Journal of Cellular Biochemistry 59:151–160 (1995)

Bone Marrow Interface: Preferential Attachment of an Osteoblastic Marrow Stromal Cell Line

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Abstract In this study, we report on the cell adhesion properties of marrow stromal cells to extracellular matrix components such as collagen and noncollagenous proteins. The osteoblastic cells and their non-osteoblastic counterparts (MBA series) from the marrow stroma differentially recognized a spectrum of extracellular matrix proteins. The osteoblastic cells, MBA-15, preferentially attached to bone matrix proteins, whereas fibroendothelial MBA-2.1 and adipocytic 14F1.1 cells did not. The MBA-15 cells demonstrated a preference in their attachment to fibronectin > mixture of collagens > bone matrix extracts > collagen type I > noncollagenous proteins. Clonal subpopulations derived from the MBA-15 cell line representing various stages along the osteogenic lineage expressed differential attachment preference. MBA-15.4, a less differentiated clonal line, was compared to MBA-15.6, a mature cell line.

Key words: stromal cells, osteoblasts, attachment, bone matrix, bone formation

The extracellular matrix (ECM) of bone has a unique composition that conveys to bone its specialized structure, thereby allowing it to function as mechanical support. However, bone matrix is also known to serve as a major regulator of cells which are in contact with it. ECM is the natural substrate that may be chemotactic and upon which cells migrate, proliferate, and differentiate in vivo. In each microenvironment, the ECM acts as a specialized coordinator for cellular growth and differentiation. The anchorage of cells to a substratum is a fundamental condition for the promotion of cellular expression at different stages of differentiation. The major components of bone matrix are mainly collagen type I and a variety of noncollagenous proteins (NCP) [Termine, 1993], including fibronectin (FN), osteopontin, bone sialoprotein, thrombospondin, bone gla-protein and others. Several bone matrix macromolecules promote cellular adhesion in vitro [Clezardin et al., 1989; Clover et al., 1992; Gehron Robey et al., 1989; Grzesik et al., 1993; Oldberg et al., 1986, 1988a,b; Somerman et al., 1987, 1989]. These and other matrix macromolecules have been shown to affect growth, morphology, and phenotypic expression of osteoblastic cells [Lucas et al., 1988; Vukicevic et al., 1990]. Several matrix components mediate the interaction with cells, in some cases through the existence of integrin receptors on the cell surface which can be specific for various matrix components. Osteoblastic cells express several subunits of the integrin family that mediate cell attachment via the RGD sequence in proteins found in the mineral compartment of bone [Clover et al., 1992; Grzesik et al., 1993]. Other matrix macromolecules, such as type I collagen, interact with different integrins by a non-RGD-mediated mechanism [Kramer and Marks, 1989; Ruoslahti, 1991], and these are those which can affect cellular events such as those occurring via non-integrin receptors [Lucas et al., 1988] or by integrin-mediated attachments [Iruela-Arispe et al., 1991]. The cellular interactions with ECM may promote or, alternatively, inhibit the osteogenic cells' maturation towards differentiation and full expression which lead to bone formation at restricted sites in the marrow compartment.

Received December 19, 1994; accepted February 9, 1995.

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An important source of osteoprogenitor cells is the marrow stromal system [Benayahu et al., 1989; Owen and Friedenstein, 1988]. The osteogenic members of the stromal cell family are intimately involved with bone formation at the endosteal surface of bone. However, there is little information concerning the regulation of osteogenesis within the medullary cavity of bones. Stimulators and/or inhibitors in the stromal compartment may affect the osteogenic functions within the marrow stroma. Identification of the potential role of matrix components in eliciting the osteogenic phenotype is of crucial importance.

In the present study, we used the MBA series of marrow stromal cells representing subpopulations that have been classified according to their morphological, biological, and biochemical properties. The various stromal subpopulations include fibroblast, endothelial, adipocytic, and osteoblastic cells. Extensive characterization of the different marrow stromal cell lines was previously undertaken and published [Benayahu et al., 1989, 1991, 1994a,b,c; Fried et al., 1993; Zipori et al., 1984, 1985a,b]. MBA-1.1 and MBA-15 cells were shown to have a fibroblastoid appearance, MBA-2.1 cells are spindle-shaped, whereas 14F1.1 have a polygonal appearance in young cultures, later followed by an adipocytic morphology. These cells exhibit variables in their expression of extracellular matrix proteins. MBA-15 produced only type I collagen [Benayahu et al., 1989] and expressed mRNA for various noncollagenous proteins [Benayahu et al., 1994a]. MBA-1 produced type I and type III collagen [Zipori et al., 1985a], and expressed various non-collagenous proteins [Benayahu et al., 1994c]. MBA-2.1 cells produced collagens type I, IV, and V. 14F1.1 cells produced types I and IV collagen [Zipori et al., 1985a,b]. Alkaline phosphatase (ALK-P) has long been correlated with osteoblast differentiation. The level of this enzyme was also shown to be variable between the stromal cell types [Benayahu et al., 1991]. Hormonal response to dexamethasone, retinoic acid, vitamin D, and growth factors modulate the ALK-P activity of these cells [Benayahu et al., 1991, 1994a]. Our studies on the cell line, MBA-15, demonstrated their ability to form osteogenic tissue when implanted in vivo within diffusion chambers [Benayahu et al., 1989], and MBA-15 and MBA-1 also formed bone under the kidney capsule [Benayahu et al., 1994b,c]. Such properties support the hypothesis that there are stem cells present within the marrow stroma giving rise to a variety of mature cell lineages necessary to establish the marrow stromal compartment. The relationships of the osteoblastic MBA-15 cells in the hemopoiesis regulation was studied in vitro [Benayahu et al., 1992] and in vivo [Benayahu et al., 1994b].

In this study, our aim was to extend the characterization of these lines by demonstrating their ability to bind to various bone ECM components. In particular, we studied the interactions of various stromal cells with specific matrix components in vitro. This knowledge may broaden our understanding of the cellular interactions leading to specific cellular changes in a specific local microenvironment. Furthermore, this experimental system may serve as a model to define cellular-matrix interactions and shed light on the nature of this relationship between the marrow stroma at the endosteal bone surfaces which occurs in situ.

MATERIALS AND METHODS

Cells

Previously described mouse bone marrow-derived stromal cell lines were included in this study: MBA-2.1 endothelial-like, 14F1.1 endothelial-adipocyte, and MBA-15 (an osteoblastic cell line) [Benayahu et al., 1989, 1991] and its clonal subpopulations MBA-15.4 and MBA-15.6 [Fried et al., 1993]. Other osteoblastic cell lines used were MC-3T3-E1 and ROS 17/2.8 cells. The various cell lines were seeded in tissue culture plates (Nunc, Intermed, Denmark) in growth medium containing high glucose DMEM and supplemented with 10% FCS (Bet-Haemek, Israel). Cultures were incubated at 37°C in a humidified atmosphere of 10% CO_2 in air. Cells used for experiments were trypsinized and counted before seeding.

Extracellular Matrix Proteins

These proteins included pepsin-solublized collagen (Coll I) from bovine dermal collagen (Vitrogen 100, Collagen Corp., Palo Alto, CA); skin collagens (S. Coll), a mixture of type I, III, and V (Collagen Corp.); fibronectin (FN) from human plasma, vitronectin (VN) from human plasma (Collaborative Research Inc., Bedford, MA); thrombospondin (TS) from human platelets (Calbiochem, San Diego, CA); bovine serum albumin (BSA), fraction V (ICN Biochemical, Irvine, CA). Serum-free conditioned medium

from confluent primary cultures of human bone cells (h OB-CM) [Gehron Robey and Termine, 1985; Grzesik and Gehron Robey, 1994] was used. Fraction A and Fraction B from rat long bone extracts were separated as described in Polyacrylamide Separation of Bone Matrix Proteins below. All stocks of proteins were stored frozen in aliquots.

Extraction and Chromatography of Bone Matrix Proteins

Normal rat long bone was trimmed free of soft tissue, cut into small pieces, and processed to a fine powder under liquid nitrogen. Milled bone was extracted in 4 M guanidine-HCl. 0.05 M Tris, 0.1 M 6-aminocaproic acid (Sigma Chemical Co., St. Louis, MO), 5 mM benzamidine HCl, pH 7.4, for 48–72 h at 4°C as previously described elsewhere [Termine et al., 1980]. Chromatography on Sepharose CL-6B (Pharmacia, Piscataway, NJ) columns as described by Termine et al. [1981] was performed, and the fractions which were monitored at 234 nm were pooled and concentrated by ultrafiltration.

Polyacrylamide Separation of Bone Matrix Proteins

For determination of composition, the proteins in extracted fractions were separated by SDS-PAGE gel electrophoresis as described by Fisher et al. [1987]. Fraction A, which was enriched in osteopontin (OPN), was separated on 10% polyacrylamide gel, and Fraction B, which was enriched in matrix gla-protein, was visualized on 15% polyacrylamide gel, both having been stained with Coomassie blue (R-250). Prestained molecular weight standards were used: a range of 106–27.5 kDa (Bio-Rad, Richmond, CA) for 10% SDS-PAGE gel and of 44.5–7.5 kDa (BRL, Gaithersburg, MD) for 15% SDS-PAGE gel.

Thrombin Digestion Assay

To determine the presence of osteopontin, a thrombine-sensitive protein analysis was performed. A freeze-dried portion of Fraction A was digested with thrombin at 37°C for 3 h in 10 mM tris/HCl buffer, pH 7.8, containing 10 mM $CaCl_2$ and 1 U thrombin. Digestion was terminated by the adding of 4-fold concentrated SDS-PAGE sample buffer [Ullrich et al., 1991].

Protein-Coated Dishes

Nontissue-culture, 96-well microtiter plates (Costar, Cambridge, MA) were treated with an aliquot of 50 μl/well at various concentrations ranging as detailed in Results. The coating proteins were diluted in PBS containing 1 mM $CaCl_2$ (coating buffer). The plates were incubated for 1 h at room temperature (RT), rinsed with the same buffer and incubated with 0.5% BSA for 30 min in order to block nonspecific adhesion sites. They were finally rinsed with PBS containing 1 mM $CaCl_2$ and 0.5% BSA (washing buffer).

Attachment Assay

The cells were dispersed using trypsin-EDTA (Beth-Haemek, Israel), washed once in growth medium and resuspended in DMEM containing 0.5% BSA and 5 μgr/ml ITS Premix (Collaborative Research Inc., Bedford, MA). They were counted and diluted to the required cell density as described in each experiment, and seeded into the protein-coated wells for specified incubation time intervals at 37°C, as described in Results. Following incubation, the unattached cells were removed by aspirating the medium, and the wells were rinsed twice with PBS. The attached cells were quantitatively determined by the uptake of methylene blue as described by Goldman and Bar-Shavit [1979]. Briefly, the attached cells were fixed with 3% paraformaldehyde, rinsed with 0.1 M borate buffer, pH 8.5, and stained with 1% methylene blue for 10 min at RT. The wells were washed four times in borate buffer to remove all unbound dye. Cell-bound dye was dissolved in 0.1 N HCl during incubation for 40 min at 37°C. The dye released from the cells was determined by its absorbance at a 550 nm wavelength using a microplate reader (vMax, Molecular Devices, Palo Alto, CA).

Chemotaxis Assay

A modified Boyden 48-well Micro Chemotaxis Chamber (Neuro Probe® Inc., Bethesda, MD) was used for the assayed proteins. Twenty-five microliters of each protein in triplicate was added to the bottom 48 wells and a nucleopore membrane filter of 8-μm holes (Nucleopore Corp., Pleasanton, CA) was then placed on top of the wells in the bottom plate. The gasket and top plate were fixed in place, and the assembly chamber containing the tested cells was incubated for 3 h at 37°C in humidified air. After incubation, the top plate,

gasket, and filter were removed, the cells on top of the filter were wiped off and the filter was fixed in methanol for 2 min. The filter was air dried on a glass slide and stained with Diff-Quick (Merz & Dade AG, Düolingen, Switzerland). The cells were counted using a Nikon microscope with a 10× objective.

RESULTS

Basic Conditions for Attachment Assay

Determination of cell density and time course. The attached cells were quantitatively determined by the uptake of methylene blue as described in Materials and Methods. The cells applied in densities ranging between 16,000–80,000 cells/well as standard curve showed a linear correlation to the OD absorbance measured (data not shown). Furthermore, microtiter plates were coated with collagen, and MBA-15 cells were applied in two cell densities, 40,000 and 80,000 cell/well, which were allowed to incubate for 1–5 h (Table I). The determination of minimal experimental time was by selecting that which would correspond to the maximal amount of attached cells. According to these results, 40,000 cells/well and 3 h of incubation were chosen for the further attachment experiments.

Protein concentration for attachment assay. Proteins were applied on microplates in a dose-dependent manner. We defined the optimal lowest protein concentration for each protein to use for coating the plastic wells which would provide the attachment of the highest number of the osteoblastic MBA-15 cells. The range of concentrations for the individual proteins examined and the final concentrations chosen are summarized in Table II. The dose response varied significantly from protein to protein, and this reflected the different numbers of binding domains for each protein. The concentrations of proteins as substrate for attachment were optimized for attachment of the osteoblastic, MBA-15 cells since they are the focus of this study. The MBA-15 cell line was further studied and compared to other osteoblastic cells lines as well as to other stromal cell clones.

TABLE I. Time Curve of MBA-15 Cell Adhesion*

Cell density/well	Time (h)	OD absorbance
40,000	1	0.24
	3	0.28
	5	0.31
80,000	1	0.45
	3	0.47

*Optimization of MBA-15 cell plating density and length of incubation for adhesion was determined and 40,000 and 80,000 cells were added to well-precoated 96-well plates with collagen in coating buffer. The cell adherence was determined after 1–5 h of incubation. The results show the optical density (OD) at 550 nm determined for the uptake of methylene blue.

TABLE II. Range of Protein Concentration Applied for Attachment Assay

Protein	Range examined	Final concentration for assay[a]
BSA	2–0.25mg/ml	0.5 mg/ml = 7.2 μM
FN	20–1μg/ml	5 μg/ml = 0.012 nM
COL I	100–1μg/ml	10 μg/ml = 0.033 μM
S. Coll	0.8–0.02mg/ml	0.1 mg/ml = 0.33 μM
LN	20–0.3μg/ml	2.5 μg/ml = 0.003 μM
VN	50–12.5ng/ml	25 ng/ml = 0.35 nM
TSP	1–0.25μg/ml	0.5 μg/ml = 0.001 μM
Fractions extracted from rat bone		
Fraction A (OPN)		
1–0.01 mg/ml		150 μg/ml
Fraction B (MGP)		
1–0.15 mg/ml		50 μg/ml

[a]The concentration chosen for each protein was the lowest at which the highest number of cells become attached using MBA-15 (40,000 cells/well).

An inhibitor of protein synthesis was added to incubated cells during the attachment assay to inhibit endogenous synthesis of adhesion proteins. The effect of cyclohexamide when using various quantities was examined in a dose-dependent manner (5–0.002 μg/ml). However, we found only minor effects of cyclohexamide on attachment capability under the final assay conditions used. The subsequent experiments were performed without the addition of this substance.

Polyacrylamide Gel Separation

Proteins were extracted from rat long bones and chromatographically separated. The fractions were collected in correspondence with the described peaks of noncollagenous proteins from human bone [Fisher et al., 1987]. The peaks were analyzed on SDS-PAGE gels. Fraction A showed a heavy band at approximately 70 kDa and a lower band at 45 kDa. The molecular weight migration could indicate the possibility of this peak being a bone sialoprotein (BSP). Using Western blot analysis, we reacted this fraction with a polyclonal antibody (LF-87) that

is specific towards BSP, and obtained a negative response. OPN, whose migration has almost the same molecular weight, was stained well with Coomassie blue whereas BSP was not stained with this dye (Fig. 1A). In addition, thrombin digestion was used to determine the protein in Fraction A. The incubation with thrombin resulted in both a loss of the 70 kDa band and the appearance a new lower band of 32 kDa (Fig. 1A). The evidence of thrombin cleavage indicates the protein as being OPN by the appearance of its Coomassie blue staining and by the location of the thrombin-sensitive site. Fraction B is a protein with a lower molecular weight, has a 14 kDa band, and is most likely a matrix gla-protein (MGP) (Fig. 1B).

Preferential Binding of Stromal Osteogenic and Non-Osteogenic Cells to Individual Matrix Components

To determine the nature of the cellular adhesion, a variety of cell lines representing osteogenic and non-osteogenic subpopulations of marrow stroma [Benayahu et al., 1991], were allowed to attach to different matrix proteins. The tested proteins, representing the major proteins in bone matrix, were analyzed according to their attachment-inducing activity. Major differences between cell subtypes were subsequently revealed. The non-osteogenic cells, MBA-2.1 and 14F1.1, expressed a low attachment ability to all matrix proteins examined (Fig. 2). On the other hand, the stromal osteoblastic cells, MBA-15, MC-3T3-E1, and ROS 17/2.8, showed binding affinity to various proteins. MBA-15 cells and, to a lesser extent, MC-3T3-E1 cells were capable of adhering to all proteins tested. The range of attachment was 2- to 17-fold over the non-specific binding determined on BSA-coated wells. The most effective binding was to FN (up to 17-fold) and to a lesser extent to skin preparations (S. Coll) containing collagen types I, III, and V (9-fold). Coll I enhanced cell attachment (4-fold) followed by VN and TS (3-fold). Other noncollagenous matrix proteins which were extracted and purified from long bone, Fraction A (OPN) and Fraction B (MGP), revealed a similar attachment pattern, and the amount of OB cells successfully binding was up to 5-fold. Serum-free conditioned medium (h OB-CM) supported attachment only of the osteoblastic cells.

Attachment Capability and Cellular Maturation Stage

In order to evaluate the attachment potential with regard to the cells' differentiation stage, we used clonal subpopulations of MBA-15. These cells have been recently characterized as representing different stages of differentiation [Fried et al., 1993]. The MBA-15.4 cell line was shown to behave as an early cell in the osteoblastic lineage, while the MBA-15.6 cell line appeared to represent a more mature stage. The parental cell line, MBA-15, and the two clonal lines, MBA-15.4 and MBA-15.6, were compared (Fig. 3). The attachment capability of these lines was exam-

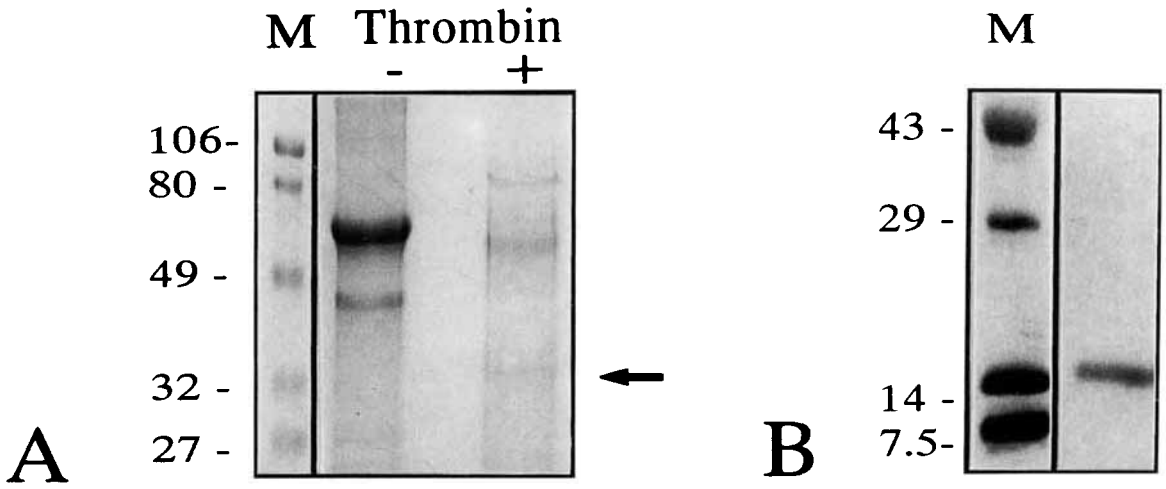

Fig. 1. SDS-gel electrophoresis of peaks from fractionated rat bone extracts. **A:** Fraction A shows two bands at approximately 70 and 45 kDa, and these proteins were reduced to 32 kDa Mr (*arrow*) following thrombin cleavage. **B:** Fraction B shows a single band of 14 kDa Mr, representing enriched matrix gla-protein. Both gels are stained with Coomassie blue (R-250).

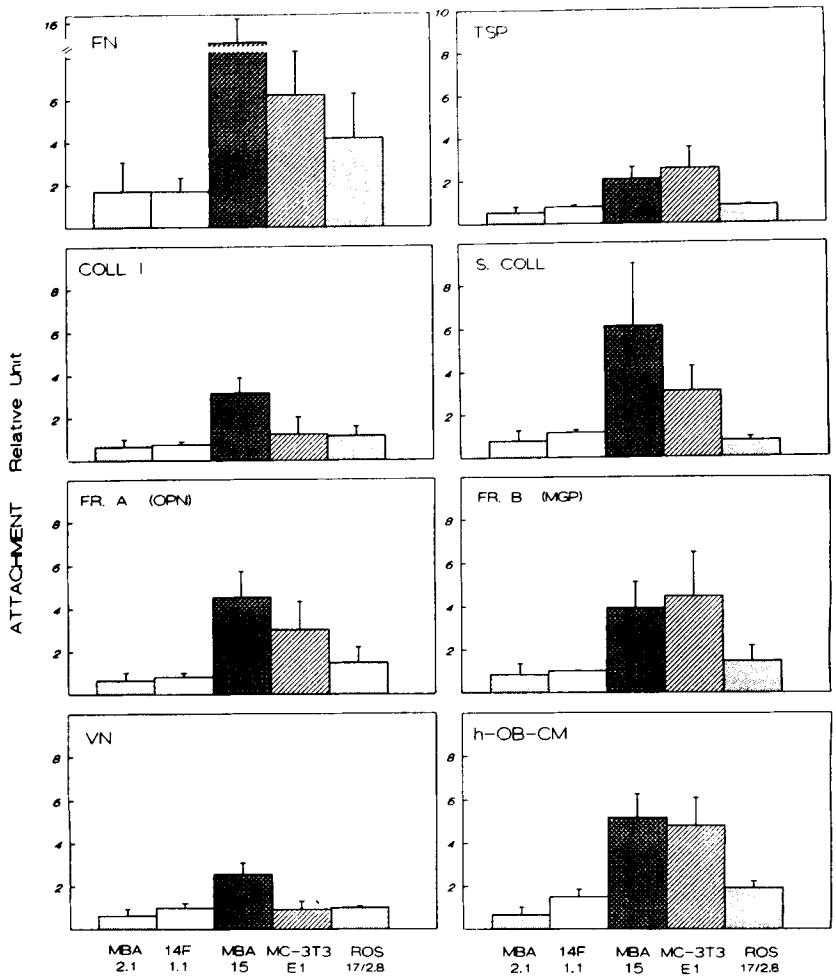

Fig. 2. The preferential attachment of various stromal and osteoblastic cells to the bone matrix proteins. Confluent MBA-2.1 fibroendothelial, 14F1.1 adipocyte, MBA-15 stromal osteoblast, MC-3T3-E1 calvaria osteoblast, and ROS 17/2.8 osteosarcoma cells were examined. The proteins were coated at the concentrations summarized in Table II. The results are expressed as relative adhesion of each protein/BSA level. The values are means ± SEM of duplicate determination of 3-4 separate experiments.

ined when cells were harvested from two different stages in culture: cells from proliferating cultures were compared to those from confluent cultures. The MBA-15.4 cells exhibited high attachment to FN and S. Coll (30-fold), to Fraction A (enriched with OPN, 8-fold), and to all other proteins but to a lesser extent. In comparison, the mature cell, MBA-15.6, expressed attachment capability to FN and collagen (from 2.5- to 7.5-fold) and had a lower binding activity with all other NCP.

Chemotaxis to Matrix Proteins

Various stromal cells were examined for their chemotaxis potential to the matrix proteins investigated in the attachment assay. We were not able to demonstrate any chemoattraction response by either the stromal cells (MBA-1.1.1, MBA-2.1, MBA-15) or MC-3T3-E1 towards these matrix proteins. However, the osteoblastic cells, MBA-15 and MC-3T3-E1, were chemoattracted towards the h OB-CM. These results may imply that the examined single ECM constituent has a role in cell adhesion alone. The chemotaxis elicited by the h OB-CM may indicate the presence of cytokines other than the matrix proteins secreted by the h OB cells.

DISCUSSION

While osteoprogenitor cells exist in the medullary cavity, their osteogenic potential is fully expressed only on endosteal bone surfaces. At this point, they interact with bone matrix, and this may induce subsequent maturation cellular

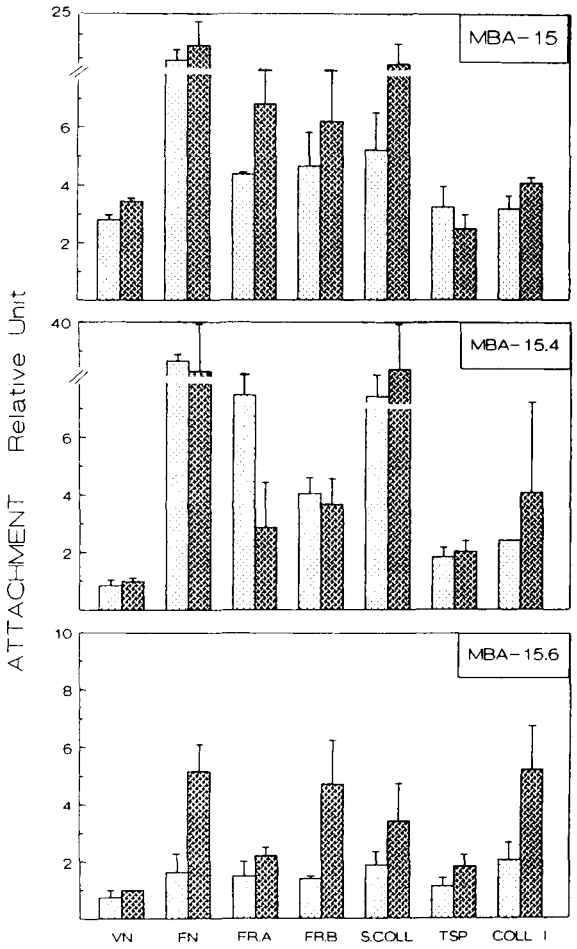

Fig. 3. Attachment capability correlated with proliferation and differentiation stages. MBA-15, compared to immature MBA-15.4 and mature MBA-15.6 cell lines, were seeded on precoated wells with various proteins as described in Figure 2. The results are expressed as relative adhesion of each protein/BSA level. Cell harvested from proliferating stock cultures (*light dotted bar*) and from confluent cultures (*dark dotted bar*) are depicted. The values are means of duplicate determination of two separate experiments.

expression. It is well recognized that the ECM proteins enhance proliferation and differentiation in vivo and that this occurs in many culture systems. The matrix plays a role in the anchorage of cells to the substrate and then may further modulate their differentiation or change their response to hormones and growth factors. The cells' interactions with a specific ECM may also specifically control the development of various tissues in organs. This study was geared to characterize the specific relation between the marrow stromal system; the cellular interactions with ECM proteins from bone. This may shed light on the cell-matrix relations occurring in situ and may reflect the interactions between the various stromal cell subtypes and bone proteins existing in the medullary cavity and the bone-marrow interface.

Bone matrix contains collagen and noncollagenous proteins. The main function of the ECM is to provide structural support for the cells that synthesize it and ultimately mineralize it [Termine, 1993]. Analysis of the functions of the matrix leads to the understanding of its principal role in bone organization. In this study, we used various stromal cells (MBA series) [Benayahu et al., 1991] which enabled us to create an experimental model whose specificity was to emphasize the cell-matrix interactions. Differences in adhesion patterns of osteoblastic and non-osteoblastic stromal cells were revealed. The attachment of osteoblastic cells was highest on FN and, to a lesser extent, on collagens and other NCP such as VN and TS. We also evaluated these proteins' potential for stromal and osteoblastic cell attraction by chemotaxis. None of these matrix proteins examined was able to induce migration of cells. The human OB-CM was chemotactic. As shown earlier, this human OB-CM was produced by osteoblastic cells which synthesized various matrix proteins and cytokines [Gehron Robey and Termine, 1985]. The chemoattraction response of the osteoblastic cells to h OB-CM may be due to soluble cytokines secreted by these cells. It is known that osteoblastic cells secrete and respond to growth factors. TGFβ, PDGF, IGF-1, and IGF-2 are known to be secreted by osteoblasts and were shown to have chemotactic attraction to various osteoblastic cells such as: MG-63, MC3T3-E1, ROS 17/2, rat calvaria, and primary cells [Padley et al., 1991; Xie et al., 1994; Panagakos, 1993; Tsukamoto et al., 1991; Hughes et al., 1992; Pfeilschifter et al., 1990]. It is believed that the growth factors' chemoattraction leads to cell migration and is followed by further enhancement of the osteoblastic cells' regulation during bone remodeling and healing. Our results indicate that the ECM constituents are actively involved with cell adhesion of osteoblasts, but not in chemotaxis of these cells. The major adhesive protein and most effective substrate shown for the examined osteogenic cell was FN. FN receptors, belonging to the family of integrins, were identified on osteoblastic cells in vitro [Brighton and Albelda, 1991; McCarthy and Gronowicz, 1991; Dedhar, 1989] and in vivo [Muschler and Horwitz, 1991; Grzesik and Gehron Robey, 1994]. The mechanism of cell adhe-

sion to FN substrata was suggested as being mediated through receptors containing RGD sequence [Majeska et al., 1993; Clover et al., 1992; Grzesik et al., 1993] or by being RGD-independent [Grzesik et al., 1993].

It was recently shown that MGP acts as an attachment protein even though this protein does not contain the RGD motive sequence [Loeser and Wallin, 1992]. Similar ability of cell attachment with a non-RGD site was expressed upon cleavage of BSP [Mintz et al., 1993] and OPN. In light of these data, it was hypothesized that the cells are expressing receptors that can bind to the RGD and/or non-RGD motifs. The presence of an alternative cell attachment site that does not involve RGD sequence is an interesting possibility; it may even serve to mediate several other pathways of cellular-matrix interactions.

Clarifying the ability of osteoblasts to bind to a certain ECM component is important in the understanding of the regulation processes leading to cell maturation only at the bone surface. The osteoblastic family contains a number of subpopulations of cells at various differentiation stages. The differences in the adhesion potential of different cell maturational stages was studied by using MBA-15.4, a less differentiated cell, in comparison to MBA-15.6, a mature cell stage of the osteoblastic lineage [Fried et al., 1993]. The latter had the ability to adhere to the NCPs more effectively, suggesting that not all cells in the osteoblastic lineage express the same receptors as has been shown in vivo [Grzesik and Gehron-Robey, 1994]. These results should stimulate a new series of investigations geared to identify and characterize the nature of the receptors expressed at different stages of bone cells as they mature. This may lead to a new view of the matrix regulation of various stages of cell differentiation in the osteogenic family. The interreaction with specific matrices is a prerequisite for osteoblast function and elucidation of the understanding of bone physiology. This may reflect new lines of treatment of metabolic skeletal disorders such as in osteoporosis. This disorder may involve deficient bone formation due to defective attachment and subsequent maturation of putative osteoblastic progenitors. Other bone disorders may be due to chemical changes in the bone matrix [Burnell et al., 1982; Katsuyuki et al., 1976] and may differentially reflect the various stages in the differentiation of osteoblastic cells. The cell-matrix relationship is also important in clinical application which requires an integration of implants in osteogenic tissue. Knowledge of the cell-matrix relationship may lead to the improvement of implant surfaces, as the matrix may serve as an osteointegrator inducer on implants used in orthopedics and dentistry [Cannas et al., 1988]. The possible use of specific or combined matrix constituents that mimic the osteoid bone surface may improve the osteogenesis occurring in specific implantation sites and reduce the incidence of non-bonding at the site of interface.

In this study, we demonstrated a variety of adhesion responses of stromal cells to ECM proteins. This cellular system may be a useful tool for promoting the study of various osteoblastic properties in vitro in order to reflect their functions in vivo.

ACKNOWLEDGMENTS

This study was supported by grants from the United States-Israel Binational Science Foundation (89-482) to S.W., and by grants from Basic Fund and The Schrieber Fund of Tel-Aviv University to D.B.

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