Journal of Cellular Biochemistry 77:499–506 (2000)

Differential Regulation of Cadherins by Dexamethasone in Human Osteoblastic Cells

Fernando Lecanda,[1] Su-Li Cheng,[1] Chan Soo Shin,[1] Mari K. Davidson,[2] Pamela Warlow,[1] Louis V. Avioli,[1] and Roberto Civitelli[1]*

[1]Division of Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, Missouri 63110
[2]Division of Dermatology, Vanderbilt University Medical School, Nashville, Tennessee 37232

Abstract Human osteoblasts express a repertoire of cadherins, including N-cadherin (N-cad), cadherin-11 (C11), and cadherin-4 (C4). We have previously shown that direct cell-cell adhesion via cadherins is critical for BMP-2-induced osteoblast differentiation. In this study, we have analyzed the regulation of cadherin expression in normal human trabecular bone osteoblasts (HOB), and osteoprogenitor marrow stromal cells (BMC), during exposure to dexamethasone, another inducer of human bone cell differentiation. Dexamethasone inhibited the expression of both C11 and N-cad mRNA in both BMC and HOB, although the effect was much more pronounced on N-cad than on C11. This action of the steroid was dose dependent, was maximal at 10^{-7} M concentration, and occurred as early as after 1 day of incubation. By contrast, expression of C4 mRNA and protein was strongly induced by dexamethasone in BMC and was stimulated in HOB. This stimulatory effect lasted for at least 2 weeks of incubation. A cadherin inhibitor, HAV-containing decapeptide only partially (~50%) prevented dexamethasone-induced stimulation of alkaline phosphatase activity by BMC, which instead was not altered by incubation with a neutralizing antibody against C4. Therefore, the pattern of cadherin regulation by dexamethasone radically differs form that observed with BMP-2. Dexamethasone effects on certain osteoblast differentiated features, such as induction of alkaline phosphatase activity are not strictly dependent on cadherin function. J. Cell. Biochem. 77:499–506, 2000.

Key words: osteoblast differentiation; cell-cell adhesion; cadherins; corticosteroids; hormonal regulation

Bone remodeling is a complex process that requires the coordinated activity of many cell types. Bone-forming cells must work in synchrony to produce mineralized matrix in an efficient and organized spatio-temporal fashion and thus maintain bone integrity. Synchronized cell activity in osteoblastic networks is mediated by both soluble factors and direct cell-cell contact and signaling. Increasing evidence indicates that cell-cell interactions via cell adhesion molecules, in particular cadherins, are critically involved in different aspects of bone remodeling, including osteoblast differentiation [Civitelli, 1998] and osteoclast precursors fusion [Mbalaviele et al., 1995]. Cadherins are a large superfamily of cell adhesion molecules that provide calcium-dependent, homophilic cell-cell adhesion [Gumbiner, 1988]. Amply distributed in most tissues, these molecules are involved in variety of biologic processes, including morphogenesis, establishment and maintenance of cell polarity, regulation of cell proliferation, and cell-cell signaling [Takeichi, 1993, 1994; Gumbiner, 1996]. Cadherins are single-chain integral membrane glycoproteins with a single transmembrane domain, a conserved intracellular tail, and a large extracellular domain that defines each individual isotype [Kemler, 1993].

We have previously shown that human osteoblasts express at least three cadherins—cadherin-11 (C11), N-cadherin (N-cad), and cadherin-4 (C4)—and that disruption of cell-cell adhesion using an inhibitory peptide al-

Grant sponsor: National Institutes of Health; Grant number: AR43470; Grant number: AR32087.

Fernando Lecanda and Su-Li Cheng contributed equally to the work reported and should be both considered first authors.

*Correspondence to: Roberto Civitelli, Division of Bone and Mineral Diseases, Barnes-Jewish Hospital of St. Louis, 216 S. Kingshighway Blvd., St. Louis, MO. 63110. E-mail: rcivitel@imgate.wustl.edu

Received 29 October 1999; Accepted 28 December 1999

This article published online in Wiley InterScience, April 2000.

most completely prevented human osteoblast differentiation, assessed as increase in alkaline phosphatase activity induced by recombinant, human BMP-2 (rhBMP-2) [Cheng et al., 1998]. We also found that the relative abundance of osteoblast cadherins changes slightly during rhBMP-2 induced osteoblastic differentiation. In particular, whereas C11 and N-cad are only marginally affected, C4 is sharply downregulated by rhBMP-2 in human models of osteogenic cells [Cheng et al., 1998]. Treatment of human bone marrow osteoprogenitor cells with rhBMP-2 recapitulates most of the steps leading to osteoblast differentiation, including induction of osteoblast-specific genes, such as type I collagen, osteocalcin, and bone sialoprotein [Lecanda et al., 1997]. Osteoblastic precursors can also be induced to differentiate into more mature osteoblastic cells by treatment with dexamethasone, which stimulates alkaline phosphatase expression and mineralization [Cheng et al., 1994a,b]. In these studies, we have monitored cadherin expression in human osteoblastic cells during exposure to dexamethasone, with the goal of finding general patterns of cadherin expression during in vitro human osteoblast differentiation. The results suggest that the relative abundance of different osteoblast cadherins changes under the action of dexamethasone, but these changes are fundamentally distinct from those produced by rhBMP-2. In addition, we found that chemical or immunological inhibition of cadherin function only partially prevents induction of alkaline phosphatase activity by the steroid in human bone cells.

MATERIALS AND METHODS

Antibodies and Chemicals

Monoclonal antibodies specific for C11 (113I and 113H) and C4 (120A) generated against extracellular domains of each cadherin have been previously described [Cheng et al., 1998]. The 120A anti-C4 antibody was tested in a cell aggregation assay [Heimark et al., 1990] using L cells transfected with C4 [Tanihara et al., 1994] for inhibitory activity on C4-dependent adhesion. At the concentration of 100 μg/ml, this antibody completely inhibited cell aggregation among C4-transfected L cells. This was measured by counting the number of particles (cell aggregates) in the absence or in the presence of the antibody, and normalizing to the initial number of particles (isolated cells) in C4-transfected L cell suspensions at the beginning of the incubation period. The number of particles was reduced 65% in the absence (indicating spontaneous cell-cell aggregation), and only 5% in the presence of 120A antibody after 90-min incubation in calcium-containing medium. The decapeptide LGAHAVDING containing the HAV sequence present in human N-cad and a scrambled peptide, VLDGANAGIH containing the same amino acids in a random order, were synthesized on solid phase and purified by reverse-phase high-performance liquid chromatography (RP-HPLC) in the Peptide and Nucleic Acid Chemistry Laboratory of Washington University (St. Louis) as described by Cheng et al. [1998]. All other chemicals and the tissue culture media were obtained from Sigma Chemical Co. (St. Louis, MO), unless otherwise indicated.

Cell Cultures

Human trabecular osteoblasts (HOB) and bone marrow osteoprogenitor stromal cells (BMC), were prepared from human surgical specimens as previously described [Lecanda et al., 1997]. BMC are believed to represent less differentiated osteoblast precursors, and they can be induced to differentiate into more mature osteoblastic cells by treatment with dexamethasone [Cheng et al., 1994a]. For these studies, some BMC cultures were incubated in 10^{-7} M dexamethasone for up to 2 weeks. After this time, most of the cells in the cultures express phenotypic characteristics of differentiated osteoblastic cells [Cheng et al., 1994a]. HOB cells derived from human trabecular bone, are believed to represent more differentiated osteoblast precursors, and they can be induced to mineralize by treatment with dexamethasone [Wong et al., 1990]. In the peptide and antibody inhibition studies, induction of osteogenic differentiation in BMC was assessed by the amount of alkaline phosphatase activity after 7 days of culture, as described [Cheng et al., 1994a].

RNA Blots

Because preliminary experiments showed that cadherin mRNA is expressed at low levels by osteoblastic cells, poly-A RNA was purified from cell extracts using the Mini RiboSep kit (Collaborative Biochemical Products, Bedford,

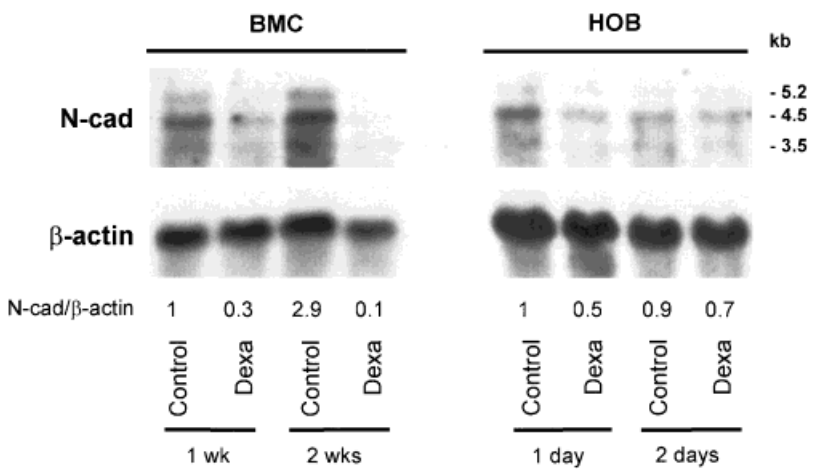

Fig. 1. Effect of dexamethasone on N-cadherin mRNA expression by human osteoblasts. Bone marrow stromal cells (BMC) and human trabecular bone osteoblasts (HOB) were cultured for the indicated periods of time in the absence (Control) or in the presence (Dexa) of 10^{-7} M dexamethasone. mRNA was isolated and hybridized with [^{32}P]-labeled cDNA probes specific for human N-cadherin (N-cad). The membrane was washed and rehybridized for human β-actin (β-actin) to control for quantity and integrity of mRNA. The N-cad/β-actin band intensity ratio determined by densitometry is also shown.

MA). Northern blot analysis was performed using the cDNA probes (1.0-kb *Eco*RI/*Pst*I fragment of C11,1.2 kb *Eco*RI/*Pst*I fragment of C4, 2.5-kb *Pst*I fragment of N-cad) and previously described methods [Cheng et al., 1998]. After each hybridization step, blots were stripped or RNA according to previously published protocols, and the absence of any carryover bands was verified by autoradiography. Relative amounts of mRNA were quantitated by densitometric analysis of autoradiographic bands after normalization for intensity of human β-actin.

Immunoblots

The procedures previously detailed [Cheng et al., 1998] were used. Briefly, cells grown in 100-cm^2 Petri dishes were extracted in a buffer containing 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 1 mM phenantroline, 50 μg/ml benzamidine, 0.5% Triton X-100, Hepes pH 7.4, and protease inhibitors. The latter extraction method ensures a good solubilization of cytoskeletal proteins, to which cadherins are tightly bound. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membranes, and incubated for 2 h at room temperature in Tris-buffered saline (TBS), containing 5% skim milk. The membranes were then incubated for 1 h at room temperature with primary antibody in TBS containing 0.05% Tween-20 (TBS-T). After extensive washing with TBS-T, the membranes were incubated for 1 h at room temperature with alkaline phosphatase-conjugated anti IgG antibody (Promega, Madison, WI) in TBS-T. After washing, the immune reaction was detected by exposing the membranes to nitroblue tetrazolium (NBT) (Sigma).

Statistical Analysis

Each experiment was performed at least in triplicate. Group means were compared by an unpaired Student's *t*-test. Unless otherwise indicated, the data are presented as mean ±SEM.

RESULTS

For these studies, we have used dexamethasone to induce differentiation of human osteoblastic cells, as we have previously described [Cheng et al., 1994a]. In the presence of 10^{-7} M dexamethasone, N-cad mRNA was sharply downregulated in BMC and HOB cells (Fig. 1). This inhibitory effect was evident within 1 week of cell exposure to the steroid and persisted for at least 2 weeks (Fig. 1). The effect was dose-related and strong downregulation was obtained with concentrations of dexamethasone of $\geq 10^{-8}$ M (Fig. 2), corresponding to the doses that induce phenotypic changes in BMC and HOB [Cheng et al., 1994a]. Similar to N-cad, C11 mRNA was also decreased by 10^{-7} M dexamethasone, an effect that was more evident in BMC than in HOB, where only a 40% inhibition was observed (Fig. 3). Similar to N-cad, down-regulation of C11 mRNA had a rapid onset (within days) and persisted for up to 2 weeks. Therefore, at least at the mRNA level, the two major osteoblast cadherins, N-cad and C11 are downregulated by dexamethasone.

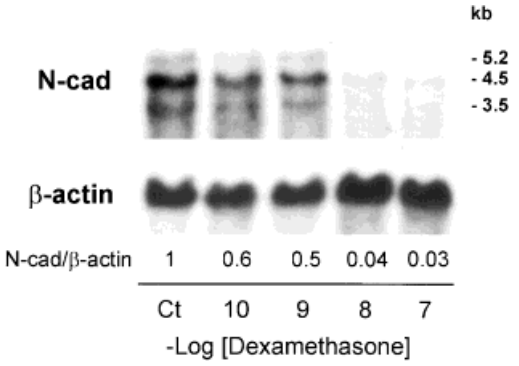

Fig. 2. Dose-response of dexamethasone effect on N-cadherin mRNA by human bone marrow stromal cells. Cells were cultured for 1 week in the absence (Ct) or in the presence of increasing concentrations of dexamethasone, as indicated. mRNA was isolated and hybridized with [^{32}P]-labeled cDNA probes specific for human N-cadherin (N-cad). The membrane was washed and rehybridized for human β-actin to control for quantity and integrity of mRNA.

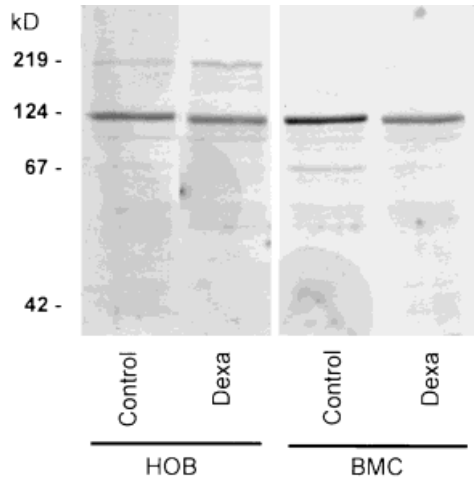

Fig. 4. Regulation of cadherin-11 protein by dexamethasone in human osteoblasts. Cultures of human trabecular bone osteoblasts (HOB) and bone marrow stromal cells (BMC) were incubated in the absence (Control) or in the presence of 10^{-7} M dexamethasone (Dexa) for 1 week. Whole cell lysates were separated by PAGE and blotted with a monoclonal antibody against cadherin-11.

Western blot analysis using monoclonal antibodies confirmed expression of C11 as a single band of ~120 kD in HOB and BMC (Fig. 4). However, there was only a marginal decrease in the intensity of the C11 band in dexamethasone-treated HOB cells as compared with control cells after 1 week incubation (Fig. 4). At this time, the steroid action in C11 mRNA was already maximal (Fig. 3). By contrast, dexamethasone decreased C11 protein abundance by approximately 50% in BMC during the same time period (Fig. 4). Unfortunately, we were unable to obtain specific reactive bands to N-cad, using commercially available antibodies in our human bone cells.

By contrast, while C4 mRNA in basal conditions were barely detectable in BMC, treatment with dexamethasone (10^{-7} M) strongly induced expression of C4 transcripts within 1 day, an effect that was maintained for 7 days (Fig. 5). The effect of the steroid was not restricted to BMC, since dexamethasone increased the abundance of C4 mRNA in HOB as well, although to a lesser degree than in BMC (Fig. 5). Interestingly, only the largest (~7 kb) and the smallest (~3.5-kb) transcripts were increased by dexamethasone, whereas the intensity of the intermediate band (~4.5 kb) observed in untreated HOB was decreased after exposure to the steroid (Fig. 5). This band may represent an N-cad transcript cross-reacting to

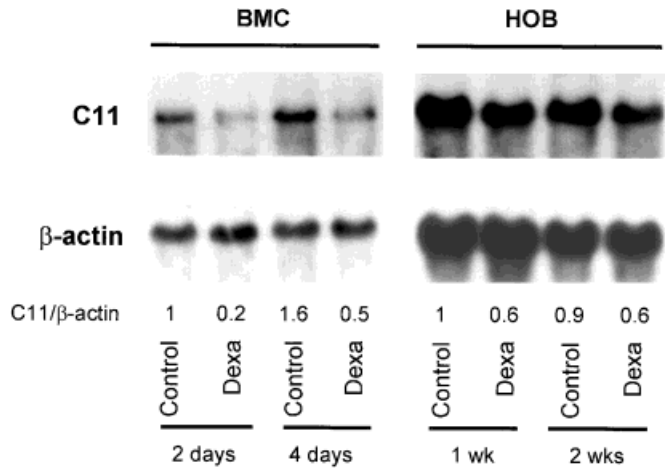

Fig. 3. Effect of dexamethasone on cadherin-11 mRNA expression by human osteoblasts. Bone marrow stromal cells (BMC) and human trabecular bone osteoblasts (HOB) were cultured for the indicated periods of time in the absence (Control) or in the presence (Dexa) of 10^{-7} M dexamethasone. mRNA was isolated and hybridized with [^{32}P]-labeled cDNA probes specific for human cadherin-11 (C11). The membrane was washed and rehybridized for human β-actin to control for quantity and integrity of mRNA. The C11/β-actin band intensity ratio determined by densitometry is also shown.

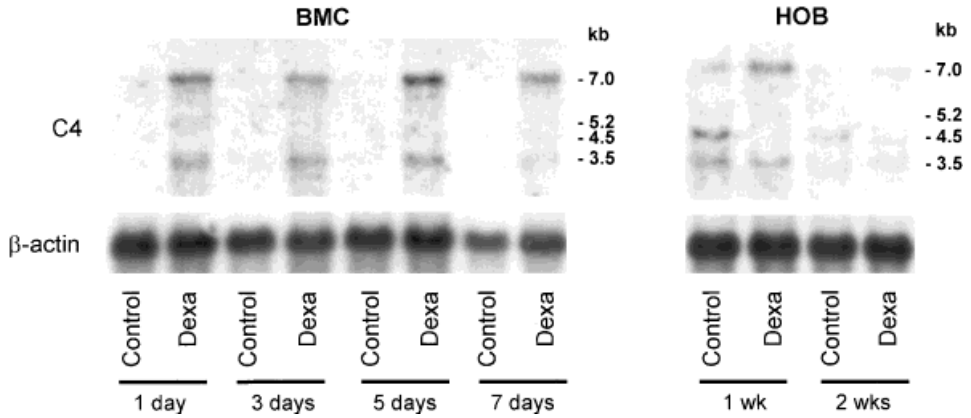

Fig. 5. Effect of dexamethasone on cadherin-4 mRNA expression by human osteoblasts. Bone marrow stromal cells (BMC) and human trabecular bone osteoblasts (HOB) were cultured for the indicated periods of time in the absence (Control) or in the presence of 10^{-7} M dexamethasone (Dexa). mRNA was isolated and hybridized with [^{32}P]-labeled cDNA probes specific for human cadherin-4 (C4). The membrane was washed and rehybridized for human β-actin to control for quantity and integrity of mRNA.

our C4 cDNA probe [Cheng et al., 1998]. Its decrease by dexamethasone, in the face of the increase of the ~7-kb and ~3.5-kb transcripts, would be consistent with this hypothesis. Alternatively, the ~4.5-kb band may represent a different splice variant of C4, which is downregulated by dexamethasone. However, Western blot analysis using a C4-specific monoclonal antibody was consistent with a stimulatory action of the steroid on C4. An immunoreactive band (~120 kD) was present in resting HOB extracts, but not in untreated BMC, whereas dexamethasone induced C4 protein expression in BMC and increased its abundance in HOB (Fig. 6).

We then tested the effects of interfering with cadherin function on dexamethasone-induced differentiation on BMC, using an HAV peptide we had previously shown to almost completely prevent stimulation of alkaline phosphatase by rhBMP-2 in the same cells [Cheng et al., 1998]. Alkaline phosphatase activity, a marker of committed osteogenic cells, was increased after 7 days of incubation with the steroid, as compared with untreated cells. As shown in Figure 7, in the presence of the HAV peptide, induction of alkaline phosphatase activity by the steroid was significantly but only partially blunted (~50% induction compared with control cells), whereas exposure to a scrambled peptide had a minor inhibitory action on dexamethasone-induced alkaline phosphatase activity, probably reflecting an unspecific effect of the high concentrations of peptide. Considering the remarkable upregulation of C4 by

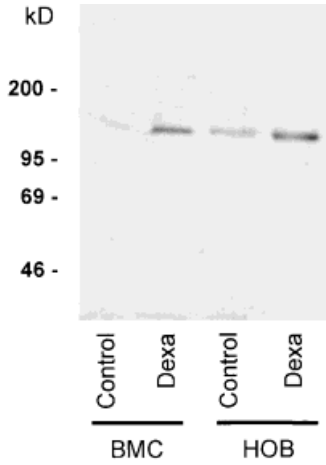

Fig. 6. Regulation of cadherin-4 protein by dexamethasone in human osteoblasts. Whole cell lysates of human bone marrow stromal cells (BMC) and trabecular bone osteoblasts (HOB), grown in the absence (Control) or in the presence of 10^{-7} M dexamethasone (Dexa) were separated by PAGE and blotted with a monoclonal antibody against cadherin-4.

dexamethasone, we then asked whether this cadherin may have a specific role in the action of the steroid on BMC differentiation. To this end, we incubated BMC in the presence of 100 μg/ml of the monoclonal anti-C4 antibody, 120A. At this concentration, this antibody is able to prevent aggregation of L cells expressing C4, thus functioning as a neutralizing antibody (see under Methods). Induction of alkaline phosphatase activity after 1 week incubation with dexamethasone was not affected by the presence of this antibody in the

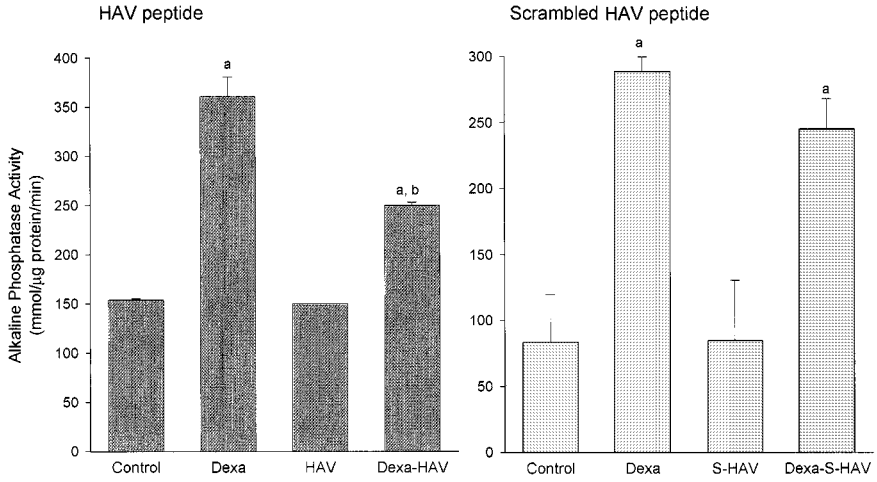

Fig. 7. Effect of an HAV peptide on alkaline phosphatase activity in human bone marrow stromal cells. Cells were seeded at 70–80% density in 24-well plates and grown for 7 days in the absence (Control) or in the presence of 10^{-7} M dexamethasone (Dexa), which was added twice during the incubation period. The HAV peptide LGHAVDING or the scrambled peptide VLDGANAGIH were added daily in fresh medium at the concentration of 0.5 mg/ml. At the end of the incubation period, alkaline phosphatase activity was determined and normalized to protein content, as an index of osteoblast differentiation. $^{a}P < 0.01$ vs. control (*t*-test for unpaired samples); $^{b}P < 0.05$ vs. Dexa alone.

culture medium (Fig. 8). Unavailability of antibodies neutralizing C11 and N-cad function precluded the use of the same approach to test the role of C11 and N-cad in this action of dexamethasone.

DISCUSSION

We have previously identified several members of the cadherin superfamily in human osteoblasts, and demonstrated that the repertoire of cadherins is regulated during rhBMP-2-induced differentiation. We now show that dexamethasone, also an inducer of human osteoblast differentiation and mineralization, alters the pattern of cadherin expression in an entirely different manner compared to that produced by rhBMP-2. Also, in contrast with rhBMP-2-induced differentiation, we found that interference with cadherin function using inhibitory peptides and antibodies only partially affects dexamethasone action on BMC.

While both dexamethasone and rhBMP-2 promote osteogenic differentiation in vitro, rhBMP-2 represents a physiological stimulator of bone formation and its actions in vitro correlate with its in vivo effects [Lecanda et al., 1997]. Although dexamethasone induces human BMC to become more differentiated and mineralize, it downregulates expression of some matrix proteins, such as collagen type I, osteocalcin, osteopontin, and bone sialoprotein

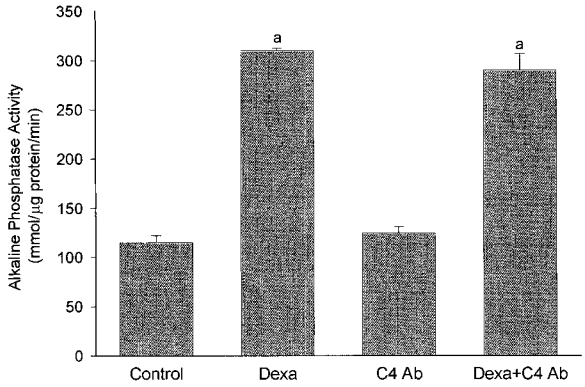

Fig. 8. Lack of effect of a neutralizing anti-C4 antibody on dexamethasone-induced alkaline phosphatase activity. Bone marrow stromal cells were grown for 7 days either in the absence (Control) or in the presence of 10^{-7} M dexamethasone (Dexa), which was added twice during the incubation period. The monoclonal anti-C4 antibody was added at the concentration of 100 μg/ml daily for 7 days. At the end of the incubation period, alkaline phosphatase activity was determined and normalized to protein content. $^{a}P < 0.01$ vs. control (*t*-test for unpaired samples).

[Cheng et al., 1994, 1996], which participate in matrix deposition and are instead stimulated by rhBMP-2 [Lecanda et al., 1997]. This discrepancy reflects radically different actions of these two agents on bone. When administered in vivo, corticosteroids ultimately depress bone formation [Luckert and Kream, 1996], whereas rhBMP-2 is a bone formation simulator. While

the molecular mechanisms by which dexamethasone induces alkaline phosphatase activity and mineralization by human osteoblastic cells in vitro remain unclear, the effects on gene expression most likely reflect the pharmacologic action of corticosteroids on bone. Some of these regulatory actions are clearly unrelated to stimulation of osteoblast differentiation. The results presented underscore this paradigm, demonstrating that osteoblast cadherins are distinctly regulated by dexamethasone, but in an opposite fashion compared to rhBMP-2. Therefore, data obtained with dexamethasone cannot be directly extrapolated to osteoblast differentiation.

The same line of reasoning can be applied to the inability of HAV peptides to completely prevent the stimulatory action of dexamethasone on alkaline phosphatase activity in BMC. Clearly, the effect of the steroid on alkaline phosphatase can be dissociated, at least partially, from cadherin function. The HAV peptide used in this study was a strong inhibitor of calcium-dependent cell-cell adhesion [Cheng et al., 1998], and it also completely prevented the upregulation of alkaline phosphatase by rhBMP-2 in the BMC [Cheng et al., 1998], yet it only partially altered dexamethasone stimulation of alkaline phosphatase. In theory, upregulation of C4 could compensate for the decreased expression of N-cad and C11 under dexamethasone action. However, this compensatory increase is not relevant to the effect of the steroid, as a neutralizing antibody could not prevent its action. The HAV peptide is effective in inhibiting adhesion mediated by type I cadherins (N-cad and C4), which possess the HAV adhesion-recognition motif [Mege et al., 1992], whereas the anti-C4 antibody is specific for C4 [Tanihara et al., 1994]. Therefore, the partial inhibitory activity of the HAV peptide in the face of lack of effect of the anti-C4 antibody indicates that either N-cad or another cadherin, perhaps C11, may participate in the effect of dexamethasone. However, C11 is a type II cadherin, and the HAV peptide may not fully inhibit its adhesive properties. Taken together, our results show that cell-cell adhesion and cadherin function are not critically required for dexamethasone induction of alkaline phosphatase in human osteoprogenitor cells.

The function of individual cadherins in osteoblasts remains to be determined. Cadherin-11 is widely expressed in mesenchymal cells [Simonneau et al., 1995]. During embryonic development, it participates in cell condensation and segregation in the head, somites, and limb buds [Kimura et al., 1995]. Cadherin-11 protein and a spliced variant are abundant in bone cells of different species [Okazaki et al., 1994; Cheng et al., 1998], and their expression does not seem to be changing substantially with differentiation [Kawaguchi et al., 1999; Tsutsumimoto et al., 1999]. Aside the modest downregulation by dexamethasone observed in this study, C11 does not appear to be significantly altered by regulators of bone formation, primarily rhBMP-2 [Cheng et al., 1998] and transforming growth factor-β_2 (TGF-β_2) (unpublished observation). These findings suggest that C11 may be a constitutive protein reflecting the mesenchymal origin of osteoblasts, perhaps contributing to define the fate of osteogenic precursors and segregate committed cells from other mesenchymal lineages. Interestingly, a recent report demonstrated that C11 abundance decreases with aging in rat bone marrow stromal cells [Goomer et al., 1998], an observation consistent with the notion of this cadherin as a constitutive marker of bone marrow stromal cells.

N-cadherin, also abundant on osteoblasts [Ferrari et al., 2000; Cheng et al., 1998], is probably more sensitive to bone remodeling regulators than C11 appears to be. In the present study, N-cad mRNA was suppressed by dexamethasone in human BMC. Downregulation of N-cad was also recently observed in MC3T3-E1 mouse osteoblastic cells after exposure to IL-1 or TNF-α [Tsutsumimoto et al., 1999]. By contrast, although N-cad was barely affected by rhBMP-2 in our previous study [Cheng et al., 1998], others investigators have found, in preliminary reports, that osteoblast N-cad is upregulated by parathyroid hormone [Suva et al., 1994] and by a constitutively active, mutated FGFR-2 receptor [Lemonnier et al., 1998]. Its regulation profile and its high expression in mature osteoblasts position N-cad as a surface molecule tightly associated with osteoblast differentiation and function.

By contrast, the function of C4 in osteoblasts remains elusive. This type I cadherin is suppressed by rhBMP-2 [Cheng et al., 1998] and is strongly upregulated by dexamethasone. As noted above, C4 expression and function are unrelated to dexamethasone action on human osteoblastic cells; in fact, its regulation seems to be inversely correlated to rhBMP-2-induced osteoblast differentiation. Perhaps C4 is linked to other cell lineages originating from bone

marrow precursors, a hypothesis we are currently exploring.

In conclusion, we have shown that dexamethasone regulates the repertoire of cadherins expressed by human osteoblastic cells. Inhibition of cadherin-dependent cell-cell adhesion only partially affects dexamethasone induction of alkaline phosphatase activity in human BMC.

ACKNOWLEDGMENTS

The authors thank Linda Halstead and Aurora Fausto for help with the human tissue cultures, and Janine Harrison and Marykay Ligocki (ICOS, Bothell, WA) with the immunoblots. Part of the material reported in this manuscript has been presented at the 17th and 18th annual meetings of the American Society for Bone and Mineral Research, Baltimore, MD, September 9–13, 1995, and Seattle, WA, September 7–11, 1996 (abstracts M253 and M326, respectively).

REFERENCES

Cheng S-L, Yang JW, Rifas L, Zhang S-F, Avioli LV. 1994a. Differentiation of human bone marrow osteogenic stromal cells in vitro: induction of the osteoblast phenotype by dexamethasone. Endocrinology 134:277–286.

Cheng S-L, Zhang S-F, Nelson TL, Warlow PM, Civitelli R. 1994b. Stimulation of human osteoblast differentiation and function by ipriflavone and its metabolites. Calcif Tissue Int 55:356–362.

Cheng S-L, Zhang SF, Avioli LV. 1996. Expression of bone matrix proteins during dexamethasone-induced mineralization of human bone marrow stromal cells. J Cell Biochem 61:182–193.

Civitelli R. 1998. Cell-cell communication in bone. In: Zaidi M, editor. Molecular and cellular biology of bone. Greenwich, CT: JAI Press. p 543–564.

Cheng S-L, Lecanda F, Davidson M, Warlow PM, Zhang S-F, Zhang L, Suzuki S, St. John T, Civitelli R. 1998. Human osteoblasts express a repertoire of cadherins, which are critical for BMP-2-induced osteogenic differentiation. J Bone Miner Res 13:633–644.

Ferrari SL, Traianedes K, Thorne M, Lafage-Proust M-H, Genever P, Cecchini MG, Behar V, Bisello A, Chorev M, Rosenblatt M, Suva LJ. 2000. A role of n-cadherin in the development of the differentiated osteoblastic phenotype. J Bone Miner Res 15:198–208.

Goomer RS, Maris T, Amiel D. 1998. Age-related changes in the expression of cadherin-11, the mesenchyme specific calcium-dependent cell adhesion molecule. Calcif Tissue Int 62:532–537.

Gumbiner BM. 1988. Cadherins: a family of Ca^{2+}-dependent adhesion molecules. Trends Biochem Sci 13:75–76.

Gumbiner BM. 1996. Cell adhesion: the molecular basis of tissue architecture and morphogenesis. Cell 84:345–357.

Heimark RL, Degner M, Schwartz SM. 1990. Identification of a Ca^{2+}-dependent cell-cell adhesion molecule in endothelial cells. J Cell Biol 110:1745–1756.

Kawaguchi J, Takeshita S, Kashima T, Imai T, Machinami R, Kudo A. 1999. Expression and function of the splice variant of the human cadherin-11 gene in subordination to intact cadherin-11. J Bone Miner Res 14:764–775.

Kemler R. 1993. From cadherins to catenins: cytoplasmic protein interactions and regulation of cell adhesion. TIG 9:317–321.

Kimura Y, Matsunami H, Inoue T, Shimamura K, Uchida N, Ueno T, Miyazaki T, Takeichi M. 1995. Cadherin-11 expressed in association with mesenchymal morphogenesis in the head, somite, and limb bud of early mouse embryos. Dev Biol 169:347–358.

Lecanda F, Avioli LV, Cheng S-L. 1997. Regulation of bone matrix protein expression and induction of differentiation of human osteoblasts and human bone marrow stromal cells by bone morphogenetic protein-2. J Cell Biochem 67:386–396.

Lemonnier J, Modrowski D, Hott M, Delannoy P, Lomri A, Marie PJ. 1998. The Ser252Trp FGFR-2 mutation in Apert syndrome selectively increases E-cadherin and N-cadherin expression in human calvaria osteoblasts in vitro and in vivo. Bone 23:S188.

Luckert BP, Kream BE. 1996. Clinical and basic aspects of glucocorticoid action in bone. In: Bilezikian JP, Raisz LG, Rodan GA, editors. Principles of bone biology. San Diego, CA: Academic Press. p 533–548.

Mbalaviele G, Chen H, Boyce BF, Mundy GR, Yoneda T. 1995. The role of cadherin in the generation of multinucleated osteoclasts from mononuclear precursors in murine marrow. J Clin Invest 95:2757–2765.

Mege RM, Goudou D, Diaz C, Nicolet M, Garcia L, Geraud G, Rieger F. 1992. N-cadherin and N-CAM in myoblast fusion: compared localization and effect of blockade by peptides and antibodies. J Cell Sci 103:897–906.

Okazaki M, Takeshita S, Kawai S, Kikuno R, Tsujimura A, Kudo A, Amann E. 1994. Molecular cloning and characterization of OB-cadherin, a new member of cadherin family expressed in osteoblasts. J Biol Chem 269:12092–12098.

Simonneau L, Kitagawa M, Suzuki S, Thiery JP. 1995. Cadherin 11 expression marks the mesenchymal phenotype: towards new functions for cadherins? Cell Adhes Commun 3:115–130.

Suva LJ, Towler DA, Harada S, LaFage M-H, Stueckle SM, Rosenblatt M. 1994. Hormonal regulation of osteoblast-derived cadherin expression in vitro and in vivo. J Bone Miner Res 9:S123.

Takeichi M. 1993. Cadherins in cancer: implications for invasion and metastasis. Curr Opin Cell Biol 5:806–811.

Takeichi M. 1994. The cadherin cell adhesion receptor family: roles in multicellular organization and neurogenesis. Prog Clin Biol Res 390:145–153.

Tanihara H, Kido M, Obata S, Heimark RL, Davidson M, St. John T, Suzuki S. 1994. Characterization of cadherin-4 and cadherin-5 reveals new aspects of cadherins. J Cell Sci 107:1697–1704.

Tsutsumimoto T, Kawasaki S, Ebara S, Takaoka K. 1999. TNF-α and IL-1β suppress N-cadherin expression in MC3T3-E1 cells. J Bone Miner Res 14:1751–1760.

Wong M-M, Rao LG, Ly H, Hamilton L, Tong J, Sturtridge W, McBroom R, Aubin JE, Murray TM. 1990. Long-term effects of physiologic concentrations of dexamethasone on human bone-derived cells. J Bone Miner Res 5:803–813.