 min, 37°C) and cells were replated at one-third density for continued passaging. The experiments described here were performed after the fourth passage.

Differentiation of MSCs

Differentiation of MSCs into either the osteoblastic lineage or adipocytes was evaluated as follows. For osteoblastic differentiation, cells were maintained during 14 days in culture medium supplemented with 100 nM dexamethasone, 10 mM β-glycerophosphate, and 50 μg/ml ascorbic acid added daily (osteogenic medium). The degree of differentiation was evaluated measuring the alkaline phosphatase activity and by the extent of calcium phosphate deposition on the cell layer [Rodríguez et al., 1999]. At selected times (0, 7, and 14 days), culture medium was collected and MMP-2 activity, type I collagen, and TGF-β produced by the MSCs were measured.

Adipogenic differentiation was assessed in cells cultured in a medium supplemented with 1 μM dexamethasone, 100 μg/ml 3-isobutyl-1-methylxanthine (IBMX) (adipogenic medium) during 14 days. Cells were stained with oil Red O for 30 min, rinsed briefly in 60% isopropanol, and counterstained with hematoxylin [Nuttall et al., 1998].

Zimogram for MSCs Interstitial Gelatinase

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis zymograms were prepared with 0.15 mg of gelatin per milliliter of gel. Serum-free conditioned medium samples from MSCs derived from control and osteoporotic donors (days 0, 7, and 14) subject to basal and osteogenic conditions were applied to the gels and subjected to electrophoresis. All samples were normalized for cell number. Gels were washed twice with 2.5% Triton X-100 for 30 min with mild shaking at room temperature and incubated in 50 mM Tris-HCl (pH 8.0), containing 10 mM calcium chloride and 50 mM benzamidine at 25°C, for 16 h. Gels containing gelatin were stained with 0.25% Coomassie blue R-250 in 50% methanol and 7.5% acetic acid for 30 min, and destained with 10% methanol in 7.5% acetic acid. The proteolytic bands were visualized by negative destaining, and the extent of the reaction was evaluated by reflectance values obtained with an imaging densitometer (Bio-Rad, Model GS-670) [Santibañez et al., 1996; Rodríguez et al., 1998]

Synthesis of Type I Collagen

Cells were cultured for 1 week until confluency. Afterwards, the medium of the confluent cultures was changed to culture medium supplemented with 50 μg/ml β-aminopropionitrile and 50 μg/ml of ascorbic acid for 24 h. The medium was then collected and the amount of type I collagen released to the culture medium as well as the collagen deposited in culture plates was measured by dot blot.

Collagen produced by MSCs from control and osteoporotic donors was measured after 14 days of culture. Cells were released from the

plates with a rubber policeman, suspended in 0.25% acetic acid, and sonicated at 23 kHz. The supernatant of each sample was incubated for 48 h at 4°C in a shaking bath to solubilize collagen. Samples were neutralized with PBS 10×, and collagen was measured by dot blot [Santibañez et al., 1996]

Dot blots were performed with a Bio-Dot microfiltration apparatus. Aliquots of a standard solution of type I collagen (concentrations ranged from 1 to 100 ng/ml) and experimental collagen samples were blotted onto 0.45-μm nitrocellulose membranes (Bio-Rad). After blocking with 1% bovine serum albumin (BSA) and 0.5% Tween-20, the membranes were incubated with monoclonal antibody anti-type I collagen (C2456, Sigma) diluted 1:750 in 1% BSA-PBS, for 1 h at room temperature. Membranes were rinsed three times with PBS-Tween and incubated with the secondary antibody, alkaline-phosphatase-conjugated goat-antimouse IgG (Calbiochem, La Jolla, CA), at a 1:500 dilution in 1% BSA-PBS for 1 h, at room temperature. The membranes were incubated with 5-bromo-4-chloro-3-indolylphosphate/tetranitroblue tetrazolium (BCIP/NBT) (Calbiochem, La Jolla, CA).

The extent of the reaction of the monoclonal antibody was evaluated by the reflectance values obtained with an imaging densitometer (Bio-Rad, Model GS-670), which converts the color intensity developed by the alkaline phosphatase reaction into digital data [Rodríguez et al., 1998].

Determination of Type I Collagen Propeptide

Type I collagen carboxyterminal propeptide (CICP), which reflects type I collagen production, was measured in the culture medium conditioned by control or osteoporotic MSCs. Cells were cultured for up to 14 days and, at selected times (0 and 14 days), cells were cultured for 24 h in Dulbecco′s Minimal Essential Medium, without FBS. The medium was collected and CICP was measured using an enzyme immunoassay (Metra Biosystems, Mountain View, CA).

Transforming Growth Factor-β Measurements

MSCs from control and osteoporotic donors were cultured in culture medium or osteogenic medium. TGF-β was measured at days 0 and 14 after confluency and after 14 days of culture in osteogenic medium. TGF-β produced by three different culture dishes was pooled and measured as follows: Cell monolayers that were subjected to basal and osteogenic conditions were disrupted with a lysis buffer containing 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM NEM, 1 ng/ml leupeptin, 1 ng/ml pepstatin, 0.02% aprotinin 0.02%, and 1 mM NaF in NP-40 0.1%. Aliquots (50 μl) of normalized proteins were applied in a dot blot apparatus and vacuum dried. Membranes were blocked with 1% BSA and incubated with chicken anti-TGF-β antibody (R & D Systems Inc., Minneapolis, MN) at 1:100 dilution for 1 h at room temperature. Membranes were rinsed and incubated with a peroxidase coupled secondary anti-chicken antibody for 1 h at room temperature. Finally, the membrane was revealed with BCIP/NBT (Calbiochem, La Jolla, CA). The reaction was quantitated by the reflectance values obtained with an imaging densitometer (Bio-Rad, Model GS-670), which converts color intensities into digital data [Rodríguez et al., 1998]. Cell numbers were evaluated in parallel dishes cultured under the experimental conditions described above.

RESULTS

Synthesis of Type I Collagen

As shown in Figure 1A, MSCs derived from both control and osteoporotic donors synthesized type I collagen. However, the amount of collagen synthesized by control cells was higher (1.3–2.0 times) than type I collagen produced by cells derived from osteoporotic donors. These results agree well with measurements of type I collagen carboxyterminal propeptide in conditioned medium of short- or long-term cell cultures (Fig. 1B). Both normal and osteoporotic cells decreased type I collagen synthesis after 14 days in culture. However, synthesis of type I collagen decreased more markedly in osteoporotic than in control cells (60% versus 40%) (Fig. 1B).

Deposition of Collagen Type I

Results presented in Figure 2 indicate that the amount of type I collagen deposited by MSCs derived from control donors is twofold greater than that deposited by MSCs from osteoporotic patients. Decreased amounts of type I collagen deposited by osteoporotic-derived MSCs correlates with decreased synthesis of this protein in these type of cells (Fig. 1A).

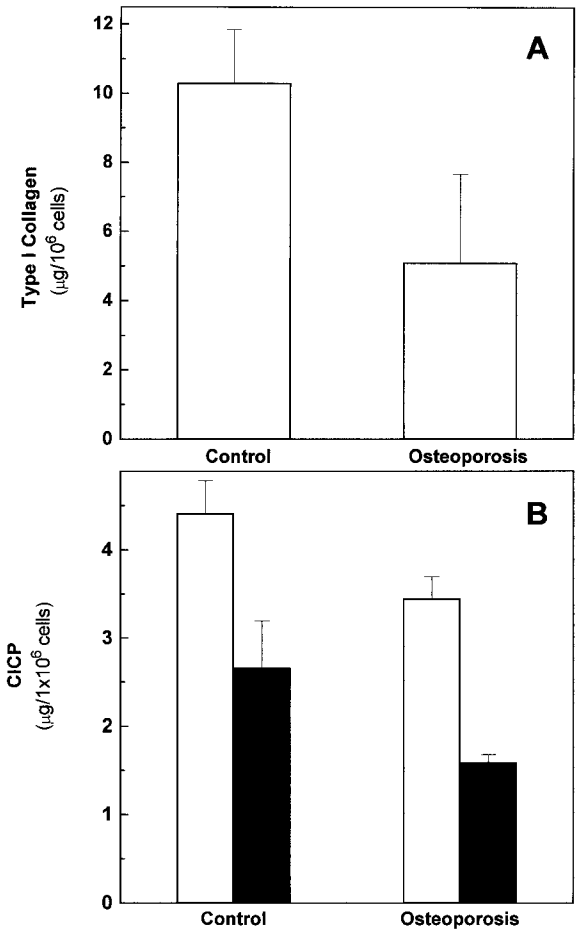

Fig. 1. Synthesis of type I collagen. MSCs from control and osteoporotic donors were cultured as described in Materials and Methods. Each experiment was performed in triplicate and type I collagen (**A**) produced in three different culture dishes and present in the culture medium was measured by dot blot using a specific monoclonal antibody. The amount of collagen was express as micrograms per 10^6 cells (**B**). The carboxyterminal propeptide of type I collagen (CICP) was measured using an enzyme immunoassay at 0 (open bar) and 14 (gray bar) days after confluency. Results were obtained from four different donors: two controls and two osteoporotic. Results were expressed as mean ± SD.

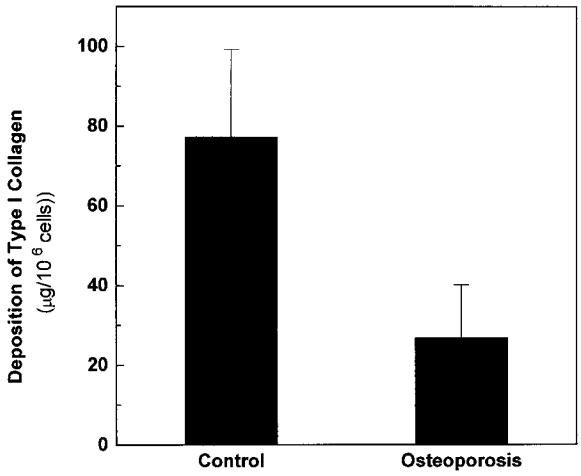

Fig. 2. Deposition of type I collagen. Collagen deposition in three different culture dishes was measured on day 14 after confluency. Collagen deposits were released and measured as described in Materials and Methods. Type I collagen deposition was measured by dot blot using a specific monoclonal antibody. The amount of collagen was expressed as micrograms per 10^6 cells. Results were obtained from four different donors: two controls and two osteoporotic. Results were expressed as mean ± SD.

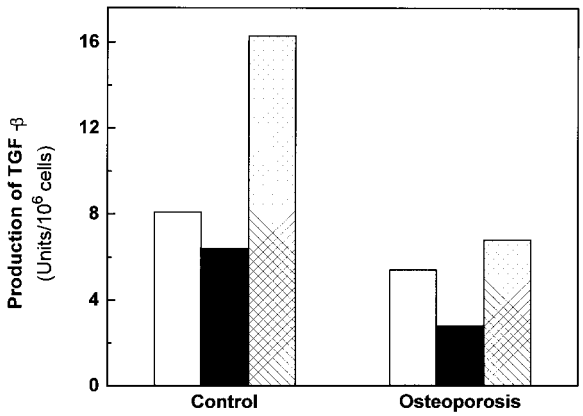

Fig. 3. Synthesis and accumulation of TGF-β. MSCs from control and osteoporotic donors were cultured as described in Materials and Methods. TGF-β was measured at 0 (open bar) and 14 days (gray bar) after confluency, and after 14 days of culture in osteogenic medium (cross-hatched bar). Each experiment was performed in triplicate, and TGF-β produced in three different culture dishes was pooled and measured. The amounts of TGF-β are expressed as optic density units per 10^6 cells.

TGF-β Synthesized by MSC

TGF-β is a potent modulator of the ECM. This is exerted by enhancement of collagen synthesis and regulation of the expression of several genes that encode MMPs, the enzymes that degrade the ECM [White et al., 2000]. Because TGF-β is one of the main regulators of ECM, we measured its production by MSCs from control and osteoporotic postmenopausal women. Measurements were performed on day 0 and at the end of the differentiation process (day 14).

As Figure 3 shows, MSCs derived from controls produce more TGF-β than cells derived from osteoporotic patients at each point in time (0 and 14 days). TGF-β level decreased in both types of cells after 14 days of culture, but this decrease was higher in osteoporotic than in control cells (50% versus 20%). At the beginning of the culture, control cells pro-

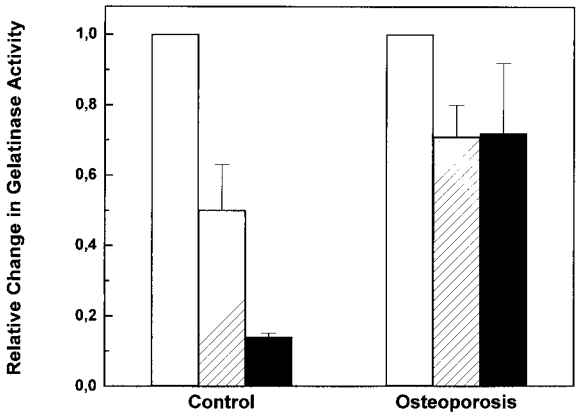

Fig. 4. Relative change in gelatinase activity. MSCs from control and osteoporotic donors were cultured as described in Materials and Methods. Gelatinase activity produced by MSCs was measured at 0 (open bar), 7 (hatched bar), and 14 days (gray bar) after confluency, in regular culture and in osteogenic medium. Gelatinase activity in osteogenic conditions is expressed in relation to gelatinase activity by cells cultured in regular culture medium. Results were obtained from four different donors: two controls and two osteoporotic. Each experiment is performed in triplicate and results are expressed as mean + SD.

duced 1.5 times the amount of TGF-β produced by osteoporotic cells; the difference increased after 14 days to 2.0–2.3 times. The differences in TGF-β production were more marked in cells cultured under osteogenic conditions. Thus, although both types of cells increased their TGF-β production in osteogenic conditions, while osteoporotic cells increase it only 1.2–1.3 times, normal cells increase it 2.0 times (Fig. 3; open and dashed bars).

Gelatinase Production by MSCs

Because decreased deposition of type I collagen by osteoporotic-derived MSCs may result from either lower amounts of type I collagen synthesis and/or increased levels of degradative enzymes, we measured gelatinase activity produced by both types of MSCs.

Control and osteoporotic MSCs released a 72-kDa type A gelatinase (MMP-2) into the culture medium. No other gelatinolytic activities (as MMP-9) were detected under our experimental conditions (data not shown). MSCs derived from osteoporotic donors produced higher levels of MMP-2 (1.5–2.0 fold) than MSCs obtained from controls. Under osteogenic stimulation, both types of cells decreased MMP-2 secretion. As shown in Figure 4, the enzymatic activity of both normal and osteoporotic cells under osteogenic conditions (7 and 14 days) was less than that under standard conditions. Thus, although gelatinase activity produced by control cells significantly diminished after osteogenic culturing (85–90% in 14 days), osteoporotic cells maintained high MMP-2 levels, throughout the culture period. Only 20–25% of inhibition of enzymatic activity was observed after 14 days of culture. The time course shown in Figure 4 represents the differential response to osteogenic stimulus of both types of cells.

MSCs Differentiation to Adipocytes

MSCs derived from control and osteoporotic donors were cultured in an adipogenic medium. In Figure 5, photomicrographs show a representative field of MSCs derived from control (Fig. 5A) and osteoporotic (Fig. 5B) donors after 14 days of culture under adipogenic conditions. Both control and osteoporotic MSCs differentiated into adipocytes, as shown by Oil Red O staining. Also shown in Figure 5 are representative culture dishes of MSCs derived from control (Fig. 5C) and osteoporotic (Fig. 5D) donors after 14 days in adipogenic conditions. These pictures show the whole cultures of both types of MSCs and demonstrate that more MSCs derived from osteoporotic than control donors cells differentiated into the adipogenic phenotype.

In an attempt to quantitate the degree of differentiation into adipocytes of MSCs derived from control and osteoporotic donors, cell layers stained with Oil Red O were analyzed with an imaging densitometer (Bio-Rad, Model GS-670). The results indicate that the intensity of the staining of osteoporotic cells is double that of control cells (9.33 versus 4.62 O.D./mm^2, respectively). It is important to notice that under adipogenic conditions, MSCs derived from controls accumulate twice as much type I collagen compared with osteoporotic cells (33.9 ± 0.52 versus 18.6 ± 0.03 $\mu g/10^6$ cells, respectively).

These results may explain the deficient ability of MSCs derived from osteoporotic donors to differentiate to the osteogenic lineage [Rodríguez et al., 1999], and their propensity to differentiate to the adipogenic phenotype. There are some evidence that shows an inverse relationship between the differentiation of osteogenic and adipocytic cell lines [Locklin et al., 1995; Gimble et al., 1996; Nuttall et al., 1998].

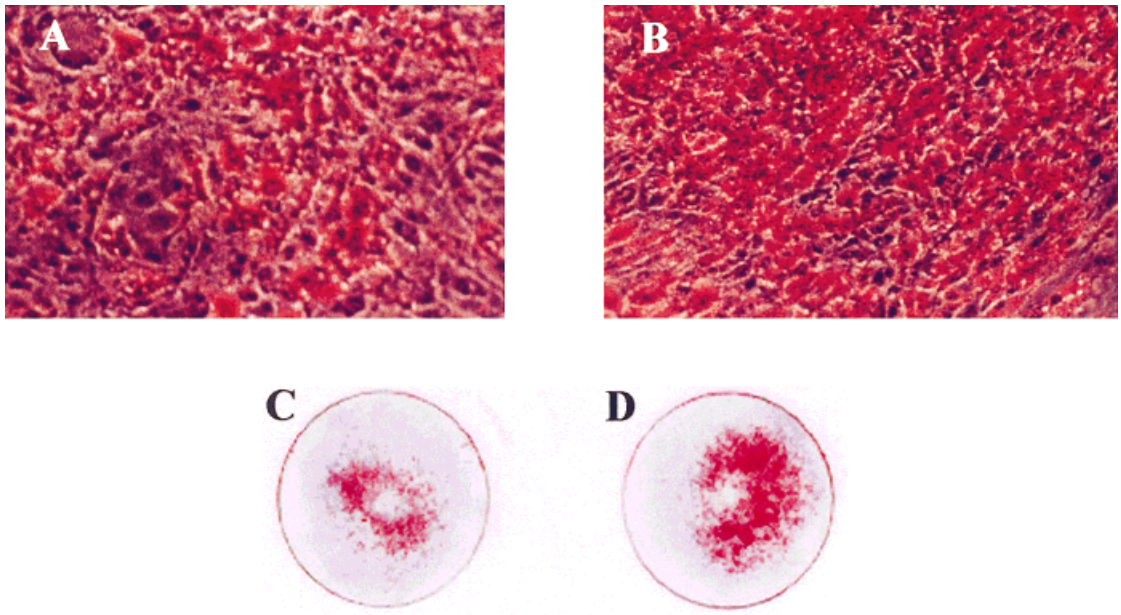

Fig. 5. Adipocyte differentiation. MSCs from control and osteoporotic donors were cultured in adipogenic medium. Photomicrographs show MSCs derived from control (**A**) and osteoporotic (**B**) donors cultured in adipogenic conditions for 14 days (original magnification ×10). **C** and **D:** The appearance of culture dishes with MSCs derived from control and osteoporotic donors after 14 days of culture. Cells were stained with Oil Red O, and counterstained with hematoxylin.

DISCUSSION

The results presented here show that MSCs derived from osteoporotic postmenopausal women have decreased capacity to generate and maintain type I collagen-rich ECM. This feature should result from the decreased potential of osteoporotic MSCs to synthesize type I collagen, from its enhanced type A gelatinase (MMP-2), activity and from the decreased production of TGF-β. It is also shows that MSCs from osteoporotic donors have increased propensity to differentiate into the adipogenic lineage than control cells. These changes may explain the decreased mineralization previously observed in these cells [Rodríguez et al., 1999].

The decreased mineralization of osteoporotic bone has been analyzed under different approaches. Some results point to the importance of the collagen matrix. Studies performed by Bailey et al. [1999] demonstrated that the quality of bone collagen in osteoporotic patients differed from that of controls. According to these authors, the differences in collagen quality could originate from posttranslational modifications of the collagen fiber [Bailey et al., 1999]. Our results indicate that osteoporotic cells express decreased levels of type I collagen, which further decline with more prolonged cultures (up to 14 days); this is proportional to the decrease of intracellular TGF-β (Figs. 1B and 3). This is supported by several studies that demonstrate that TGF-β plays a determinant role in the osteogenic phenomenon by stimulating type I collagen and proteoglycan synthesis, as well as the expression of alkaline phosphatase [Locklin et al., 1999]

MMPs play an important role in the turnover of collagenous bone matrix [Johansen et al., 1992]. In cultured osteoblastic cells, different soluble growth factors generated by bone cells during maturation of ECM can modulate the production of MMPs and their natural tissue inhibitors of metallo proteases (TIMPs) [Panagakos and Kumar, 1995]. Our results (Fig. 4) show that in control cells, osteogenic stimulation induced a considerable decline in MMP-2 activity that could correlate with the establishment of a functional ECM. Conversely, osteoporotic cells maintain high MMP-2 levels during in vitro osteoblastic differentiation. Culture of MSCs in osteogenic culture medium affected both types of cells differently, as well as TGF-β accumulation and MMP-2 production. Although control cells cultured for 14 days in osteogenic medium exhibited enhanced

TGF-β production, the same experimental conditions did not increase TGF-β production by cells derived from osteoporotic patients (Fig. 3). On the other hand, the osteogenic medium significantly decreased MMP-2 activity released to the culture medium by control cells. This was not observed in cells derived from osteoporotic patients. These results allow us to conclude that an inverse relationship between TGF-β level and MMP-2 activity exists, which agrees with results previously reported by White et al. [2000].

The plasticity of human MSCs allows their differentiation along the osteogenic, chondrogenic, adipogenic, and marrow stroma lineages [Bruder et al., 1997; Dennis et al., 1999; Pittenger et al., 1999]. Both osteoblast and adipocyte pathways appear to be more closely related [Gori et al., 1999]. Our results confirm the decreased ability of MSCs derived from osteoporotic donors to differentiate into the osteogenic lineage [Rodríguez et al., 1999] as well as a concomitant increase of their potential to differentiate as adipocytes. There is evidence showing an inverse relationship between the differentiation of osteogenic and adipocytic cell lines [Locklin et al., 1995; Gimble et al., 1996; Nuttall et al., 1998; Gori et al., 1999]. Clinical and in vitro observations indicate that in osteoporotic patients, increased bone marrow adipose tissue correlates with decreased trabecular bone volume [Gimble et al., 1996]. In addition, some observations suggest that changes in bone cell dynamics causing osteoporosis are caused by adipose replacement of the marrow cell population [Meunier et al., 1971]. More recently, it has been demonstrated that products of low-density lipoprotein oxidation promote osteoporotic loss of bone by directing progenitor marrow stromal cells to adipogenesis instead of osteogenic differentiation [Parhami et al., 1999]. It has also been demonstrated that osteoblast and adipocyte differentiation are reciprocally regulated processes. Thus, it has been reported that bone morphogenetic-2 protein shunts uncommitted marrow stromal precursor cells from the adipocyte to the osteoblast differentiation pathway, enhancing osteoblast commitment and inhibiting late adipocyte maturation [Gori et al., 1999]

It is not known exactly what role ECM plays in adipogenesis, as well as the mechanisms whereby TGF-β inhibits differentiation [Bortell et al., 1994; Locklin et al., 1995; Gagnon et al., 1998]. However, it has been proposed that among the molecular mechanisms involved, the adipogenic process may be strongly affected by ECM composition [Bortell et al., 1994] and by the bioavailability of TGF-β [Locklin et al., 1995]. Our results show an inverse relationship between collagen levels and adipocyte formation and are in agreement with those reported previously [Bortell et al., 1994], demonstrating that an increase in adipogenesis seems to be associated with a decrease in collagen synthesis. Recent data support the idea that TGF-β may stimulate osteogenic differentiation and inhibits the adipogenic pathway [Locklin et al., 1999].

Our results agree with those cited above in the sense that osteoporotic MSCs produced an ECM whose quality and quantity of growth factors favor adipogenic rather than osteogenic differentiation. It is difficult to propose the sequence of events that operate in this case, because it is not known whether the primary event that commands the differentiation process is decay of TGF-β production or the cumulative decrease of type I collagen deposition.

Specific inhibition of marrow adipogenesis and a concomitant enhancement of osteoblastogenesis from common precursor cells may provide a novel therapeutic approach for treatment of osteopenic disorders, such as postmenopausal osteoporosis. Factors that control the balance between adipocytic and osteogenic differentiation need to be investigated in future studies.

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