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Retinoic Acid Stimulates Expression of the Functional Osteoclast Integrin $\alpha_v\beta_3$: Transcriptional Activation of the β_3 but Not the α_v Gene

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Abstract The capacity of osteoclasts to resorb bone depends, in part, on the surface expression of the integrin $\alpha_v\beta_3$. We have investigated whether the steroid hormone retinoic acid, known to stimulate bone resorption, regulates the appearance of the $\alpha_v\beta_3$ complex in avian osteoclast presursors. Using surface labeling, followed by immunoprecipitation with a $\alpha_v\beta_3$-specific antibody, we show that retinoic acid increases surface expression of the heterodimer in a dose- and time-dependent manner. Northern analysis reveals that the high basal steady-state levels of α_v mRNA do not change, while those for β_3 rise significantly from their initially low levels. Nuclear run-on studies confirm that steroid treatment stimulates transcription of the β_3, but not the α_v gene. Osteoclast precursors treated with retinoic acid exhibit increased multinucleation and expression of the osteoclast marker enzyme tartrate-resistant acid phosphatase. However, the fused cells do not have an increased capacity to resorb bone. In summary, multinucleated cells generated in this study do not represent fully differentiated bone-resorbing polykaryons. These results suggest that treatment of osteoclast precursors with retinoic acid is necessary, but insufficient, for expression of the mature osteoclast phenotype.

Key words: integrin, $\alpha_v\beta_3$, retinoids, gene transcription, osteoclast

The integrins are a family of heterodimeric transmembrane receptors whose external domains recognize specific peptide sequences in matrix or cell-bound proteins. These interactions result in cell–matrix or cell–cell attachment [reviewed in Hynes, 1992; Albelda and Buck, 1990; Hemler, 1990]. Adding complexity to their biological repertoire, integrins are now known to also participate in bidirectional signaling across the plasma membrane [Leavesley et al., 1993; Juliano and Haskill, 1993; Schwartz and Lechene, 1992; Miyauchi et al., 1991; Kornberg et al., 1991; Ingber et al., 1990; Guan et al., 1991]. This latter function apparently involves communication between integrin cytoplasmic tails and either cytoskeletal proteins and/or one of a number of kinases [Sastry and Horwitz, 1993; Damsky and Werb, 1992]. The signaling capacity of integrins may be particularly important, since it provides a mechanism by which extracellular matrix can modulate events such as differentiation, proliferation, migration, metastasis, and morphogenesis. Given the diversity and biological relevance of integrins, regulation of their expression at their site of function, namely the plasma membrane, is an issue of interest.

A number of cytokines are known to modulate integrin expression. For example, treatment of cells with tumor necrosis factor and interferon-γ, in combination, leads to a 70% diminution of $\alpha_v\beta_3$ on human endothelial cells [Defilippi et al., 1991]. This downregulation of $\alpha_v\beta_3$ is specific, since expression of β_1 integrins is not altered. Likewise, when human monocytes are treated with granulocyte-macrophage colony-stimulating factor there is increased expression

Abbreviations used: bp, base pair(s); BSA, bovine serum albumin; kb, kilobase(s); FBS, fetal bovine serum; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; RA, retinoic acid; SDS, sodium dodecyl sulfate; sulfo-NHS biotin, sulfosuccinimidobiotin; TCA, trichloracetic acid; TRAP, tartrate-resistant acid phosphatase.

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of the integrin $\alpha_v\beta_3$ [De Nichilo and Burns, 1993]. While not as extensively studied as cytokines, steroid hormones also regulate integrins. Retinoic acid (RA) treatment of the murine embryonal cell line P19 enhances appearance of $\alpha_v\beta_1$, and to a lesser degree, $\alpha_v\beta_3$ [Dedhar et al., 1991]. Similarly, RA stimulates expression, by PC12 cells, of the laminin receptor $\alpha_1\beta_1$, apparently reflecting increased levels of α_1 mRNA [Rossino et al., 1991].

Bone resorption is an event in which expression of $\alpha_v\beta_3$ on osteoclasts is a critical and rate-limiting step [Fisher et al., 1993; Ross et al., 1993; Horton et al., 1991; Chambers et al., 1986]. In keeping with its ability to stimulate osteoclastic bone degradation, we find that the steroid hormone 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) enhances surface expression of this heterodimer [Mimura et al., 1994]. Moreover, induction of $\alpha_v\beta_3$ by $1,25(OH)_2D_3$ is accompanied by transactivation of both gene subunits [Medhora et al., 1993; Mimura et al., 1994].

RA, another member of the steroid hormone superfamily [Wahli and Martinez, 1991], also stimulates bone resorption [Hough et al., 1988]. We report here that, like $1,25(OH)_2D_3$, this compound enhances expression of $\alpha_v\beta_3$ by avian osteoclast precursors. In contrast to $1,25(OH)_2D_3$, RA transactivates only the β_3 and not the α_v gene. Thus, at least in the case of RA, regulation of $\alpha_v\beta_3$ surface expression appears to be controlled via the modulation of the β_3 subunit.

MATERIALS AND METHODS

Cell Isolation and Culture

Avian osteoclast precursors were isolated and cultured as described previously [Mimura et al., 1994]. Briefly, bone marrow cells from laying hens maintained on a calcium-free diet for 2–3 weeks were fractionated on Ficoll-Hypaque, and the mononuclear fraction was cultured overnight on Falcon plastic cell culture dishes. The nonadherent cells were re-isolated and cultured for varying periods of time at 4–6 $\times$ 10^6 cells/ml in α-MEM/5% FBS + 5% chicken serum, with the addition of 10^{-5}–10^{-8} M all-*trans* RA in ethanol (at a final concentration of $<0.1\%$). In specific experiments, cells from the same bird were treated with either 10^{-6} M all-*trans* RA or 10^{-8} M $1,25(OH)_2D_3$ for 3 days. All sera used for culture had been charcoal-stripped to remove endogenous steroids.

Surface Labeling and Immunoprecipitation

In most instances, cells were labeled with the water-soluble biotin reagent sulfosuccinimidobiotin (sulfo-NHS-biotin, Pierce Chemical, Rockford, IL), using minor modifications of a published method [Miyake et al., 1990]. Briefly, adherent cells were rinsed free of culture medium with PBS and then labeled for 1 h at room temperature with the reagent at 0.2 mg/ml in 100 mM Hepes, at pH 8.0. Following removal of the labeling solution, cells were lysed into buffer containing 10 mM Tris, at pH 7.2, 150 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 0.025% NaN_3, 5 mM iodoacetamide, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 4 mM PMSF, and 0.25 TIU aprotinin per ml. The lysate was precleared with protein G-Sepharose (Pharmacia, Piscataway, NJ) and immunoprecipitated with the monoclonal antibody LM609, shown previously to recognize the avian integrin complex $\alpha_v\beta_3$ [Mimura et al., 1994]. The immune precipitate, recovered with excess protein G-Sepharose, was boiled with electrophoresis sample buffer and subjected to SDS–PAGE in 6% nonreducing minigels. The separated proteins were transferred to nitrocellulose (Nitro ME, MSI, Westbro, MA) with a semidry blotter, using the manufacturer's instructions (BioRad, Richmond, CA) and the blot was probed with 0.1% streptavidin–horseradish peroxidase (Boehringer-Mannheim, Indianapolis, IN) in PBS, with color development with 4-chloronaphthol at 2 mg/ml. In selected studies, 150-mm plates of adherent cells were labeled with 1 mCi ^{125}I, using the lactoperoxidase method as described [Mimura et al., 1994]. Rinsed plate contents were lysed with a minimal volume of lysis buffer, following which equal numbers of TCA-precipitable counts were immunoprecipitated with the antibody LM609 as described above. Analysis was performed by separation in nonreducing 24 cm 6% SDS–PAGE gels, which were dried and exposed at −70°C to Kodak X-Omat film (Eastman Kodak, Rochester, NY), prior to development.

Analysis of Steady-State α_v and β_3 mRNA Levels

Osteoclast precursors were treated for up to 3 days with vehicle, 10^{-5}–10^{-8} M all-*trans* RA or 10^{-8} M $1,25(OH)_2D_3$, without change of medium. At the relevant times medium was removed with PBS and total RNA was isolated with RNAzol, according to the manufacturer's instructions (Teltest, Friendswood, TX). Equal

amounts of RNA were treated with formaldehyde and separated on 1% agarose gels, followed by transfer to a nylon membrane (Hybond-N, Amersham, Arlington Heights, IL) using a vacuum blotter. A full-length cDNA for the avian β_3 gene product, cloned in our laboratory [Mimura et al., 1994], was labeled by random priming and hybridized to filters overnight at 42°C in 5× SSPE, 5× Denhardt's solution, 50% formamide, 0.1% SDS, and 10% background quencher (Teltest). The filters were washed three times at 55°C with 1× SSPE, 0.1% SDS, and exposed to film, prior to development. In some experiments in which cells had been treated with RA or $1,25(OH)_2D_3$, the membranes were stripped by boiling in 0.1% SDS in RNase-free water, followed by washing in the same water. The membranes were then reprobed in the same manner with a cDNA probe, provided by Dr. Louis Reichardt, which represents 2.2 kb of the coding region of the avian α_v cDNA [Bossy and Reichardt, 1990].

Measurement of the Rates of β_3 and α_v mRNA Synthesis

Nuclear run-on assays were performed as follows. Nuclei were isolated from cells treated with either vehicle or 10^{-6} M RA, using an established method [Mimura et al., 1994]. Briefly, cells were lysed in 10 mM Tris pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, and 0.5% NonIdet P-40. Nuclei were isolated by centrifugation at 400 rpm and stored at −80°C at a concentration of 10^8 cells per ml in 50 mM Hepes, pH 8.0, 5 mM $MgCl_2$ 0.5 mM DTT, 1 mg/ml BSA, and 25% glycerol. To measure new RNA synthesis, approximately 5×10^7 nuclei were thawed and mixed with an equal volume of 2× reaction buffer (100 mM Hepes, pH 8,0 4 mM $MgCl_2$, 6 mM MgOAc, 4 mM DTT, 2 mg/ml BSA, 300 mM NH_4Cl, 1 mM each ATP, CTP, and GTP, 20% glycerol, and 100 μCi of ^{32}P-UTP. Incubation was carried out for 15 min at 37°C, following which total RNA was extracted as described above. Equal amounts of TCA-precipitable counts were slot hybridized to a nitrocellulose membrane (Biodot, BioRad, Richmond, CA), to which 10 μg of linearized plasmid DNAs coding for avian α_v and β_3 had been applied in 20× SSC. As controls, a cDNA coding for LEP, an avian lysosomal protein [Fambrough et al., 1988], and plasmid DNA were applied in adjacent slots. The labeled RNA was hybridized at 55°C for 48 h in 5× SSC, 50% formamide, 2× Denhardt's solution, 20 μg tRNA, 50 mM NaH_2PO_4, 0.1% SDS, and 1× Background Quencher (Teltest). Membranes were subjected to three 5-min washes at 45°C with 2× SSC, 0.1% SDS for 5 min, followed by three 15-min washes at 55°C with 0.2× SSC, 0.1% SDS. Membranes were dried, exposed at −70°C to Kodak Scientific Imaging film, which was developed after appropriate times.

Analysis of Cell Morphology and Enzyme Content

Adherent cells were fixed with methanol and stained with Giemsa prior to photography. To detect the presence of TRAP activity cells were fixed with acetone/methanol/30 mM citrate pH 5.4 (6:1:3) and stained using the Sigma kit #387-A, according to the supplier's instructions.

Measurement of Bone Resorption

Nonadherent osteoclast precursors were treated with either vehicle (0.1% ethanol) or 10^{-6} M RA for 3 days. At this time, medium was replaced, together with a suspension of 100 μg of ^{3}H-labeled rat bone particles, prepared as described previously [Blair et al., 1986]. After 3 days, supernatant from the cultures was removed carefully and counted for the presence of radiolabeled proline/hydroxyproline, as a quantitative index of bone resorption [Blair et al., 1986]. This assay was recently shown to be highly correlated with that in which osteoclasts generate pits on slices of bone [Hiura et al., 1995].

RESULTS

Retinoic Acid Treatment of Osteoclast Precursors Increases Surface Expression of the Integrin $\alpha_v\beta_3$

We have demonstrated that $1,25(OH)_2D_3$ increases surface expression of $\alpha_v\beta_3$ by avian osteoclast precursors [Mimura et al., 1994]. Having recently identified a putative RA response element in the 5′-flanking region of the β_3 gene [Cao et al., 1993a,b], we asked whether RA, like $1,25(OH)_2D_3$, also modulates appearance of the integrin in these cells. The result, shown in Figure 1, which reflects surface labeling of cells with ^{125}I, confirms the agonistic effect of RA and, in addition, demonstrates that this steroid is more potent than the maximally stimulatory concentration of $1,25(OH)_2D_3$ in this regard. Because of the large amounts of ^{125}I required for

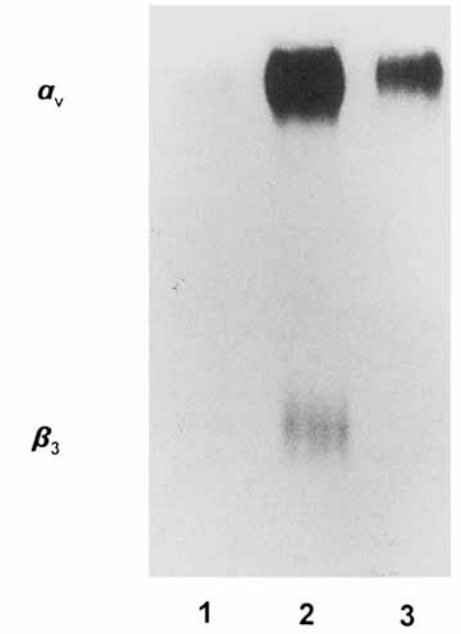

Fig. 1. Retinoic acid, like $1,25(OH)_2D_3$, induces expression of the integrin $\alpha_v\beta_3$ on avian osteoclast precursors. Precursor cells were treated with vehicle (*lane 1*), 10^{-6} M retinoic acid (*lane 2*), or 10^{-8} M $1,25(OH)_2D_3$ (*lane 3*) for 3 days. Cells were surface iodinated and equal amounts of TCA-precipitable protein were immunoprecipitated with excess LM 609, a monoclonal antibody known to recognize the integrin complex. The immune complex was analyzed by SDS–PAGE and autoradiography. Since LM609 recognizes only the intact heterodimer, the molar amounts of each subunit must be the same. The different degree of α_v and β_3 labeling (*lanes 2, 3*) reflects the lower tyrosine content of the α_v chain.

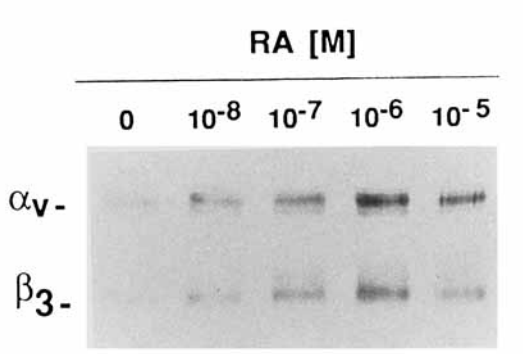

Fig. 2. Induction of $\alpha_v\beta_3$ by retinoic acid is dose dependent. Cells were treated for 3 days with varying doses of retinoic acid or vehicle. At this time, cells were surface labeled with sulfo-NHS-biotin and equal amounts of lysate protein were immunoprecipitated with excess LM 609. The immune complex was separated by SDS–PAGE, transferred to nitrocellulose, and visualized with streptavidin-HRP and a chromogen.

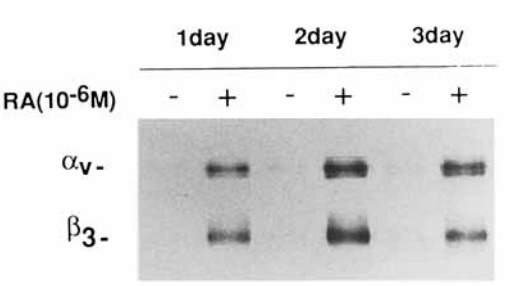

Fig. 3. Induction of $\alpha_v\beta_3$ by retinoic acid is time dependent. Cells cultured for various times with 10^{-6} M retinoic acid or vehicle were surface labeled and analyzed for $\alpha_v\beta_3$ expression as described in Figure 2.

more extensive studies, we turned to labeling the cells with biotin for the performance of the time- and dose-dependency studies. As shown in Figure 2, RA modulates $\alpha_v\beta_3$ expression in a dose-dependent manner, an effect seen at 10^{-8} M, which is within the range reported for the serum levels of this hormone [Eckhoff and Nau, 1990], with the maximal result at 10^{-6} M. Similar to the result for $1,25(OH)_2D_3$, the protein appears on the cell surface within 24 h of hormone treatment (Fig. 3).

Retinoic Acid Increases β_3 But Not α_v mRNA Levels

Since $1,25(OH)_2D_3$ increase both α_v and β_3 steady-state mRNA levels in osteoclast precursors [Medhora et al., 1993; Mimura et al., 1994], we asked whether similar changes occur when cells are exposed to RA. As shown in Figure 4, such treatment prompts a dose-dependent enhancement of β_3 mRNA, with the effect once again apparent at concentrations of the steroid within the physiological range. However, unlike $1,25(OH)_2D_3$, RA fails to increase steady-state levels of α_v mRNA. Indeed, in several experiments we noted a small decrease in steady-state levels of α_v mRNA following RA treatment (not shown). In a result that parallels surface expression of $\alpha_v\beta_3$, changes in β_3 message levels are seen within 24 h of hormone treatment (Fig. 5).

Change in β_3 mRNA Levels Arises from Increased Transcription of β_3 Gene

We reported that $1,25(OH)_2D_3$-enhanced α_v and β_3 mRNA levels in osteoclast precursors reflects transactivation of both genes [Medhora et al., 1993; Mimura et al., 1994]. Thus, we asked whether the same mechanism mediated the increased levels of β_3 mRNA in cells treated with RA. The data in Figure 6 confirm that, like $1,25(OH)_2D_3$, RA increases transcription of the β_3 gene. By contrast, but consistent with our finding of generally unaltered α_v mRNA levels, the rate of transcription of this gene is the same

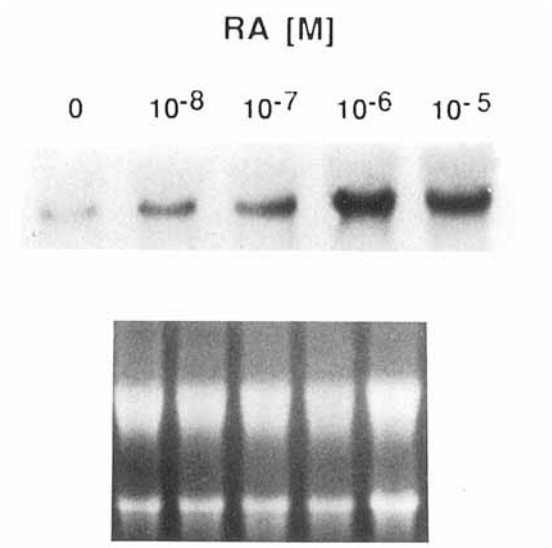

Fig. 4. Retinoic acid increases β_3, but not α_v, steady-state mRNA levels in a dose-dependent manner. Cells were treated with varying levels of retinoic acid for 3 days, at which time total RNA was isolated and subjected to sucessive Northern analysis with avian-specific β_3 and α_v cDNA probes, with removal of the labeled probe prior to re-analysis. The lower bands reflect 28S RNA levels, demonstrating equal loading of the gel.

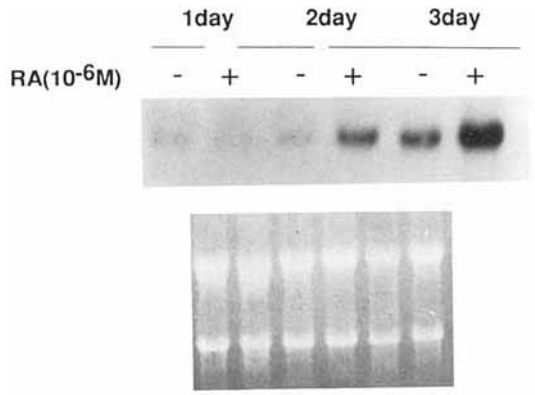

Fig. 5. Induction of β_3 mRNA levels by retinoic acid is time dependent. Cells were treated with 10^{-6} M retinoic acid for up to 3 days and Northern analysis was performed as described in Figure 4. The lower bands reflect 28S RNA levels, demonstrating equal loading of the gel.

in both control and RA-treated cells. As an additional control, we measured the rate of synthesis of LEP, a lysosomal protein known not to respond to $1,25(OH)_2D_3$ [Billecocq et al., 1990], and found minimal alteration in this parameter.

Treatment of Osteoclast Precursors With Retinoic Acid Stimulates Production of TRAP-Positive Multinucleated Cells

Avian osteoclast precursors grown in culture multinucleate, forming TRAP-positive osteo-

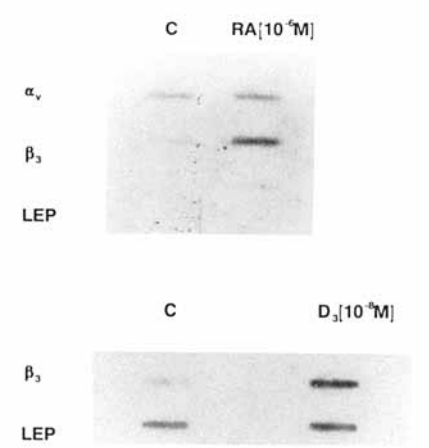

Fig. 6. Retinoic acid stimulates transcription of the avian β_3, but not α_v, gene. Nuclei were from cells treated for 3 days with vehicle (C), 10^{-6} M retinoic acid, or 10^{-8} M $1,25(OH)_2D_3$ were used in nuclear run-on assays, as described in Methods and Materials. As a positive control $1,25(OH)_2D_3$-induced transcription of the β_3 gene was measured, while measurement of the rate of transcription of the avian LEP gene served as a negative control.

clast-like cells capable of resorbing bone [Alvarez et al., 1991]. We find that treatment of the precursors for 3 days with 10^{-6} M RA increases the extent of multinucleation and the TRAP content of individual cells (Fig. 7a). However, when equivalent numbers of precursors are exposed to either vehicle or RA, followed by incubation with radiolabeled bone, there is no difference in resorption by control and treated cells (Fig. 7b).

DISCUSSION

Steroid hormones play a central role in regulating osteoblasts and osteoclasts, the two major cells present in bone. Osteoblasts contain receptors for glucocorticoids, estrogens, and $1,25(OH)_2D_3$ and respond to these hormones by modulating expression of a variety of functionally important proteins, including osteopontin, osteocalcin, bone sialoprotein, collagen, alkaline phosphatase, neutral collagenase, and insulin-like growth factor 1 [Oliva et al., 1993; Li and Sodek, 1993; Kesterson et al., 1993; Henrichs et al., 1993; Subramaniam et al., 1992; Meikle et al., 1992; Heath et al., 1992; Chen et al., 1991; Candeliere et al., 1991; Craig and Denhardt, 1991; Ng et al., 1989].

Mature osteoclasts have estrogen receptors [Oursler et al., 1993a], and the sex steroid inhibits their resorptive activity [Oursler et al., 1993a,b]. By contrast, avian osteoclast precursors, but not fully differentiated polykaryons,

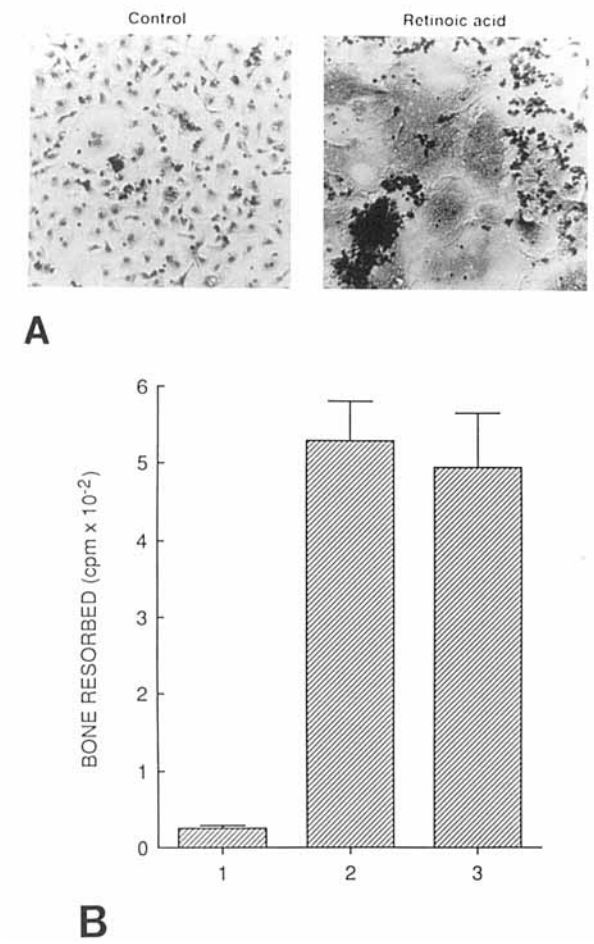

Fig. 7. Retinoic acid treatment of avian osteoclast precursors stimulates multinucleation and TRAP activity, but fails to increase bone resorption. Precursor cells treated for three days with either vehicle or 10^{-6} M retinoic acid were fixed and stained for TRAP (**A**) or used in a standard bone resorption assay (**B**). *Lane 1,* labeling bone plus no cells; *lane 2,* labeling bone plus cells treated with vehicle; *lane 3,* labeled bone plus cells treated with 10^{-6} M retinoic acid.

express receptors for $1,25(OH)_2D_3$ [Merke et al., 1986], promoting differentiation along the macrophage pathway [Clohisy et al., 1987; Bar-Shavit et al., 1985]. Since fully differentiated osteoclasts do not contain receptors for $1,25(OH)_2D_3$ [Merke et al., 1986], yet the hormone stimulates bone resorption, its effects are probably mediated through enhanced maturation of osteoclast precursors.

RA is a member of the steroid hormone superfamily [Wahli and Martinez, 1991] and directly influences several aspects of bone cell biology (reviewed in Gudas et al., 1994). The hormone regulates both chondrocyte function [Pacifici et al., 1991; Benton, 1990; Takishita et al., 1990] and expression of osteoblastic alkaline phosphatase [Heath et al., 1992]. Rats treated, in vivo, with retinol, the inactive precursor of RA, exhibit increased osteoclastic activity [Hough et al., 1988]. Most importantly in the current context, the retinoid stimulates formation, by avian osteoclasts, of podosomes [Oreffo et al., 1988], structures found in osteoclast adhesion plaques [Zambonin-Zallone et al., 1988]. These plaques are believed to arise from clustering of integrins as they associate with elements of the cytoskeleton [Turner and Burridge, 1991].

We and others have shown that blocking the integrin $\alpha_v\beta_3$ on mature osteoclasts decreases their ability to resorb bone [Fisher et al., 1993; Ross et al., 1993; Horton et al., 1991; Chambers et al., 1986]. We also demonstrated that avian osteoclast precursors contain low levels of the integrin $\alpha_v\beta_3$ and that treatment with $1,25(OH)_2D_3$ increases expression of this important heterodimer [Mimura et al., 1994]. We have identified a canonical vitamin D response element in the 5′ region of the avian β_3 promoter and have shown that the β_3 gene is activated transcriptionally by $1,25(OH)_2D_3$ [Cao et al., 1993a]. This result explains, at least in part, the increase in surface expression of $\alpha_v\beta_3$ induced by the hormone.

We recently cloned the avian β_3 promoter and found that, when linked to a reporter gene, it is transactivated by RA and contains a putative RA response element [Cao et al., 1993a,b]. This observation, and the known role of retinoids in stimulating bone resorption, prompted us to ask whether RA regulates the integrin $\alpha_v\beta_3$ on osteoclast precursors. Our results demonstrate that, like $1,25(OH)_2D_3$, RA increases surface expression of the heterodimer by mechanisms involving enhanced transcription and mRNA steady-state levels of the β_3 gene. The effect of RA on β_3 mRNA synthesis and $\alpha_v\beta_3$ expression is time- and dose-dependent, with the alterations in protein expression reflecting changes in β_3 mRNA levels. In contrast to $1,25(OH)_2D_3$ [Mimura et al., 1994], RA neither accelerates α_v transcription nor augments its mRNA steady-state levels. The most reasonable explanation of these results is that the β_3, and not the α_v, gene regulates $\alpha_v\beta_3$ surface expression. Since, in cells other than platelets, β_3 associates only with α_v, while α_v combines with a number of different β chains, our observations are in keeping with the developing paradigm [Sheppard et al., 1992; Santala and Heino, 1991; Heino et al., 1989] that it is the monogamous, and not the promiscuous subunit,

that governs integrin subunit expression on the plasma membrane.

The net effect, in vivo, of excess systemic retinol is stimulated bone resorption [Hough et al., 1988], but the underlying mechanism remains unclear. In addition, in vitro studies in several models on the resorptive effects of RA, the active form of retinol, are paradoxical. Similar to our avian experiments, RA treatment of human bone marrow cultures increases multinucleation with no effect on bone resorption [Thavarajah et al., 1991]. By contrast, when marrow cells from adult rats are cultured with $1,25(OH)_2D_3$ and RA, the latter compound inhibits the $1,25(OH)_2D_3$-stimulated formation of multinucleated cells. Combined hormone treatment alters multinucleated cell morphology, yielding a phenotype that may represent activated osteoclasts [Scheven and Hamilton, 1990]. However, the addition of RA to fetal rat long bones cultured in vitro increases osteoclast number and bone resorption, a result ascribed to the capacity of the hormone to enhance differentiation of osteoclast precursors [Scheven and Hamilton, 1990]. Furthermore, in the murine calvarial culture system, RA treatment increases bone resorption [Togari et al., 1991]. This result may be explained by the finding that RA treatment of the total cellular fraction isolated from adult mouse bone marrow increases both the number of osteoclast-like cells generated and bone resorption, measured by the pit-forming assay [Kaji et al., 1995]. These same investigators also demonstrate that RA stimulated expression of osteopontin mRNA by freshly isolated rabbit osteoclasts. This latter finding, if confirmed at the level of osteopontin protein, would result in increased availability of a bone matrix protein capable of supporting attachment by $\alpha_v\beta_3$-bearing osteoclasts.

Two contradictory reports have appeared concerning treatment of isolated avian osteoclasts with retinoids. One study concludes that RA decreases formation of resorption lacunae [O'Neill et al., 1992], while the other states that retinol treatment increases bone degradation [Hough et al., 1988]. Expression by the same cells of TRAP, an osteoclast marker enzyme, is also enhanced following dosing with either retinol or RA [Hough et al., 1988]. The reason for these diametrically opposed results is unclear, but it may involve subtle differences in cell isolation or culture conditions, or both. Irrespective of differences, in vitro studies must eventually explain the finding that systemic retinoid excess enhances osteoclastic bone resorption [Hough et al., 1988]. The complexity of this issue may be reflected in our findings that, despite increases in both the extent of multinucleation and expression of two osteoclast-related markers, namely TRAP and surface expression of the integrin $\alpha_v\beta_3$, RA treatment of avian osteoclast precursors does not enhance bone resorption. While conjectural, these observations suggest that the steroid alone promotes osteoclast precursor differentiation, but not to the stage of the fully mature resorptive polykaryon.

In summary, exposure of avian osteoclast precursors to the osteoclastogenic hormone RA is necessary, but insufficient, for expression of the mature osteoclast phenotype. This treatment leads to the generation of polykaryons that, while incapable of resorbing bone, bear several markers of the osteoclast phenotype, including the integrin $\alpha_v\beta_3$. The retinoid stimulates expression of the integrin by increasing transcription of the β_3, but not α_v, gene. Thus, at least in the case of RA, regulation of $\alpha_v\beta_3$ surface expression appears to be controlled via the modulation of the β_3 subunit.

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