Integrin-extracellular Matrix Interactions in Connective Tissue Remodeling and Osteoblast Differentiation

R.K. Globus\textsuperscript{1,2}, A. Moursi\textsuperscript{3}, D. Zimmerman\textsuperscript{3}, J. Lull\textsuperscript{1,2}, and C. Damsky\textsuperscript{3}

Department of \textsuperscript{1}Medicine and \textsuperscript{3}Stomatology, University of California San Francisco, CA and \textsuperscript{2}NASA-Ames Research Center Moffett Field, CA.

ABSTRACT

The differentiation of bone cells is a complex multistep process. Bone is somewhat unusual in that it is very actively and continually remodeled in the adult and that maintenance of its mass in the mature organism is exquisitely sensitive to mechanical as well as chemical signals. Bone is also unique because it consists of a very large amount of extracellular matrix (ECM) that is mineralized. The integrin family of ECM receptors has been shown to play an important role in tissue morphogenesis in several systems. Our studies on the regulation of matrix remodeling enzymes by integrins in rabbit synovial fibroblasts show that two b1 integrin fibronectin (FN) receptor complexes (\(\alpha 5\beta 1\) and \(\alpha 4\beta 1\)) cooperate in detecting subtle changes in the composition of the ECM. As a result of signal transduction by these integrins, the levels of mRNA and protein for several members of the metalloproteinase family are regulated in these cells. We have also used antibody and RGD peptide perturbation studies to determine the significance of cell/ECM interactions to normal osteogenesis. We found that interactions between the cell binding domain of FN and integrins are required for both normal morphogenesis and gene expression in cultured osteoblasts that differentiate to form bone-like tissue in culture. These data lead us to propose that b1 integrins play an important role in osteoblast differentiation as well as in bone remodeling.

INTRODUCTION

Osteoblasts are responsible for producing a complex ECM needed to provide structural support for the organism. Thus, osteoblasts must be responsive both to changes in mechanical loading as well as to systemic demands for the minerals stored in bone’s matrix. To help accomplish these tasks, osteoblasts interact directly with both growth factors and adhesive ligands that reside in the ECM. The ECM of bone contains a vast assortment of growth factors, including members of the transforming growth factor-\(\beta\), fibroblast growth factor, platelet derived growth factor and insulin-like growth factor families, which regulate the proliferation and differentiation of osteoblasts and their precursors (Hauschka et al., 1986; Canalís et al., 1993). In addition, interactions between adhesion receptors and abundant structural components of the ECM, such as collagen and FN, are likely to play key regulatory roles in normal modeling and remodeling processes as well as in disuse, injury and disease.

Integrins are an important class of transmembrane proteins that mediate adhesive interactions between cells with one another and with components of the ECM (Yamada, 1991; Damsky and Werb, 1992; Hynes, 1992). As shown in Figure 1, integrins are heterodimeric proteins consisting of one \(\alpha\) and one \(\beta\) subunit that together bind ECM components and determine ligand specificity. The \(\beta\) subunit interacts with the cytoskeleton, serving to provide a direct physical link between matrix proteins in the extracellular space and cytoskeletal components and signaling molecules inside of the cell. Ligation of integrins initiates signaling cascades shared with growth factors and hormones, including cytoplasmic alkalinization, tyrosine phosphorylation, and increased intracellular calcium (Damsky and Werb, 1992). Integrin activation also causes cytoskeletal reorganization and cell shape changes that may play a role in the cellular adaptation to mechanical forces (Ingber, 1991). Integrins may bind more than one matrix component, and several different integrins are capable of interacting with a single ligand (Figure 1); this complexity permits cellular responses to be fine-tuned to the precise composition of the matrix.

The control of differentiation, morphogenesis, and remodeling by integrin ligation are active areas of investigation that are also highly relevant to the problem of how osteoblasts adapt to mechanical loading (Ashkenas et al., 1994). Cells in the osteoblast lineage are known to express a variety of different integrin subunits, including \(\alpha 1\), \(\alpha 2\), \(\alpha 3\), \(\alpha 4\), \(\alpha 5\), \(\alpha V\) and \(\beta 1\), \(\beta 3\) and \(\beta 5\) (Albelda and Buck, 1990; Brighton and Albelda, 1992; Clover et al., 1992; Hughes et al., 1993; Grzesik and Robey, 1994; Saito et al., 1994). However, very little is known about the functional role of integrins in osteoblasts. Integrins have been shown to mediate adhesion of osteoblasts to specific ligands that comprise the ECM of bone (Brighton and Albelda, 1992; Majeska et al., 1993; Grzesik and Robey, 1994). In addition, transformed human osteoblasts selected for increased expression of the \(\alpha 5\beta 1\) integrin demonstrate enhanced ability to differentiate and to mineralize their ECM (Dedhar et al., 1987; Dedhar et al., 1989). Furthermore, function-blocking antibodies against \(\alpha 5\beta 1\) prevent interleukin 1β-stimulated increases in alkaline phosphatase, an early marker of osteoblast differentiation (Dedhar, 1989). These results indicate that \(\alpha 5\) and \(\beta 1\) may be important in regulating early stages of osteoblast differentiation.

In addition to effects on osteoblast differentiation, integrin-ECM interactions may also participate in the regulation of ECM remodeling in bone. This hypothesis

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Figure 1. Integrin-ECM Receptors. The integrin superfamily of heterodimeric receptors comprises at least 8 β and 16 α subunits. The β1 and αv families contain integrins that interact with ECM components; some integrins interact with more than one ligand, as shown. The integrin binding domains of the ligands are in parentheses.

arises from studies using rabbit synovial fibroblasts (RSF), in which we have demonstrated regulation of matrix metalloproteinase (MMP) genes by integrin-ECM interactions (Werb et al., 1989; Tremble et al., 1993; studies, diagrammed in Figure 2, RSF express either basal or elevated levels of MMP depending on the composition of their ECM. Attachment of RSF to intact FN, vitronectin, or collagen results in a low (basal) level of interstitial collagenase (MMP-1), stromelysin (MMP-3) or the 92 kDa gelatinase-B (MMP-9). However, if RSF are plated on a fragment of FN containing the large central cell binding domain (FN 120), or on substratum-bound peptides containing the RGD sequence, increased levels of MMP are induced via the α5β1 FN receptor (Werb et al., 1989; Huhtala et al., 1995). These data indicate that information in FN outside the cell binding domain is able to override the induction of MMP by α5β1-FN 120 interaction and restore basal expression. When different FN fragments are co-coated on the substrate along with FN 120, only fragments containing the CS-1 peptide from the IIICS region of FN are able to suppress the induction of MMP by FN 120 (see Figure 3A for the structure of FN). The ability of CS-1 to suppress FN 120 induction of MMP is mediated by α4β1, which is strongly expressed by RSF (Figure 2 and Huhtala et al., 1995). Thus, RSF respond to very subtle changes in ECM, and these experiments show that a complex pattern of integrin ligation can regulate specific remodeling processes in connective tissue cells. Clearly then, defining the functionally relevant repertoire of integrins and their ligands in a connective tissue such as bone is an important first step to identify the molecular mechanisms regulating both the synthesis and degradation of the ECM.

We therefore initiated studies to define which matrix ligands and integrins participate in the progressive differentiation of osteoblasts, concentrating our attention on candidates that may mediate the response to mechanical stimuli. Immunocytochemical studies of embryonic rat calvaria revealed that FN was enriched along the periosteal surface and was co-localized with one of its receptors, α5β1. Based on this result, along with the finding that radial bone growth at the periosteal surface is acutely sensitive to inhibition by skeletal unloading and immobilization (Morey-Holton et al., 1994), we tested if either function-perturbing antibodies or competitive inhibitors of FN/integrin interactions affected the differentiation of cultured osteoblasts. We show that cellular interactions with FN appear to be required for the formation of mineralized nodules by cultured osteoblasts and that this response appears to be localized to the central cell binding domain that interacts with several integrins, including α5β1.

METHODS

Cell Culture

Osteoblasts were isolated from 21-day old fetal rat calvariae, as described by Bellows et al. (1987), with several modifications. Cells were combined after a preliminary 10 min. treatment with 570 U/ml collagenase (Worthington Biochemical Corp., Freehold, NJ) at 37°C from four consecutive 20 min. digestions then filtered through both 100 μm and 37 μm Nitex filters. Cells were plated at 25,000 cells/cm² on plastic dishes and incubated overnight. Attached cells were then removed using 0.53 mM ethylenediaminetetraacetic acid with 0.05%
Figure 3. FN Structure and Inhibition of Nodule Formation by Factors Added to Osteoblast Cultures. A. Schematic diagram of intact plasma FN. Binding activities of distinct FN domains are indicated. See text for further details. Sy = synergy domain in IIIβ. B. Cells purified from calvariae of 21-day old fetal rats grew to confluence within 3d. Thereafter, the medium was supplemented with 50 µg/ml ascorbic acid and 3 mM β-glycerophosphate to induce differentiation (see Methods). Various factors were added continuously to the culture medium; shaded bars depict antibodies to the displayed regions of FN and open bars depict peptides that correspond to the displayed regions or sequences of FN. The antibodies were tested for their ability to block nodule formation whereas the peptides added to the culture medium were assessed for their ability to act as competitive agonists for integrin binding to the corresponding region of intact FN within the ECM. Nodule formation was assessed by the appearance of clusters of refractory round cells that, once formed, typically matured into bone-like nodules containing abundant, banded type I collagen (unless treated with ORGDSPK). Inhibitory activity of the various added components was assessed visually and confirmed by quantification of nodule surface area (see Methods).

Histology

Calvaria from 21 day old rat fetuses (similar to those from which osteoblasts were isolated for cell culture) were imbedded in Tissue-Tek O.C.T. (Miles, Inc., Elkhart, IN) then 8 mm thick sections cut using a cryostat. These tissue sections were then fixed in 4% paraformaldehyde in phosphate buffered saline for 30 minutes. Cells grown on plastic slides were fixed with 4% formaldehyde, permeabilized with cold methanol, rinsed three times with phosphate buffered saline (0.01 M sodium phosphate, 0.98% NaCl, pH 7.4; PBS) then either prepared for staining or incubated with 5% sucrose in PBS then carefully removed as an intact sheet, imbedded in O.C.T., frozen in isopentane in a liquid nitrogen bath then sectioned into 8 mm thick sections.

Immunofluorescence

Fixed tissue or cells were washed with PBS, then
treated with 0.1M glycine (Sigma Chem. Co., St. Louis, MO) in PBS. Non-specific interactions were blocked by incubating with 0.5% casein (Sigma Chem. Co., St. Louis, MO), 5.0% bovine serum albumin (Sigma Chem. Co., St. Louis, MO), 0.1% Tween 20 (Fisher Scientific, Fair Lawn, NJ) and 2.0% donkey serum (Jackson ImmunoResearch, West Grove, PA). Primary antibody was incubated on the slides overnight at 4°C, washed, then rhodamine-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA) were added for 30 minutes at room temperature. Slides were then washed and coverslips placed with Aqua Polymount (Polysciences Inc., Warrington, PA).

**ECM Ligand and Antibodies**

Synthetic peptides consisting of the amino acid sequences, GRGDSPK and GRADSP, the 120kD fragment of fibronectin (120 FN) and the rabbit anti-rat fibronectin antibody were purchased from Gibco/BRL (Gaithersburg, MD). Rabbit anti-mouse α5 subunit antibodies were kindly provided by Dr. P. Ross (Jewish Hospital, Washington University, St. Louis, MO). Antibodies to the 70kDa amino-terminial fragment of FN were provided by Dr. D. Mosher (University of Wisconsin, Madison, WI). For staining, mouse anti-rat FN antibody was kindly provided by Dr. P. Johnson (University of California, San Francisco). CS-1 peptide (DELPQLVTLPHNLHGPILDPVSTP) and the CS-1C (DELPQLVTLPHNLHGPDSPVPL) and CS-1S (LTEHTHQLLEPVDGVLSDGDPPPL) control peptides were synthesized by the Howard Hughes Medical Institute (University of California, San Francisco). The ovalbumin-conjugated forms were produced as described previously (Huhtala et al., 1995). The IIF-10 FN peptide was a kind gift from Dr. H. Erickson (Duke University, Durham, NC).

**Quantification of Nodule Formation**

To calculate the surface area of nodules, photographic images were captured with a ScanMaker 1850S scanner (Microtek, Tiawan, Rep. of China) linked to a Macintosh IIci computer (Apple Computer Inc., Cupertino, CA) then analysed with NIH Image version 1.57 (National Institutes of Health, public domain). Surface areas were calculated from the average of at least three separate samples.

**Northern Analysis**

Total RNA (10 μg/lane) was fractionated on 1% agarose/formaldehyde gels, blotted to a nylon membrane (Hybond N, Amersham, Arlington Heights, IL), and incubated at 68°C with an [α-32P]CTP-labeled cDNA fragment of rat alkaline phosphatase (500 base pairs; Noda et al., 1987) in hybridization solution (QuickHyb, Stratagene, La Jolla, CA) for one hour. The filters were then washed at high stringency according to the manufacturer's instructions. The filters were exposed to film (X-OMAT AR, Kodak) for one to four days. Uniformity of sample loading was confirmed by comparing by densitometry the intensity of 28S ribosomal RNA bands on the gel stained with acridine orange prior to transfer. The steady-state expression of alkaline phosphatase mRNA was assessed by digitizing the bands on the autoradiogram using a video camera, then analysed with NIH Image version 1.57 (NIH, public domain). The data shown are representative of two experiments.

**RESULTS**

**Distribution of Specific ECM Ligands and Integrins in Embryonic Bone**

We first examined the pattern of expression in 21-day old embryonic rat bone of the ECM components, FN, type I collagen, and osteopontin, that serve as ligands for several different integrins (see Figure 1). As shown in Figure 4, staining for FN appeared particularly intense in periosteal and endosteal tissue and at the surface of bone, although FN staining appeared quite faint in the mineralized matrix. In contrast, Type I collagen is found throughout the periosteal and mineralized tissue, while another ECM protein, osteopontin, was evident only in fully mineralized bone (data not shown). Thus, expression of these ECM components appeared to be spatially restricted. Staining for the α2 and α5 integrins, receptors that recognize collagen and fibronectin, respectively, was evident on cells in periosteal, endosteal, and mineralized tissue. Thus, the expression of α2 and α5 integrin subunits in cells of the osteoblast lineage correspond to the expression of their ligands; type I collagen is distributed throughout mineralized tissue, whereas FN appears concentrated along periosteal sites where cells are actively differentiating into mature osteoblasts.

**Osteoblasts Differentiate in Culture and Express a Pattern of Integrins and Ligands Similar to Intact Bone**

To evaluate the functional role of interactions between components of the ECM and integrins, we used a well-characterized model of cultured fetal rat calvarial osteoblasts (Arnow et al., 1990; Owen et al., 1990). As shown in Figure 5, the cultured cells proliferate, and following treatment with ascorbic acid (50 μg/ml) and β-glycerophosphate (3 mM), they produce an extensive matrix (Bellows et al., 1986). Discrete nodules form and progressively mature and mineralize in culture. Three regions can be distinguished in these cultures: interstitial regions, distinct multilayered regions at the periphery of each nodule, and the nodule proper, which has a more pronounced multilayering of cells and becomes mineralized, as judged by intense von Kossa staining (Figure 5). The mineralized nodules formed in culture contain banded...
**Figure 4.** Distribution of Integrin and Ligand Expression in Fetal Rat Calvariae. Calvariae from 21-day old fetal rats were examined by indirect immunofluorescence using antibodies directed against α5 (A), FN (B), α2 (C), type I collagen (D) or control IgG (E). α2 and α5 were expressed throughout the osteoblast lineage, whereas FN was concentrated at periosteal surfaces and type I collagen was distributed throughout the mineralized tissue. P periosteum; E endosteum; MN mineralized tissue; MS marrow space.

**Figure 5.** Cultured Fetal Rat Osteoblasts Are a Model of Osteogenic Differentiation. Cells purified from calvariae of 21-day old fetal rats grew to confluence within 3d. Thereafter, the medium was supplemented with 50 μg/ml ascorbic acid and 3 mM β-glycerophosphate to induce differentiation. Cells typically initiated nodule formation within 6 to 10 days, characterized by the appearance of clusters of round, refractile cells that, once formed, matured into bone-like nodules containing abundant, banded type I collagen. Mature nodules stained positively by the von Kossa method for calcification within 14-20 days in culture.
type I collagen (results not shown) and resemble the type of immature woven bone that is formed during development (Bellows et al., 1986).

To determine if the pattern of integrin and ligand expression in osteoblasts that differentiate in culture is similar to intact bone, cultures were analyzed using immunochemical staining (data not shown). FN staining appeared abundant throughout the tissue, except in the center of the nodule. Type I collagen was present both within and outside the nodules, whereas osteopontin staining was restricted to the nodules. The α5 and α2 integrin subunits were expressed on all cells in the cultures. Since the spatially restricted pattern of ECM components evident in intact bone was retained in the osteoblast cultures, these cells provide an appropriate model to study integrin/matrix interactions involved in osteogenesis.

Interactions with FN are Required for Nodule Formation

To study the functional role of FN, osteoblast cultures were treated continuously with polyclonal rabbit anti-rat FN IgG or non-immune control IgG, starting with d3 when they became confluent. Anti-FN antibody inhibited the formation of immature nodules, reducing both nodule number (to 10% of IgG controls) and surface area (to 2% of controls) after 11d in culture, as depicted in Figure 6. Histologic and transmission electron microscopy analyses revealed that nodules in control cultures consisted of rounded cells lining an abundant and dense ECM of banded type I collagen. In contrast, cells in cultures treated with FN antibody appeared more fusiform, and produced limited amounts of fibrillar ECM and type I collagen (Figure 6).

Since FN antibodies inhibited nodule morphogenesis, we evaluated whether FN/cell interactions also regulate gene expression. Previous studies have shown that the steady-state expression of mRNA for alkaline phosphatase is highest at early stages of osteoblast differentiation in culture, and that the activity of alkaline phosphatase is both elevated in cells that possess osteogenic potential and closely associated with mineralization of the skeletal ECM (Owen et al., 1990; Wlodarski and Reddi, 1986; Rodan and Noda, 1991). Results in Figure 7 and Table 1 show that FN antibody suppressed steady-state expression of mRNA for alkaline phosphatase in cultures treated with anti-FN antibodies from d3 to d9 to a level even below that of confluent cultures at 3d. After removal of antibody from the medium, nodules formed within 4 days (data not shown), and alkaline phosphatase expression increased markedly (Figure 7, Table 1), showing that the antibody did not exert toxic effects on those cells capable of forming nodules.

To identify which regions in FN were involved in the inhibition of nodule formation (Figure 3B), domains of FN with defined activities in other systems (Hynes, 1990; Huhtala et al., 1995), were added in solution to the medium of confluent osteoblast cultures on day 3 after plating. The 120 kD central cell binding fragment (spanning approximately the region from FN type III repeat 3-11), which interacts with the α5β1 and αV integrins, inhibited the formation of immature nodules to the same extent as the antibody to FN, most likely by interfering with osteoblast-integrin binding to the intact FN associated with the ECM. Similarly a smaller cell binding fragment corresponding to FN type III repeats 7-10, that encompasses both the RGD and synergy sites required for optimal binding via α5β1 (Aota et al., 1994), inhibited nodule formation strongly. When the small RGD peptide GRGDSPK was added, which mimics the major cell binding sequence in FN and several other ligands found in bone matrix, initial formation of nodules was not blocked. However, their maturation was delayed, and the immature nodules regressed after several days of continuous treatment with GRGDSPK. Since the numbers of cells in cultures treated with GRGDSPK or the control peptide, GRADSP, were not different (data not shown), this dose of GRGDSPK did not cause cell detachment or block cell replication. Furthermore, when GRGDSPK was removed from the treated cultures, nodules rapidly re-formed and matured. The partial effect of GRGDSPK suggests that sites within the cell binding region in addition to the RGD sequence might be required for the full osteogenic effects of FN.

In contrast to the inhibitory effects of fragments from the central cell binding region of FN, addition of the CS-1
Table I. Densitometric Analysis of Northern for Expression of Alkaline Phosphatase in Rat Osteoblasts. Cells purified from calvariae of 21-day old fetal rats grew to confluence within 3d. Thereafter, the medium was supplemented continuously with 50 µg/ml ascorbic acid and 3 mM β-glycerophosphate to induce differentiation and with either anti-FN IgG or non-immune IgG as a control. After 9d, the anti-FN IgG was omitted from the culture medium to assess the ability of the cultures to recover from inhibition. Total RNA was recovered at the indicated times in culture, and analyzed by Northern blotting for expression of alkaline phosphatase (Figure 7), then the autoradiograms were scanned by densitometry (see Methods). The 28S ribosomal RNA band on the gel served as control for sample loading.

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Figure 7. Anti-FN Antibodies Reversibly Reduce the Steady-State Expression of mRNA for Alkaline Phosphatase in Cultured Osteoblasts. Cells purified from calvariae of 21-day old fetal rats grew to confluence within 3d. Thereafter, the medium was supplemented continuously with 50 µg/ml ascorbic acid and 3 mM β-glycerophosphate to induce differentiation and with either anti-FN IgG or non-immune IgG as a control. After 9d, the anti-FN IgG was omitted from the culture medium to assess the ability of the cultures to recover from inhibition. Total RNA was recovered at the indicated times in culture, and analyzed by Northern blotting for expression of alkaline phosphatase.

peptide from the IIICS region which recognizes the α4β1 integrin, did not interfere with either the initiation or maturation of nodules. This peptide was active in the collagenase suppression assay in RSP (see Introduction). Similarly, a function-blocking antiserum against the N-terminal 70 kDa region of FN, which regulates binding to collagen and assembly of a fibrillar FN matrix (Mosher et al., 1991) had no effect on nodule formation by cultured osteoblasts. Taken together, these results indicate strongly that integrin-mediated interaction of osteoblasts with the cell binding region of FN are required for the organization and maturation of mineralized nodules.

DISCUSSION

FN was shown in this study to play an essential role in the progressive differentiation of cultured osteoblasts. FN was localized to sites where precursors differentiate into mature osteoblasts, both in intact bone and in developing bone nodules in culture. This result is consistent with the finding that FN is present surrounding osteoblasts during endochondral bone formation induced by implanted bone matrix (Weiss and Reddi, 1981) Function-blocking antibodies to FN produced striking morphogenetic effects on nodule formation. FN antibodies caused a marked
decrease in nodule initiation and maturation, and caused the cells to assume a fusiform, fibroblast-like morphology. Ultrastructural analysis revealed that cells treated with antibody did in fact produce typical banded type I collagen fibers, although the collagen was sparse and not organized into nodules as in controls. Consistent with these morphogenic effects, FN Ab strongly reduced the steady-state expression of mRNA for alkaline phospha-
tase. The ability of the cultures once treated with FN antibody to recover both nodule formation and alkaline phosphatase gene expression demonstrates that the treatment was not cytotoxic. Instead, these results support the proposal that FN functions as a signaling molecule required for a cell to progress from a precursor to a mature osteoblast capable of forming new bone.

Since FN possesses a complex array of cellular and matrix binding activities (Hynes, 1992), we sought to identify those domains capable of conferring the ability to form nodules (Figure 3). A fragment corresponding to the cell binding domain of FN (7-10 type III repeats) that includes both the tripeptide, RGD, and proposed synergy sites for optimal ligand binding (Aota et al., 1994; Bowditch et al., 1994), conferred complete inhibitory activity. In contrast, a shorter peptide which lacks the synergy region, GRGDSPK, inhibited the maturation of nodules, but did not prevent the initial morphological changes characteristic of nodule initiation. Therefore, additional sequences within the 7-10 type III repeat region may be required for the full activity of FN in this nodule formation assay. The ability of RGD to inhibit nodule maturation is consistent with the finding that GRGDSPK causes disruption of the collagensous matrix in organ culture of calvaria, in addition to inhibiting bone resorption (Gronowicz and Derome, 1994). A conjugated CS-1 peptide that binds α4β1 failed to affect nodule formation, an expected result since these cells do not appear to express α4β1 as assessed by immunocytochemical and immunoprecipitation methods (unpublished observations).

Interactions between integrins and the ECM are likely to play an important role in the response of osteoblasts and their precursors to mechanical forces generated by weightbearing, although more evidence is needed to support this hypothesis. Integrins have already been shown to mediate contractile forces generated by the cell on a collagensous matrix. α2β1 is required for contraction of collagen gels by mesenchymal cells and overexpression of α2 confers the de novo ability to contract collagen gels (Schiro et al., 1991), demonstrating that mechanical forces exerted by the cell to the surrounding matrix requires α2β1.

Integrin/ECM interactions may be crucial both for the perception of mechanical signals that arise from the outside of the cell and in mediating the cellular responses to such stimuli. We speculate that integrin-mediated interactions of osteoblasts with the central cell binding region of FN, may be responsible for transmitting signals generated by mechanical forces to osteoblast precursors, whereas integrin/collagen I interactions may be involved in executing the cellular responses to mechanical stimuli. As part of the cellular signaling response, mechanical stimuli may cause reorganization of integrin/cytoskeletal interactions. Mechanical stimulation of MG63 osteosarcoma cells by compression at physiologic levels causes a rapid increase in the cell surface expression of α3β1 (Haskin and Cameron, 1993). Furthermore, direct twisting of β1 integrins using magnetic beads coated with either FN peptide sequences (RGD) or anti-β1 antibody, triggers cytoskeletal stiffening and focal contact formation (Wang et al., 1993). We have found that α5β1 is expressed in cells at the periosteal surface of bone where FN appears to be concentrated and where the inhibitory effects of immobilization and skeletal unloading on bone formation are obvious (Morey-Holton et al., 1994). Together, these results provide circumstantial evidence in favor of the proposal that β1 integrin interactions with FN may transmit mechanical signals generated by weightbearing in osteoblast precursors.

Evidence to support the idea that integrins might also mediate downstream cellular responses to mechanical stimulation is rapidly accumulating in various systems. Wilson et al. (1993) report that the hypertrophic response of vascular smooth muscle cells to stretch is abolished by RGD peptides and is also dependent on the specific substrate components. This response to mechanical stimulation by stretch is mediated by increased transcription of platelet-derived growth factor (PDGF); the rapidity of transcriptional activation and the ability of RGD peptides to block stretch-induced PDGF gene transcription, implicate a possible role for integrins in transmitting mechanical signals in vascular smooth muscle cells. Finally, both collagenolytic enzymes and RGD peptides abolish the gravity-induced polarity of cytoplasmic streaming in characean internodal cells, leading to the proposal that integrin-like proteins participate in gravity sensing in plants (Wayne et al., 1992). Our results show that α2β1 integrins are expressed on mature osteoblasts; whether they mediate response of osteoblasts to mechanical stimulation has not yet been determined.

Thus, integrins may participate in regulating the functional responses of osteoblasts to a variety of mechanical stimuli including gravity, stretch and compression; in the case of bone, mechanical stimulation would be expected ultimately to lead to changes in the amount of bone formed. We have identified FN/integrin interactions as potentially crucial for the formation of new bone, which may also be involved in sensing and conveying mechanical stimuli to osteoblast precursors. Integrins may act alone or in combination with other molecules implicated in mechanogenic signaling, including ion channels, prostaglandins, and growth factors (Burger and Veldhuijzen, 1993). Experimental models potentially useful for exploring the functional significance of integrin/ECM interactions and mechanical loading include cell culture models of mechanical stimulation such as fluid shear,
stretch and compression. Another potentially powerful approach would be to evaluate the dynamics of bone development, growth and remodeling as they relate to mechanical factors using transgenic animals that express altered levels of specific integrins.

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ABBREVIATIONS

- ECM: Extracellular Matrix
- FN: Fibronectin
- FN 120: 120kD FN fragment containing the large central cell binding domain
- MMP: Matrix Metalloproteinase
- PBS: Phosphate Buffered Saline
- PDGF: Platelet-derived Growth Factor
- RGD: tripeptide, Arginine-Glycine-Aspartate
- RSF: Rabbit Synovial Fibroblasts

REFERENCES


