J Musculoskelet Neuronal Interact 2008; 8(1):43-49

Original Article

Hylonome

Cellular isolation, culture and characterization of the marrow sac cells in human tubular bone

L.X. Bi[1,2], E.G. Mainous[2], D.A. Yngve[1], W.L. Buford[1]

[1]Department of Orthopaedic Surgery and Rehabilitation; [2]Department of Surgery,
Division of Oral & Maxillofacial Surgery, University of Texas Medical Branch, Galveston, TX, USA

Abstract

The goal of this study is to characterize the epithelioid-like human marrow sac cells that separate the myeloid and osteoblast populations *in situ* and to determine if they express osteoblast cytoplasmic markers. Tubular segments of femoral diaphyseal bone were obtained from healthy young (4-8 yr) male and female patients undergoing femoral shortening surgeries. The interface between bone and marrow was examined by scanning (SEM) and transmission electron microscopy (TEM). The marrow sac cells were isolated and cultured in a-MEM medium with and without dexamethasone, glycerophosphate, and ascorbic acid [DGPA]. Alkaline phosphatase (ALP), bone morphogenetic protein-2 (BMP-2) and osteocalcin were evaluated. In the SEM, the marrow sac presented a distinctive pattern of large overlapping cells. TEM studies showed that marrow sac was one or two cells thick, which were attenuated with elongated nuclei, few cellular organelles, and appeared to display intercellular gap junctions. In culture, the marrow sac cells stained positively for ALP and BMP-2, and their expression was enhanced two- to three-fold when the cells were grown in DGPA. DGPA did not enhance osteocalcin expression. The cells of the human marrow sac reside proximate to endosteal osteoblasts and express osteoblastic markers. It is possible that these stromal cells constitute an osteoprogenitor pool from which replacement osteoblasts are recruited, and that they are involved in normal bone formation and in bone diseases (e.g., osteoporosis and osteopenia).

Keywords: Marrow Sac, Marrow Stromal Cell, Preosteoblast, Bone-marrow Interface

Introduction

Bone marrow contains stromal mesenchymal cells that are capable of differentiating into osteoblast cells, fibroblasts, fibroendothelial cells, chondroblasts, adipocytes, and muscle cells[1]. The differentiation process and mechanism are influenced by multiple factors. Dexamethasone singularly induces bone marrow stromal cells to differentiate into cells exhibiting the osteoblast phenotype[2].

While clonal techniques have shown that bone marrow stromal cells comprise several physiologic populations that are not equal in rate of growth and osteogenic activity, those studies do not reveal the anatomical distribution and relative frequency of these populations[3]. One would anticipate that the putatively osteogenic stromal cells would be those that lay closest to the lining of endosteal osteoblasts. Scanning and transmission electron microscopic studies in laboratory animals revealed that the marrow and endosteal bone cells were each effectively compartmentalized by an intervening condensed surface layer of epithelial-like mesenchymal stromal cells which have been called marrow sac cells[4-5]. These cells were uniformly thin and attenuated with elongated nuclei, few small round mitochondria, and sparse rough endoplasmic reticulum. Because it is well known that there are populations of mesenchymal stromal cells in marrow which are preosteoblasts and bear osteogenic cell markers, the sac cells on the outer surface of the medullary compartment proximal to endosteal osteoblasts might be an osteogenic reserve cell population.

In the present study, we have identified the human marrow sac cells, which separate the osteoblast cells from bone marrow elements. The electron-microscopic morphology and its expression of BMP-2, osteocalcin and ALP in culture are described.

The authors have no conflict of interest.

Corresponding author: Lian Xiang Bi, M.D., Department of Orthopaedic Surgery and Rehabilitation, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-0174, USA
E-mail: lbi@utmb.edu

Accepted 8 February 2007

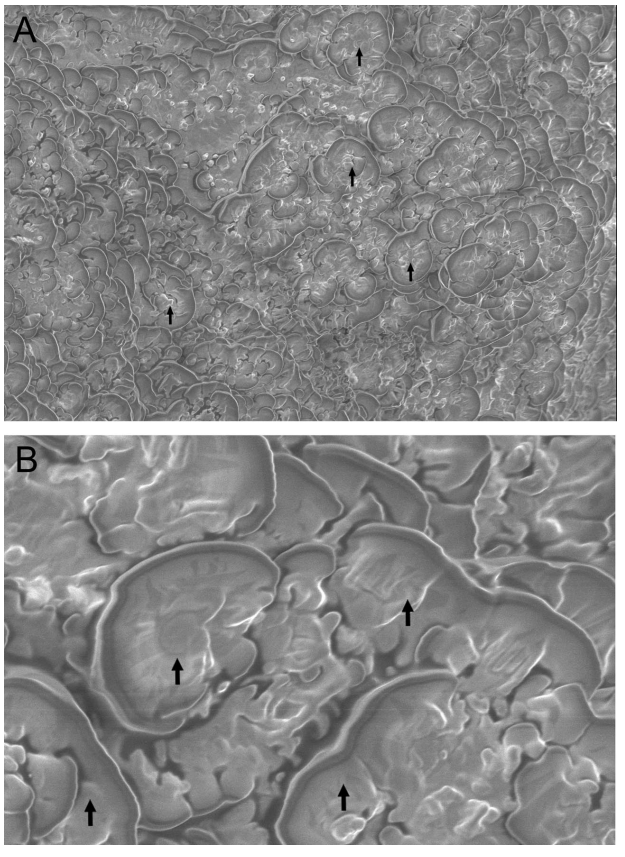

Figure 1. SEM showing a segment of human femoral marrow (A 300X, B 1200X). The outer surface of the medullary compartment is covered by a cellular sheet of flattened overlapping lobulate cells (arrows).

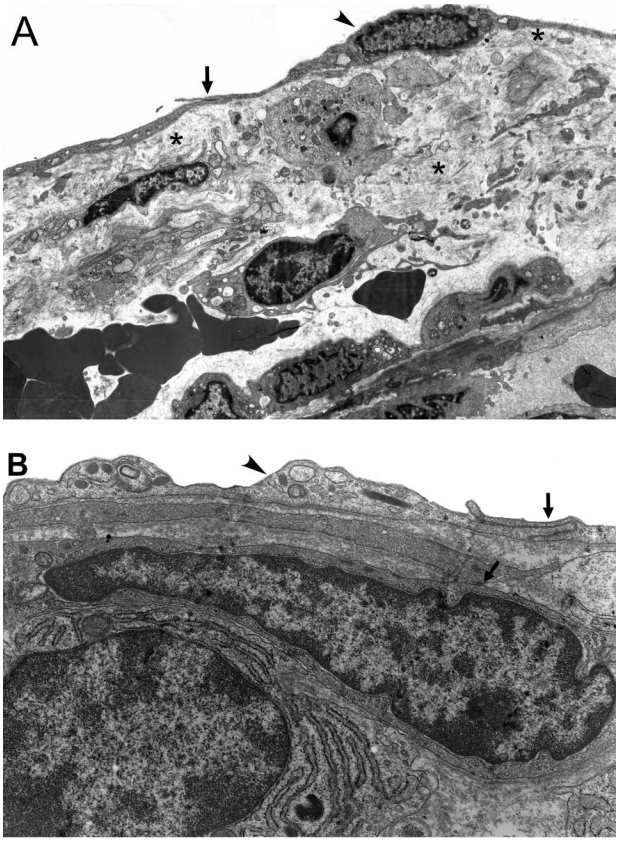

Figure 2. TEM of a transverse section of human femoral marrow showing that the marrow sac (arrowhead) was 1 (A 3000X) or 2 (B 12000X) cells thick, that the cells were attenuated with elongated nuclei, few round mitochondria and appeared to display intercellular gap junctions (filled arrow). The cells contained few organelles and no basal laminae. The concentrations of collagen fibrils (asterisks) were found in association with their deep medullary surfaces. B detail of gap junctions between marrow sacs and between marrow sac cell and bone marrow cell (filled arrows).

Materials and methods

Discarded tubular segments of femoral diaphyseal bone were obtained from healthy young (4-8 yr) male and female patients undergoing femoral shortening surgeries. The bone fragments were 1.5-2.5 cm in length.

The samples were split longitudinally. In most cases, there was a clean separation of marrow and bone. Free marrow samples were fixed for 3-4 days in cold 0.08M sodium cacodylate buffered 2% glutaraldehyde (pH 7.4). All specimens were then postfixed in 1% osmium tetroxide, rinsed in 0.08M sodium cacodylate buffer, dehydrated in a graded ethanol series, and critical point dried.

Scanning electron microscopy

Plugs from the bone marrow and bone samples were mounted on SEM specimen studs and oriented to show the character of the cells at their interface. The tissues were sputter coated with a thin layer of gold, and they were examined in an S-3500N scanning electron microscope (HITACHI, Ltd, Japan).

Transmission electron microscopy

Fixed marrow samples were cut into 1.0 mm^3 blocks for TEM investigation. They were fixed in glutaraldehyde for an additional 24 hours, postfixed in 1% osmium tetroxide, dehydrated, and embedded in Spurr epoxy resin by routine technique. Transverse sections cut from the blocks were stained with uranyl acetate and lead citrate, and examined in an H-7500 TEM (HITACHI, Ltd, Japan).

Isolation of marrow sac cells

Fresh isolated intact marrow plugs were obtained, and only the outer surface of bone marrow was gently pressed several times against a dry, sterile, polysterene, 100 mm plastic petrie dish (VWR, West Chester, PA) coated with poly-

d-lysine to cover the entire surface with marrow sac cells using the technique of touch imprint cytology[6-7]. Then, the cells were cultured in α-MEM (Atlanta Biologicals, Norcoss, GA) containing antibiotics [penicillin (100U/ml), streptomycin sulphate (100ug/ml)] and 10% fetal bovine serum (FBS) (Atlanta Biologicals, Norcoss, GA) in a humidified incubator at 37°C under an atmosphere of 5% CO_2 and 95% air. The medium was changed every other day.

After 10 days of culture, the adherent cells were collected by trypsinizing with trypsin 0.05%-EDTA 0.53 mM in Hanks' balanced salt solution without calcium and magnesium (Atlanta Biologicals, Norcoss, GA) at 37°C for 5 min. and resuspended in α-MEM with 10% FBS. The passages 4-8 were used in this study. The cells were plated at a density of 5 x 10^3 cells/well and cultured for 7 days on glass coverslips coated with Poly-d-lysine in 24-well plates using fresh medium with or without dexamethasone (10^{-8} M), β-glycerophosphate (10 mM) and ascorbic acid (50 ug/ml) [DGPA]. Then the cells were examined for the expression of alkaline phosphatase, BMP-2 and osteocalcin.

Immunocytochemistry

The avidin-biotin-immunoperoxidase method was used by following the manufacturer's procedure (Vectastain E Elite ABC kit, Vector Laboratories, Inc. Burlingame, CA). The expression of BMP-2 (monoclonal antibody h4b2/5.10.24, Genetics Institute, MA) and osteocalcin (monoclonal antibody, Zymed Laboratories, Inc. CA) were examined.

The cells on cover slips were washed with cold phosphate buffered saline (PBS), fixed in acetone for 10 minutes at -20°C, washed with PBS and then treated with 0.3% H_2O_2 in methanol for 30 minutes to deplete any endogenous peroxidase activity. The cells were pre-incubated for 20 minutes in diluted normal horse serum (3 drops of horse serum in 10 ml PBS) at room temperature. Subsequently, cells were incubated with primary antibody (10 μg/ml in PBS) overnight at 4°C, followed by secondary biotinylated horse anti-mouse IgG (one drop of stock in 10 ml PBS) for 30 minutes at room temperature, and avidin-biotin-horseradish peroxidase complex for 30 minutes at room temperature. After washing with PBS, cells were exposed to diaminobenzidine (DAB) for 5 minutes and then counterstained with Harris' hematoxylin. Cells treated without the primary antibody served as negative controls.

Alkaline phosphatase

The level of alkaline phosphatase activity was determined using a commercial kit (Cat. # FBS-25, Sigma Chemical Co. St. Louis, MO). The cells on cover slips were washed with cold phosphate buffered saline, fixed in citrate buffered acetone for 30 seconds at room temperature, washed with deionized water, then exposed to alkaline-dye mixture and incubated at room temperature for 30 minutes.

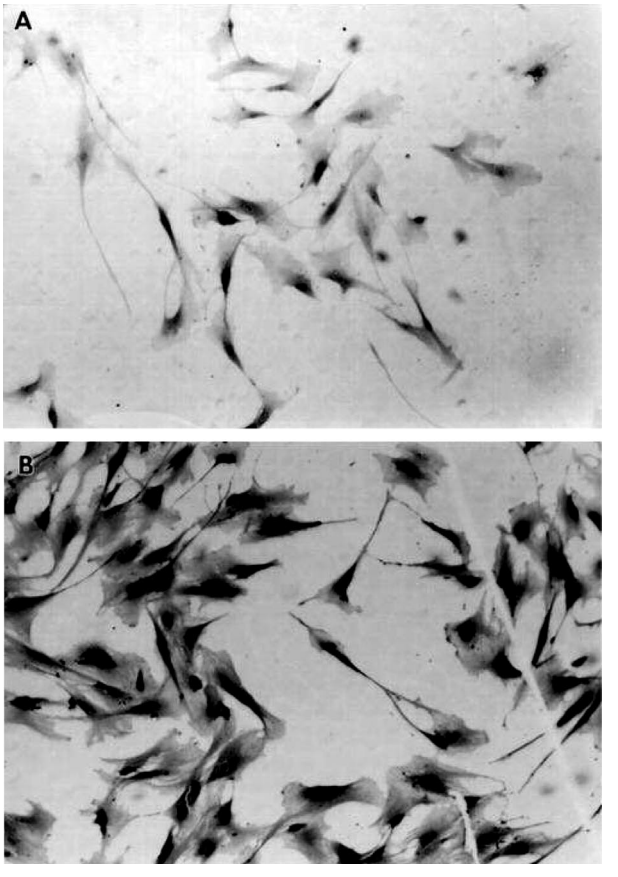

Figure 3. Immunocytochemical detection of BMP-2 in the human marrow sac cells grown in basal medium (A) or medium with DGPA (B) after 7 days of culture. The staining intensity increased in cells grown in medium with DGPA. 160X.

Results

Scanning electron microscopy

Endosteal bone surfaces were completely covered by a hexagonally packed layer of plump osteoblasts. Their surfaces were studded with many tiny microvillar-like projections, and they displayed numerous intercellular cytoplasmic processes. No osteoclast-like cells were identified.

The marrow myeloid elements were always invested in, and separated from, the endosteal osteoblast lining by a continuum of flattened sac cells. In the SEM, the sac cells appeared as flattened overlapping lobulate cells (Figure 1). TEM preparations showed that the marrow sac was 1 or 2 cells thick, that the cells were attenuated with elongated nuclei, and that they appeared to display intercellular gap junctions (Figure 2). The cells contained few small round mitochondria, few lysosomal bodies and filaments, and sparse rough endoplasmic reticulum. Such cells lacked basal laminae, but concentrations of collagen fibrils were found in association with their deep medullary surfaces.

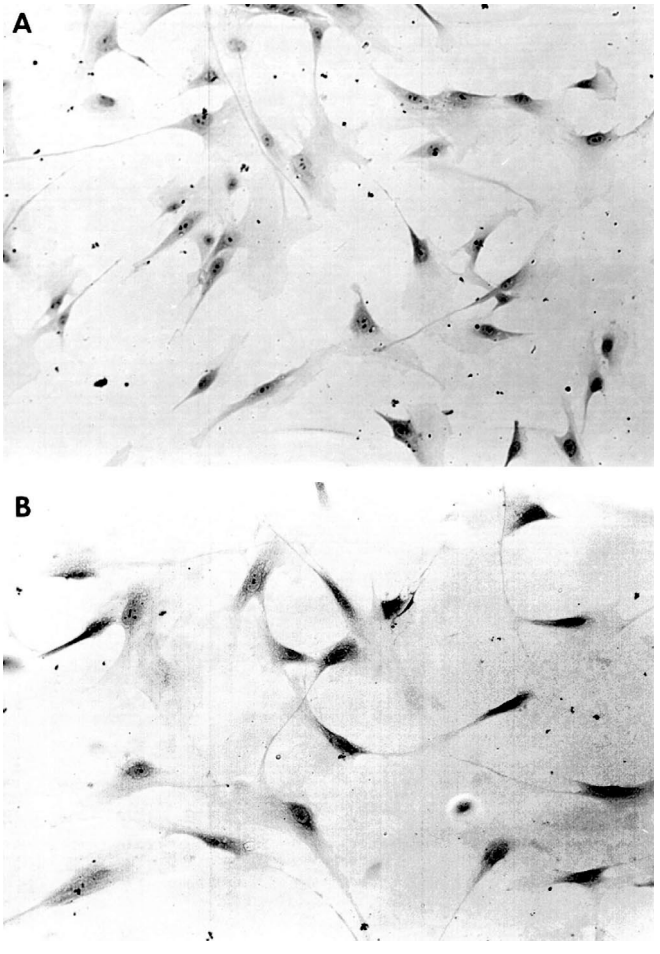

Figure 4. Expression of ALP in the human marrow sac cells grown in basal medium (A) or medium with DGPA (B) after 7 days of culture. Expression of ALP was enhanced while they were cultured in medium containing DGPA. 160X.

Immunocytochemistry and enzyme histochemistry

The question of whether marrow sac cells exhibit an osteoblast-like character was approached immunocytochemically and histochemically. Cells cultured for 7 days in basal a-MEM medium stained only weakly for BMP-2, ALP and osteocalcin (Figures 3-5A), but the expression of BMP-2 and ALP was enhanced approximately 3-fold and 2-fold, respectively when they were cultured in medium containing DGPA (Figure 3B, 4B). Osteocalcin levels remained unchanged (Figure 5B).

Discussion

The present study shows that the core of hemopoietic bone marrow in the femurs of growing children is contained within a condensed layer or sac of overlapping flattened cells. The sac cells also lie subjacent to the osteogenic cells which line the bony endosteum. Similar observations have been recorded in bone marrow from a number of commonly used laboratory mammals and in pigeons[4,5]. However, differences do exist in the organization and morphology of marrow sac cells from different species. Rats, cats and sheep have a seamless arrangement of marrow sac cells which resemble a thin, flat, simple squamous epithelium with few intercellular cytoplasmic processes. The rabbit and pigeon sacs are composed of a more woven, multilayered fabric of broadly elongated, flat, fibroblast-like cells that display many intercellular processes. In contrast, the cells in the human sac display a distinctive pattern of overlapping lobular cells which, while they send off few processes, are equipped with a conspicuous number of gap junctions which are similar to the intercellular junctions described by Yamazaki and Miller et al.[8,9]. Conventional wisdom suggests that they might be associated with the regulation of intercellular calcium transport[10] in response to hormonal, cytokine and electrochemical signals that determine cell differentiation and co-ordinate metabolic activities[11]. Otherwise, all marrow sac cells share a paucity of endoplasmic reticulum and organelles, such as mitochondria and lysosomal bodies. The architectural similarities between the species suggest that, if there were differences in sac cell function, they might well be quite trivial.

The marrow sac cells expressed BMP-2 immunocytochemically. They also could be induced by DGPA to differentiate into an osteoblastic phenotype by increasing expression of BMP-2. The BMPs represent some 13 members of the TGF-β superfamily; they are characterized by the distinctive pattern of seven cysteine residues in their carboxyl termini[12,13]. BMPs 2-12 have osteoinductive potential[14-16]. They are produced by bone marrow stromal cells and osteoblasts, and stored in bone in an inactive state. Immunocytochemistry has localized BMP to collagen fibers in osteoid and mineralized bone[17]. BMPs also play a role in embryogenesis, and have been shown to be expressed by cells in developing limbs, lung, kidney, adrenal, and urinary bladder and demonstrated to be associated with embryonic morphogenesis[18-20]. Postnatally, however, their significance lies in their capacity to mobilize osteoprogenitor cells, thereby promoting the osteoblastic differentiation processes[21]. The original *in vivo* studies, which showed that implants of BMP-rich demineralized bone and dentine preparations could induce cartilage-bone formation *in situ*, have now been confirmed using recombinant human BMP preparations[22]. *In vitro*, various osteoblastic cell lines and their mesenchymal progenitors in bone marrow [ROB-C26, W-20-17] respond to rhBMP-2 and -4 by increasing their production of mineralizable bone collagen and alkaline phosphatase and their cAMP signature following PTH stimulation[23]. BMP-2 appears to acts in both an autocrine and paracrine fashion to stimulate bone cell differentiation and bone formation. Therefore, the marrow sac cells expressing BMP-2 may play a role in bone formation in an autocrine and/or paracrine manner.

Alkaline phosphatase is well known as a classical marker of osteoblastic bone formation that reflects osteoblastic activity, and as Turksen and others have affirmed, dexamethasone induces the differentiation of AP-positive cells that

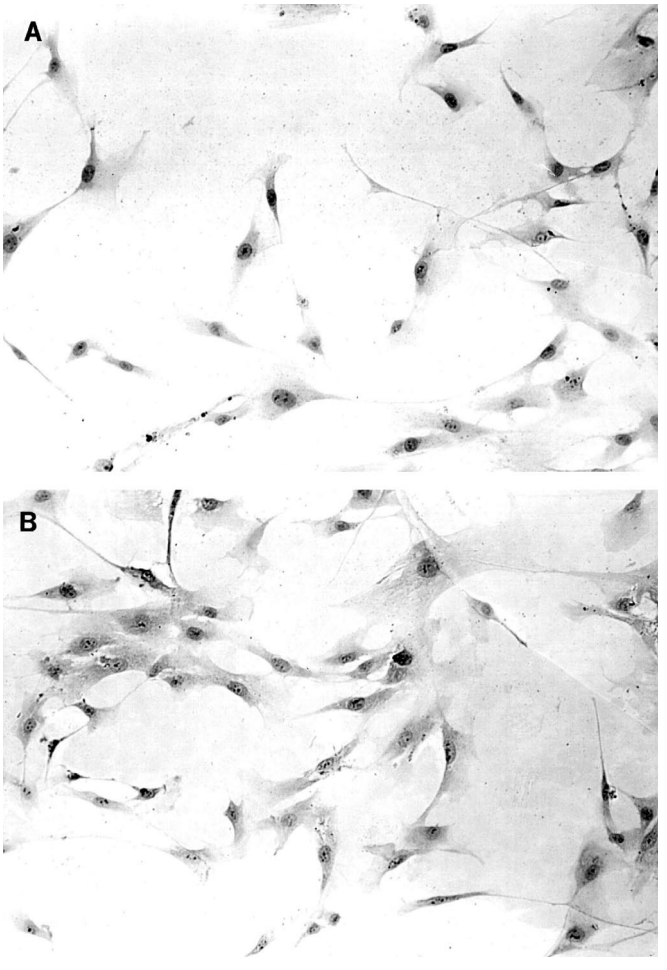

Figure 5. Immunocytochemical detection of osteocalcin in the human marrow sac cells grown in basal medium (A) or medium with DGPA (B) after 7 days of culture. Osteocalcin was detectable and no difference in this early stage. 160X.

possess the ability to form mineralizable collagen nodules[24-27]. Hauge et al.[28] showed alkaline phosphatase is displayed by osteoblasts and marrow lining cells, which are next to endosteal osteoblasts in human cancellous bone. In the present study, the marrow sac cells weakly expressed alkaline phosphatase in culture. However, the expression of alkaline phosphatase was strikingly elevated in medium containing DGPA. The marrow sac cells could be induced to differentiate into the osteoblastic cell lineage.

Osteocalcin, originally termed bone Gla-protein, is a small non-collagenous protein that is specific for bone tissue and dentin. Osteocalcin also may play an important regulatory role during osteoblast differentiation[29-31]. In the present study, the marrow sac cells expressed osteocalcin *in vitro* similar to that of marrow lining cells expressing osteocalcin *in vivo*, reported by Hauge et al.[28]. The marrow sac cells might change their metabolic state when they are activated at the beginning of bone remodeling *in vivo*[32].

The marrow sac cells separate the bone from marrow in cortical bone. This structure is similar to that that Hauge et al. described in cancellous bone[28]. The bone remodeling compartment in cancellous bone is lined on its marrow side by flattened cells, referred to as bone lining cells, and on its osseous side by the remodeling bone surface[28]. And at some areas, the bone surfaces are covered directly by bone lining cells[9,28] or capillaries which are associated with bone-forming surfaces[9,33] and osteogenesis during bone development and bone remodeling[34-38]. The lining cells express the alkaline phosphatase, osteocalcin, osteonectin[28], IGFs, TGFβ, bFGF, OPG and RANKL[39-41]. The levels of these markers vary with the bone metabolic state. These osteoblast and osteoclast differentiation factors might be associated with local regulation of pre-osteoblast recruitment and osteoblast differentiation. Conventionally, the cells close to osteoblasts are anticipated to be pre-osteoblasts. These cells may be marrow sac cells lining on the surface of bone marrow in trabecular and cortical bone with an osteogenic potential or they may be the population of cells most important to osteoblast renewal. In *in vivo* studies, the marrow sac cells could be activated by intermittent parathyroid hormone treatment[43] or by mechanical loading to form bone[32]. We show that the marrow sac cells express the osteoblast markers alkaline phosphatase, BMP-2, and osteocalcin *in vitro*, suggesting that they have the bone cell phenotype.

In summary, in the present study, the marrow sac cells are shown anatomically closest to the lining layer of endosteal osteoblasts that actively form bone. Their morphology is that of an attenuated cell with an elongated nucleus and few organelles. These cells express ALP, BMP-2 and osteocalcin, and can be induced to increase expression of ALP and BMP-2 by DGPA. The marrow sac cells may represent an osteogenic pool to recruit pre-osteoblasts, themselves differentiate into osteoblasts and, in case, play an important role in normal bone formation and bone diseases (osteoporosis, osteopenia etc.).

Acknowledgements

The authors express their appreciation to Dr David J. Simmons and Kristi Overgaard for their critical reading of the manuscript and Randal P. Morris for his preparation of photomicrographs.

References

1. Yamaguchi A. Regulation of differentiation pathway of skeletal mesenchymal cells in cell lines by transforming growth factor-beta superfamily. Cell Biol 1995;6:165-73.
2. Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whittes MJ, Hewik R, Kriz R, Wang EA. Novel regulators of bone formation: molecular clones and activity. Science 1988;242:1528-34.
3. Simmons DJ, Bi LX, Seitz PK, Yang SF, Wong CE, Krukowski MK, Mainous E. Identification of osteogenic rat marrow stromal cells (F-CFU). J Bone

Miner Res 1997;12(Suppl.1):S211.
4. Bi LX, Simmons DJ, Hawkins HK, Cox RA, Mainous EG. Comparative morphology of the marrow sac. Anat Rec 2000;260:410-5.
5. Menton DN, Simmons DJ, Orr BY, Plurad SB. A cellular investment of bone marrow. Anat Rec 1982; 203:157-64.
6. Gentry JF. Pelvic lymph node metastases in prostatic carcinoma. The value of touch imprint cytology. Am J Surg Pathol 1986;10:718-27.
7. Hasenburg A, Ledet SC, Ardaman T, Levy T, Kieback DG. Evaluation of lymph nodes in squamous cell carcinoma of the cervix: touch imprint cytology versus frozen section histology. Int J Cancer 1999;9:337-41.
8. Yamazaki K, Eyden BP. A study of intercellular relationships between trabecular bone and marrow stromal cells in the murine femoral metaphysis. Anat Embryol (Berl) 1995;192:9-20.
9. Miller SC, Bowman BM, Smith JM, Jee WS. Characterization of endosteal bone-lining cells from fatty marrow bone sites in adult beagles. Anat Rec 1980;198:163-73.
10. Talmage RV. Morphological and physiological consideration in new concept of calcium transport in bone. Am J Anat 1970;129:467-76.
11. Simmons DJ. The *in vivo* role of bone marrow fibroblast-like stromal cells. Calcif Tissue Int 1996;58:129-32.
12. Hattersly G, Hewick R, Rosen V. *In situ* localization and *in vitro* activity of BMP-13. J. Bone Miner Res 1991; 10(Suppl.1):S163.
13. Jones CM, Lyons KM, Hogan BLM. Involvement of BMP-4 and Vgr-1 in morphogenesis and neurogenesis in the mouse. Development 1991;111:531-42.
14. Celeste AJ, Jannazzi JA, Taylor RC, Wozney JM. Identification of new transforming growth factor beta family members present in bone-inducing protein purified from bovine bone. Proc Natl Acad Sci USA 1990; 87:9843-7.
15. Bi LX, Li J, Ling ZF. Repair of the bone defect of tibia with rotated muscle flap reacted with bovine bone morphogenetic protein in sheep. Chin J Pediatr Surg 1994; 15:169-71.
16. Luyten FP, Cunningham NS, Reddi AH. Purification and partial amino acid sequence of osteogenin, a protein initiating bone differentiation. J Biol Chem 1989; 264:13377-80.
17. Kou CH, Hsueh CI, Chih T. Distribution of bone morphogenetic protein in human bone and tooth germs: analysis of specificity of monoclonal antibody against bone morphogenetic protein. J Stomatology 1992; 27:102-3.
18. Vukicevic S, Latin V, Chen P, Batorsky R, Reddi AH, Sampath TK. Localization of osteogenic protein-1 during human embryonic development: high affinity binding to basement membranes. Biochem Biophys Res Commun 1994;198:693-700.
19. Carrington JL, Yanagishita M, Reddi AH. Osteogenin stimulates cartilage formation by chicken limb bud cells *in vitro*. Dev Biol 1991;146:406-15.
20. Francis PH, Richardson MK, Brickell PM, Tickle C. Bone morphogenetic protein and a signaling pathway that controls patterning in the developing chick limb. Development 1994;120:209-18.
21. Harris SE, Sabatini MA, Harris MA, Feng JQ, Wozney J, Mundy GR. Expression of bone morphogenetic protein messenger RNA in prolonged cultures of fetal rat calvarial cells. J Bone Miner Res 1994;9:389-94.
22. Wang EA, Rosen V, D'Alessandro JS, Brandy M, Cordes P, Harada T, Israel DI, Nakashima M, Nakasawa H, Yamada Y, Reddi AH. Regulatory role of transforming growth factor beta, bone morphogenetic protein-2 and protein-4 on gene expression of extracellular matrix proteins and differentiation of dental pulp cells. Dev Biol 1994;162:18-28.
23. Balk ML, Bray J, Epplerly M, Grenburger J, Evans CH, Niyibizi C. Effect of rhBMP-2 on osteogenic potential of bone marrow stromal cells from an osteogenesis imperfecta mouse (oim). Bone 1997;21:7-15.
24. Katagiri T, Yamaguchi A, Komaki M, Abe E, Takahashi N, Ikada T, Rosen V, Wozney JM, Fujisawa-Sehara A, Suda T. Bone morphogenetic protein-2 converts the differentiation pathway C2C12 myoblasts into the osteoblast lineage. J Cell Biol 1994; 127:1755-1766.
25. Worldarski KH, Reddi AH. Alkaline phosphatase as a marker of osteoinductive cells. Calcif Tissue Int 1986; 39:382-5.
26. Turksen K, Aubin JE. Positive and negative immunoselection for enrichment of two classes of osteoprogenitor cells. J Cell Biol 1999;114:373-84.
27. Bi LX, Simmons DJ, Mainous E. The expression of BMP-2 by rat bone marrow stromal cell in culture. Calcif Tissue Int 1999;64:63-8.
28. Hauge EM, Qvesel D, Eriksen EF, Mosekilde L, Melsen F. Cancellous bone remodeling occurs in specialized compartments lined by cells expressing osteoblastic markers. J Bone Miner Res 2001;16:1575-82.
29. Green E, Todd B, Heath D. Mechanism of glucocorticoid regulation of alkaline phosphatase gene expression in osteoblast-like cells. Eur J Biochem 1990; 188:147-53.
30. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, Shimizu Y, Bronson RT, Gao YH, Inada M, Sato M, Okamoto R, Kitamura Y, Yoshiki S, Kishimoto T. Targeted disruption Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell 1997;89:755-64.
31. Kozawa O, Hatakeyama D, Uematsu T. Divergent regulation by p44/p42 MAP kinase and p38 MAP kinase of bone morphogenetic protein-4-stimulated osteocalcin synthesis in osteoblasts. J Cellular Biochem 2002;84:583-9.
32. Chow JW, Wilson AJ, Chambers TJ, Fox SW. Mechanical loading stimulates bone formation by reactivation of bone lining cells in 13-week-old rats. J Bone

Miner Res 1998;13:1760-7.
33. Burkhardt R, Bartl R, Frisch B, Jager K, Mahl G, Hill W, Kettner G. The structural relationship of bone forming and endothelial cells of the bone marrow. In: Alert J, Ficat RP, Hungerford DS (eds) Bone Circulation. Williams and Wilkins, Baltimore; 1984:2-14.
34. Winet H, Bao JY, Moffat R. A control model for tibial cortex neovascularization in the bone chamber. J Bone Miner Res 1990;5:19-30.
35. Hunter WL, Arsenault AL, Hodsman AB. Rearrangement of the metaphyseal vasculature of the rat growth plate in rickets and rachitic reversal: a model of vascular arrest and angiogenesis renewed. Anat Rec 1991;229:453-61.
37. Alam AS, Gallagher A, Shankar V, Ghatei MA, Datta HK, Huang CL, Moonga BS, Chambers TJ, Bloom SR, Zaidi M. Endothelin inhibits osteoclastic bone resorption by a direct effect on cell motility: implications for the vascular control of bone resorption. Endocrinology 1992;130:3617-24.
38. Wootton R, Tellez M, Green JR, Reeve J. Skeletal blood flow in Paget's disease of bone. Metab Bone Dis Relat Res 1981;3:263-70.
39. Qvesel D, Melsen F, Eriksen EF. Paratrabecular endothelial cells as osteoblast precursors: immunohistochemical description of sinusoidal structure in human bone biopsies. Calcif Tissue Int 1999;64(Suppl.):S61.
40. Qvesel D, Eriksen EF, Melsen F. Formation of paratrabecular sinusoidal structures initiates bone remodeling: detachment, and not retraction of lining cells from the bone surface is the primary event. J Bone Miner Res 1999;14(Suppl.):S141.
41. Eriksen EF, Qvesel D, Hauge EM, Melsen F. Further evidence that vascular remodeling spaces are lined by cells of osteogenic origin: characterization of a possible coupling structure. J Bone Miner Res 2005;15(Suppl.):S371.
42. Eriksen EF, Eghbali-Fatourechi Gz, Khosla S. Remodeling and vascular spaces in bone. J Bone Miner Res 2007;22:1-6.
43. Dobnig H, Turner RT. Evidence that intermittent treatment with parathyroid hormone increases bone formation in adult rats by activation of bone lining cells. Endocrinology 1995;136:3632-8.