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Phorbol Myristate Acetate Transactivates the Avian β_3 Integrin Gene and Induces $\alpha_v\beta_3$ Integrin Expression

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Abstract 1,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) transactivates the avian β_3 integrin gene whose promoter contains at least two vitamin D response elements, one of which is in close proximity to a candidate AP1 site (TGACTCA). Since *fos/jun* and steroid hormones interact to regulate gene expression, we asked whether phorbol-12-myristate-13-acetate (PMA), which stimulates binding of *fos/jun* to AP1 sites, transactivates the avian β_3 integrin gene and, if so, does the phorbol ester modulate $1,25(OH)_2D_3$ induction of the gene. We find the candidate AP1 sequence comigrates with the consensus AP1 sequence on electromobility shift assay when incubated with recombinant c-*jun* protein. Furthermore, PMA prompts expression of β_3 integrin mRNA in the avian monocytic line, HD11. The increase in message reflects transactivation of the β_3 gene and is mirrored by plasma membrane appearance of the integrin heterodimer $\alpha_v\beta_3$. Moreover, attesting to the functional significance of PMA-enhanced $\alpha_v\beta_3$ expression, cells treated with concentrations of the phorbol ester that induce the β_3 gene, spread extensively on plastic, an event blocked by an anti-α_v antibody and a peptide mimetic known to inhibit $\alpha_v\beta_3$-mediated cell attachment. Interestingly, co-addition of $1,25(OH)_2D_3$ and PMA prompts greater expression of $\alpha_v\beta_3$ than when the cells are exposed to either agent alone and PMA enhances $1,25(OH)_2D_3$-induced β_3 integrin mRNA expression. Thus, PMA and $1,25(OH)_2D_3$ impact on the avian β_3 integrin gene independently and in combination.

Key words: cell attachment, gene transcription, AP1 site; fos/jun, $1,25(OH)_2D_3$, macrophage-like cells

The integrin $\alpha_v\beta_3$ is a transmembrane matrix receptor that recognizes proteins containing the amino acid motif, Arg-Gly-Asp (RGD) [Ruoslahti, 1991]. While this heterodimer is expressed by a number of native and transformed cells, $\alpha_v\beta_3$ is particularly prominent in those of the monocyte/macrophage family in which the complex mediates osteoclastic bone resorption [Ross et al., 1993] as well as matrix recognition by non-osteoclastic mononuclear phagocytes [Kitazawa et al., 1995].

Given the biological significance of $\alpha_v\beta_3$, attention has turned, in recent years, to its regulation. For example, we find the heterodimer induced, in avian macrophages, by the steroid hormone $1,25(OH)_2D_3$ [Mimura et al., 1994]. This observation is significant in light of the fact that $1,25(OH)_2D_3$ prompts differentiation of monocytic precursors [Clohisy et al., 1987] and macrophage-like cell lines [Bar-Shavit et al., 1985; Perkins et al., 1991].

Phorbol esters also prompt monocytic differentiation and, in some circumstances, target transformed macrophages in a manner not dissimilar to $1,25(OH)_2D_3$ [Barnea et al., 1990]. Furthermore, an intimate relationship often exists between the phorbol ester-induced transcription factors c-*fos* and c-*jun*, and steroid hormone receptors [Hyder et al., 1994].

Given these observations, we asked if the phorbol ester, PMA, modulates $\alpha_v\beta_3$ expression by an avian monocytic line and, if so, is this phenomenon is impacted by $1,25(OH)_2D_3$? We find the tumor promoter accelerates transcription of the β_3 integrin gene, an event reflected by plasma membrane appearance of the heterodimer.

Furthermore, the phorbol ester progressively enhances $1,25(OH)_2D_3$-induced expression of β_3 integrin mRNA, an event once again mirrored by $\alpha_v\beta_3$ surface expression. Thus, PMA, probably through *fos/jun* induction, interacts with

Abbreviations: $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; PMA, phorbol-12-myristate-13-acetate; FCS, fetal calf serum.

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$1,25(OH)_2D_3$ to stimulate expression of a functionally important integrin.

MATERIALS AND METHODS

Cell Culture

The avian macrophage-like cell line HD11 (a gift from Dr. T. Graf, EMBO, Heidelberg, Germany) was cultured in monolayers in RPMI 1640 medium (Gibco-BRL Life Technologies, Gaithersburg, MD) supplemented with charcoal-stripped 5% fetal calf serum (FCS) and 5% chicken serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Cells treated with phorbol-12-myristate-13-acetate (PMA) (1–240 ng/ml) or 1,25-dihydroxyvitamin D_3 (a gift from Dr. Milan Uskokovic, Hoffman-LaRoche, Nutley, NJ) (10^{-8} M), or appropriate vehicle (DMSO for PMA; ethanol for $1,25(OH)_2D_3$) were maintained for up to 2 days without change of medium.

Immunoprecipitation

HD11 cells treated for 2 days with PMA (60 ng/ml), $1,25(OH)_2D_3$ (10^{-8}M), a combination of both agents, or vehicle (30 μm DMSO for PMA, 0.05% ethanol for $1,25(OH)_2D_3$) were surface-labeled with ^{125}I-lactoperoxidase and lysed in a buffer containing 2% Renex detergent, using previously described methods [Li et al., 1995]. Aliquots of lysate containing equal numbers of trichloroacetic acid (TCA)-precipitable counts were precleared with nonimmune serum plus protein–A Sepharose followed by immunoprecipitation with excess monoclonal antibody LM 609, specifically recognizing the avian integrin complex $\alpha_v\beta_3$ [Cheresh and Spiro, 1987]. The immune complex was bound to protein A–Sepharose and the antigen recovered by boiling the beads in electrophoresis sample buffer for 10 min. Radiolabeled proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) in 7% gels, and the gels dried and subjected to autoradiography at −80°C.

RNA Extraction and Northern Blot Analysis

HD11 cells treated for with various concentrations of PMA, $1,25(OH)_2D_3$ (10^{-8} M), combinations of both agents or vehicle, were washed three times with PBS, and total RNA was isolated using TRIZol reagent (Gibco-BRL). Equal amounts of total RNA (10–15 μg/lane) were denatured and loaded on a 1% agarose gel containing 0.6% of a 37% formaldehyde solution and 0.5 μg/ml ethidium bromide. Electrophoresis was carried out in 20 mM MOPS, pH 6.8, 5 mM sodium acetate, and 1 mM EDTA, at 10 V/cm^2. RNA was transferred to a nitrocellulose membrane by vacuum blotting and then crosslinked under ultraviolet (UV) light (Gene Linker, BioRad, Richmond, CA). Filters were hybridized with a ^{32}P-labeled full-length avian β_3 integrin cDNA cloned in our laboratory [Mimura et al., 1994]. Labeling was performed with the RandomPrimed DNA Labeling Kit (Boehringer Mannheim GmbH, Germany). After hybridization overnight at 42°C, the membrane was washed twice in 2× SSPE–0.1% SDS at 26°C, twice in 0.1× SSPE-0.1% SDS at 26°C, and exposed at the −80°C for autoradiography. Prior to transfer, each gel was photographed and the ethidium bromide-stained band at 28S examined to confirm equal RNA loading.

Nuclear Run-On Transcription Assay

HD11 cells were cultured for 2 days in the presence of PMA (60 ng/ml) or $1,25(OH)_2D_3$ (10^{-8} M) or a combination of both agents or vehicle, were washed three times with phosphate-buffered saline (PBS) containing 10 mM Tris, pH 8.0, 1 mM EDTA; 5 ml of the same solution was added to each dish. Cells were isolated by scraping with a plastic lifter and centrifuged in 15-ml tubes, at which time they were resuspended in 5 ml of lysis buffer (10 mM Tris–HCl (pH 7.5), 10 mM NaCl, 3 mM $MgCl_2$ and 0.5% NP-40). After gentle vortexing, the preparation was incubated on ice for 5–10 min and spun to isolate nuclei. Purified nuclei were collected by the addition of 100 μl of buffer (50 mM Hepes, pH 8.0, 5 mM $MgCl_2$, 0.5 mM DTT, 1 mg/ml BSA and 25% (v/v) glycerol) to each 15-ml tube, with pooling of the aliquots into a 1.5-ml Eppendorf tube. All procedures above were performed at 4°C. For synthesis of labeled nascent α_v and β_3 integrin mRNA, 100 μl of fresh nuclei were mixed with an equal volume of 2× transcription reaction buffer (100 mM Hepes, 4 mM $MgCl_2$, 6 mM MgOAc, 20% glycerol, 300 mM NH_4Cl, 5 mM DTT and 2 mg/ml BSA). The reaction was initiated by the addition of 10 μl each of cold 100 mM ATP, CTP, GTP (Pharmacia, Piscataway, NJ), and, finally, 12 μl (120 μci) of α-^{32}P-UTP (ICN; >3,000 Ci/mmol). The tubes were mixed and incubated at 32°C for 30 min.

Nuclei were spun down in a microcentrifuge at 15,000 rpm for 1–2 min and total RNA, isolated by the TRIZol method described above, was dissolved in the relevant volume of RNAse-free water.

The percent incorporation of (α-^{32}P)-UTP into specific RNA species was measured as follows. Equal amounts of trichloracetic acid-precipitable counts from freshly transcribed RNA were hybridized to nitrocellulose filters onto which excess (10 μg) cloned DNA fragments had been denatured in TE buffer containing 0.4 M NaOH and 10 mM EDTA. Filters were prehybridized at 42°C for 6 h in 50% formamide, 4× SSC, 2× Denhardt's solution, 20 μg of rRNA per ml, 0.1% SDS, 50 mM Na_2HPO_4, pH 7.5. Hybridization was performed in the same mixture at 42°C for 36–48 h. Filters were washed twice at room temperature in 2× SSC–01% SDS for 10 min each, followed by three washes for 15 min each at 0.2× SSC–0.1% SDS. The nitrocellulose filters were dried and autoradiographed at −80°C for 3–5 days.

Electromobility Shift Assay

A 29-bp DNA fragment containing a putative AP1 site (box) included in 24 bp (−97 to −120) derived from the avian β_3 integrin gene (underlined) was generated by annealing the synthetic oligonucleotides 5′GG CCAACCCGAGTGACGCAGCGTAGCCGC3′ and 5′GGCTACGCTGCGTCACTCGGG TTGGCCGC3′. These two, as well as a 21-bp consensus AP1 oligonucleotide (Promega) were end-radiolabeled with T4 polynucleotide kinase (Promega, Madison, WI) and [γ-^{32}P]ATP (5,000 Ci/mmol) (Amersham, Arlington Heights, IL). Labeled probe was purified by Sephadex G-25 spin-column (Boehringer Mannheim) chromatography. Binding reactions were preincubated for 20 min at 22°C with two footprinting units of recombinant human AP1 (c-*jun*) (Promega) in 10 mM Tris–HCl, pH 7.6, 100 mM KCl 1.0 mM dithiothreiotol, 15% glycerol, 2 mg of BSA, and 50 μg/ml poly(deoxyinosine-doxycysodine) in a volume of 19 μl. One μl of DNA probe (0.5 ng, 50,000–100,000 dpm) was added, and the incubation was continued for an additional 20 min at 22°C. The incubate was subjected to nondenaturing electrophoresis on a 4% polyacrylamide gel (acrylamide/bisacrylamide, 80:1) in 23 mM Tris borate, 0.5 mM EDTA (0.25 × TBE) at 10 mA/gel. Dried gels were autoradiographed at −80°C.

Cell-Spreading Assay

HD11 cells (5×10^5/well) were plated, in 12-well cluster dishes (Costar), in RPMI 1640 medium containing 10% fetal calf serum. The latter is known to be rich in the $\alpha_v\beta_3$ ligand, vitronectin, which in serum-containing medium, mediates attachment of bone cells to plastic [Steele et al., 1993]. At initiation of culture, PMA in varying concentrations, or carrier (4 μl DMSO) was added to each well. In some circumstances the anti-avian α_v blocking antibody CHAV [Neugebauer et al., 1991] (provided by Dr. Louis Reichart, San Francisco, CA), an irrelevant monoclonal antibody (mAb), or SC56631 (Searle, Skokie, IL), a peptide mimetic known to block $\alpha_v\beta_3$-mediated cell attachment [Nickols et al., 1995] were also added. The cultures were photographed by phase microscopy, 14 h later.

RESULTS

We have noted that nucleotides −104 to −110 within the 5′ regulatory region of the avian β_3 integrin gene, comprises a putative AP-1 recognition sequence (TGACGCA) [Cao et al., 1993]. To characterize this motif further, we performed gel-shift analysis using as ^{32}P-labeled probes, a consensus AP-1 sequence (TGACTCA) and the candidate sequence derived from the β_3 promoter. As shown in Figure 1, both the consensus and candidate sequences bind purified c-jun protein producing mobility shifts of each probe to the same position.

Recognition of c-*jun* by a putative AP-1 site in the β_3 integrin promoter suggested phorbol esters, which induce *fos*/*jun* expression [Gilman, 1988], may transactivate the gene. Thus, the avian monocytic line HD11 was exposed, with time, to PMA (60 ng/ml). RNA was isolated and probed with a full-length avian β_3 integrin cDNA.

As we have previously reported, avian β_3 mRNA contains two major transcripts migrating at 3.6 and 4.5 kb, respectively [Mimura et al., 1994]. Figure 2 demonstrates that PMA augments β_3 mRNA steady-state levels. While prompting appearance of both detectable transcripts, the compound exerts its major impact on the 3.6-kb species. β_3mRNA is increased within 1–3 h of PMA exposure and persists for at least 45 h. Induction of β_3 message by the phorbol ester is dose dependent, extant at concentrations as low as 3.75 ng/ml (Fig. 3).

We next asked if PMA induction of β_3 mRNA is reflected by protein expression. To this end,

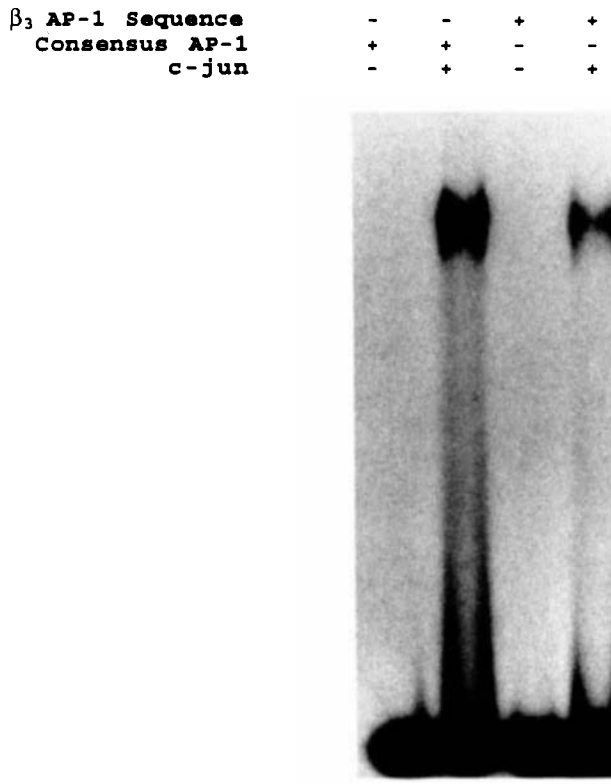

Fig. 1. The putative β_3 AP-1 site binds c-*jun*. Radiolabeled oligonucleotides containing the β_3 (TGACGCA) or consensus (TGACTCA) AP-1 sequences were incubated with or without recombinant human c-*jun* and subjected to electromobility shift assay. In the presence of c-*jun*, the probes co-migrate.

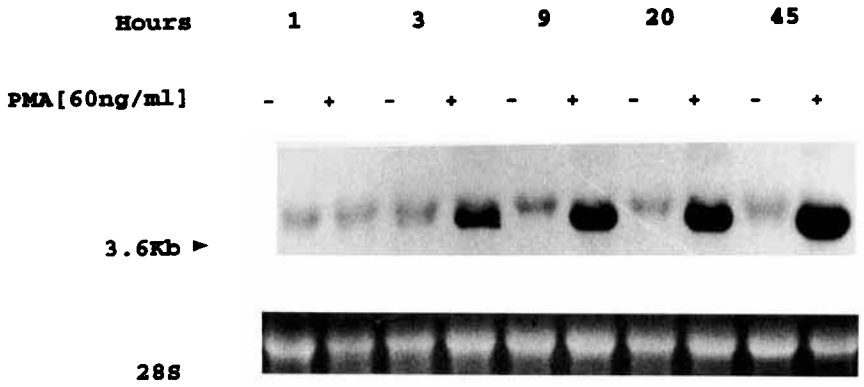

Fig. 2. PMA induces β_3 mRNA expression in a time-dependent manner. HD11 cells were incubated with PMA (+) or vehicle (−). RNA was extracted with time and subjected Northern analysis using an avian β_3 cDNA. β_3 mRNA induction is apparent by 3 h of PMA exposure.

we treated HD11 cells with PMA for 48 h and surface-labeled them with ^{125}I. The cells were lysed and the lysate immunoprecipitated with monoclonal antibody (MAb) LM609, recognizing the external domain of the intact $\alpha_v\beta_3$ heterodimer. We find, mirroring β_3 mRNA levels, PMA enhances plasma membrane $\alpha_v\beta_3$ capacity (Fig. 4).

Expression of $\alpha_v\beta_3$ is known, in other cells, to induce cytoskeletal alterations leading to spreading [Wayner et al., 1991]. As shown in Figure 5, treatment of HD11 with PMA prompts dose-dependent cell spreading occurring at concentrations as low as 1.0 ng/ml. Consistent with an $\alpha v\beta_3$-driven mechanism, PMA-induced spreading is blunted by a MAb raised against the

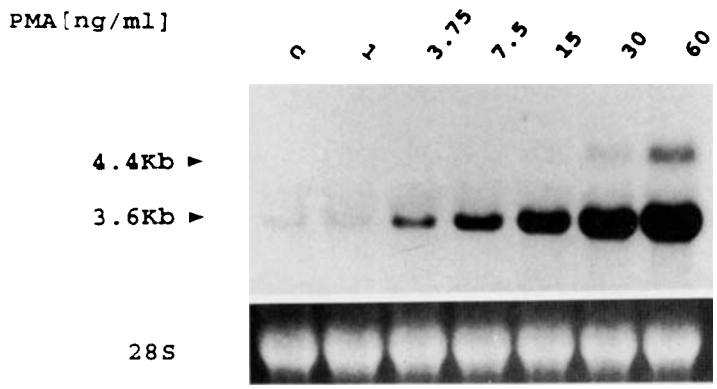

Fig. 3. PMA induces β_3 mRNA expression in a dose-dependent manner. HD11 cells were incubated for 40 h in the presence of various concentrations of PMA or vehicle (0). RNA was extracted and subjected to Northern analysis using an avian β_3 cDNA. β_3 mRNA induction is apparent at 3.75 ng/ml PMA.

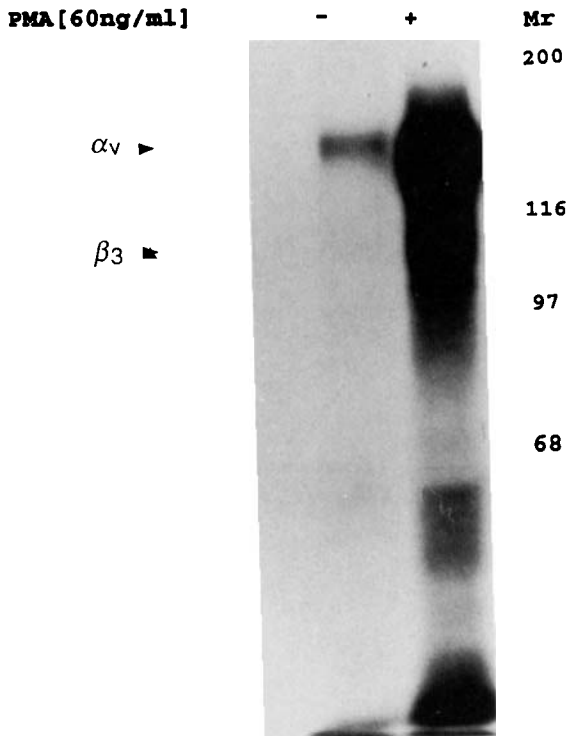

Fig. 4. PMA induces $\alpha_v\beta_3$ expression. HD11 cells were incubated for 48 h with PMA (+) or vehicle (−), surfaced labeled with ^{125}I, lysed, and the lysate immunoprecipitated with anti-$\alpha_v\beta_3$ antibody. PMA markedly upregulates plasma membrane expression of the integrin.

extracellular domain of the avian α_v integrin [Neugebauer et al., 1991] (Fig. 6). By contrast, an irrelevant monoclonal antibody has no such effect. Additional support of the contention that PMA-induced cell spreading is an $\alpha_v\beta_3$-directed event comes from the observation that such spreading is inhibited by an RGD peptide mimetic we have shown blocks cell-matrix recognition mediated by the integrin heterodimer [Nickols et al., 1995].

$1,25(OH)_2D_3$ induces differentiation of macrophages [Clohisy et al., 1987] and various macrophage lines [Bar-Shavit et al., 1985; Perkins et al., 1991]. Given the interaction of phorbol esters and D_3 in other cells [Meenakshi et al., 1993], we asked if PMA impacts on D_3-induced β_3 expression. Thus, cells were incubated with D_3 (10^{-8} M) in the presence of increasing amounts of PMA. Figure 7 shows that the steroid alone induces β_3 mRNA. Moreover, PMA, in concentrations as low as 7.5 ng/ml, enhances D_3 induction of the integrin subunit message. β_3 transcripts increase within 2–4 h of D_3/PMA exposure and the effect persists at least 45 h (Fig. 8). Most importantly, the steroid and the maximum inductive concentration of the phorbol ester, when added in combination, prompts surface expression of $\alpha_v\beta_3$ in amounts greater than when the cells are exposed to either agent alone (Fig. 9).

Finally, we turned to the mechanisms of β_3 gene transactivation by PMA and D_3. Nuclear run-on experiments show the integrin subunit is transactivated, in HD11, by both PMA and D_3 (Fig. 10). Reflecting mRNA and $\alpha_v\beta_3$ protein levels, a combination of agents accelerates transcription more than either alone.

DISCUSSION

Differentiation of native and transformed macrophages is a complex process impacted by a variety of agents, including steroid hormones and cytokines. For example, metabolites of vitamin D_3 promote maturation of bone marrow-derived monocytic precursors [Clohisy et al., 1987; Perkins and Teitelbaum, 1991] and a vari-

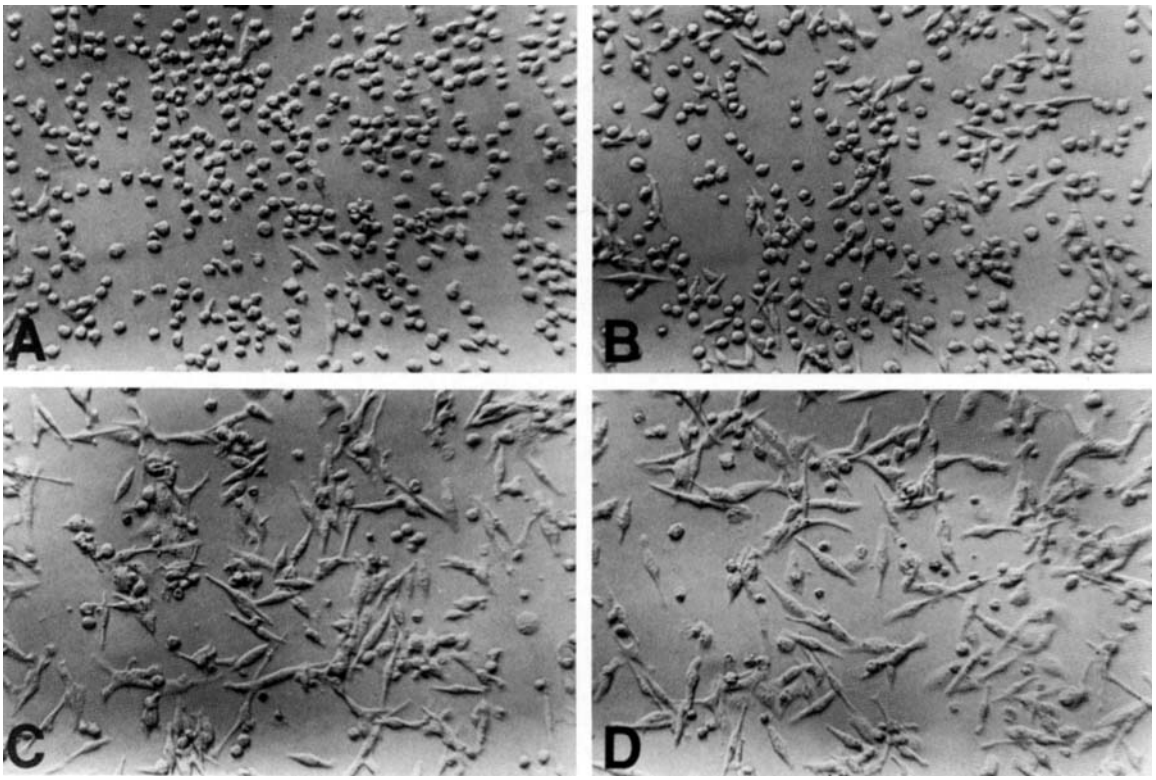

Fig. 5. PMA dose-dependently induces spreading of HD11 cells. HD11 cells plated in the presence of 10% fetal calf serum were exposed to vehicle (**A**) or PMA at 1.0 (**B**), 5.0 (**C**), or 60.0 (**D**) ng/ml for 14 h. The cells were then photographed under phase microscopy. Spreading is induced at 1.0 ng/ml PMA and maximizes at 5.0 ng/ml.

ety of macrophage cell lines [Bar-Shavit et al., 1985; Perkins et al., 1991]. Interestingly, in some cases such as the human promyelocytic leukemia line HL-60, PMA [Honda et al., 1990], like D_3 [Bar-Shavit et al., 1985] prompts selective differentiation along a monocytic pathway. Thus, D_3 and phorbol esters are both agents capable of promoting macrophage maturation.

The integrin $\alpha_v\beta_3$ is a transmembrane matrix attachment heterodimer recognizing the amino acid motif RGD [Ruoslahti, 1991]. We have shown this integrin essential to the bone resorptive function of osteoclasts, which are terminal members of the monocyte/macrophage family [Ross et al., 1993]. In this regard, appearance of marrow macrophages, as they differentiate, is prompted by the osteoclastogenic steroid hormone $1,25(OH)_2D_3$ [Mimura et al., 1994]. Expression of this heterodimer by differentiated members of the monocyte/macrophage family is not limited to osteoclasts, as it is effectively induced, in the mouse, by the anti-osteoclastogenic cytokine interleukin-4 (IL-4) [Kitawaza et al., 1995].

Our finding that $1,25(OH)_2D_3$ is a potent modulator of $\alpha_v\beta_3$ expression, an event associated with β_3 transactivation, led us to clone the regulatory region of the avian β_3 integrin gene [Cao et al., 1993]. In this regard, we identified, in the 5′ flanking region, two sequences of DNA, each of which is transactivated by the steroid. The first such sequence is a characteristic vitamin D response element consisting of two hexanucleotide repeats separated by a 3 base spacer [Cao et al., 1993]. The second response element, consisting of a complex series of hexanucleotide repeats, is in proximity (-135 to -150) [Cao et al., 1994] to a putative AP-1 recognition site (-104 to -110) [Cao et al., 1993] differing from the consensus AP-1 sequence by only one nucleotide [Angel et al., 1987]. The fact that the candidate β_3 AP-1 element recognizes purified c-*jun* protein by electromobility shift analysis prompted us to ask whether the gene is transactivated by PMA, known to induce *fos/jun* [Fisch et al., 1987].

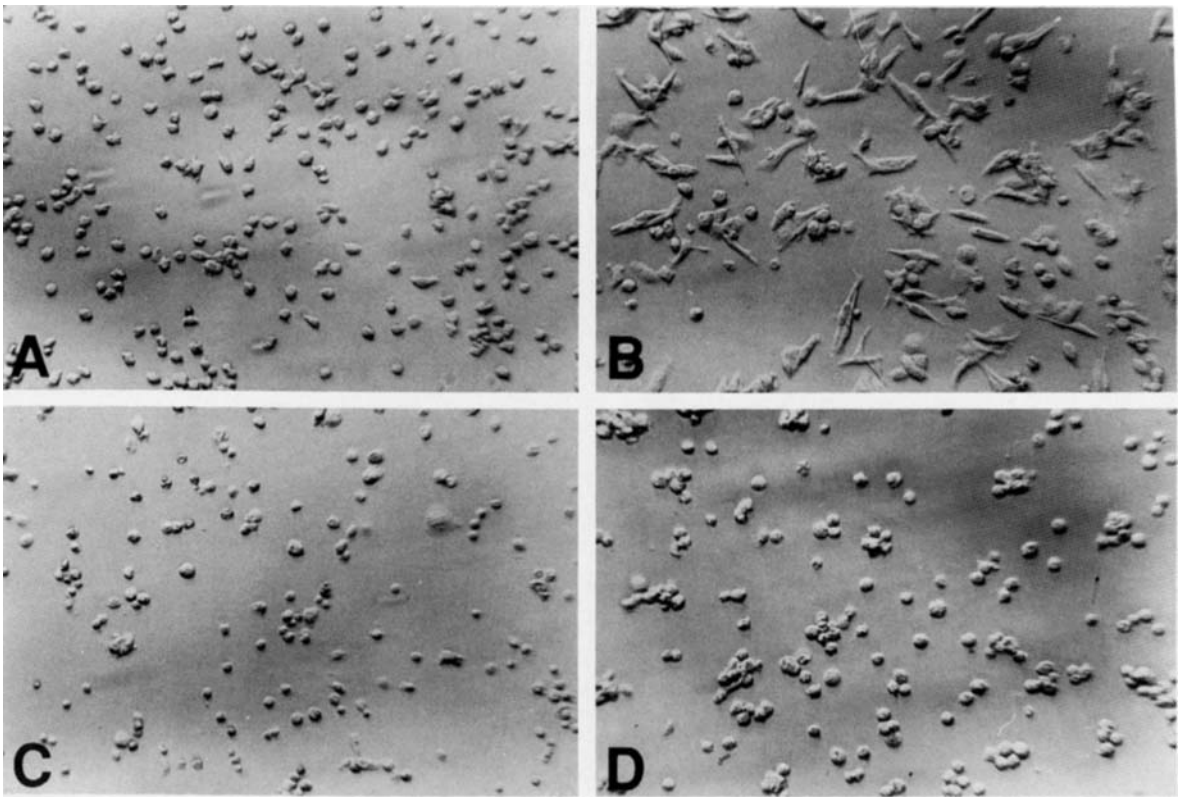

Fig. 6. $\alpha_v\beta_3$ antagonists block PMA-induced HD11 spreading. HD11 cells were plated in 10% fetal calf serum with vehicle (**A**) or 5.0 ng/ml PMA (**B–D**); 200 μg of irrelevant (**B**) or anti-avian α_v MAb, CHAV (**C**) or 40 μm $\alpha_v\beta_3$-inhibiting peptide mimetic, SC56631 (**D**) were added to the cells immediately prior to plating. Both the anti-α_v and peptide mimetic block PMA-induced spreading.

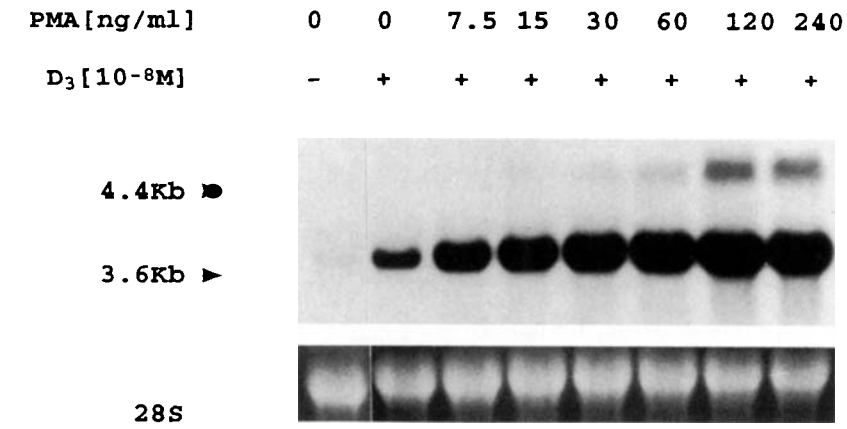

Fig. 7. PMA dose-dependently enhances $1,25(OH)_2D_3$-induced β_3 mRNA expression. HD11 cells were incubated for 48 h in the presence of $1,25(OH)_2D_3$ with or without various concentrations of PMA. RNA was extracted and subjected to Northern analysis using an avian β_3 cDNA. $1,25(OH)_2D_3$ induces β_3 mRNA expression, which is progressively enhanced by PMA.

To this end we turned to the transformed avian monocytic line, HD-11, known to contain molecular machinery capable of mediating $1,25(OH)_2D_3$ transactivation of the β_3 integrin promoter, in the context of a reporter construct [Cao et al., 1993]. We find that relatively low concentrations of PMA promote $\alpha_v\beta_3$ expression by these cells. Mirroring protein, β_3 mRNA is induced quickly and its appearance reflects transactivation of the β_3 gene. By contrast, and simi-

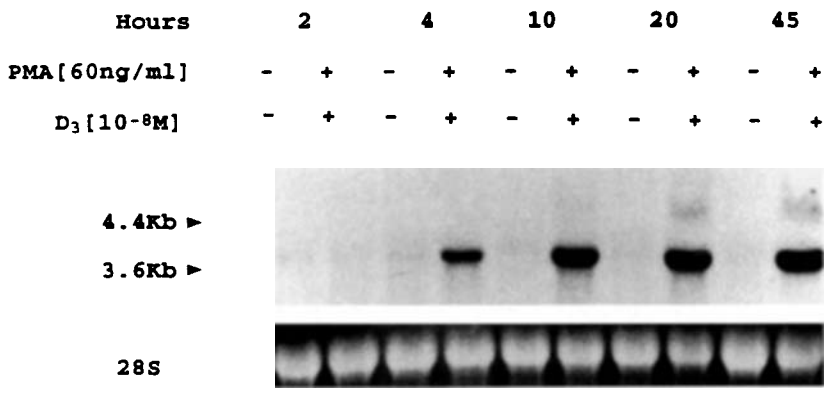

Fig. 8. PMA enhances $1,25(OH)_2D_3$-induced β_3 mRNA expression in a time-dependent manner. HD11 cells were incubated with $1,25(OH)_2D_3$ and PMA (+) or vehicle (−). RNA was extracted with time and subjected to Northern analysis using an avian β_3 cDNA. Induction of β_3 mRNA by both agents appears by 4 h of exposure.

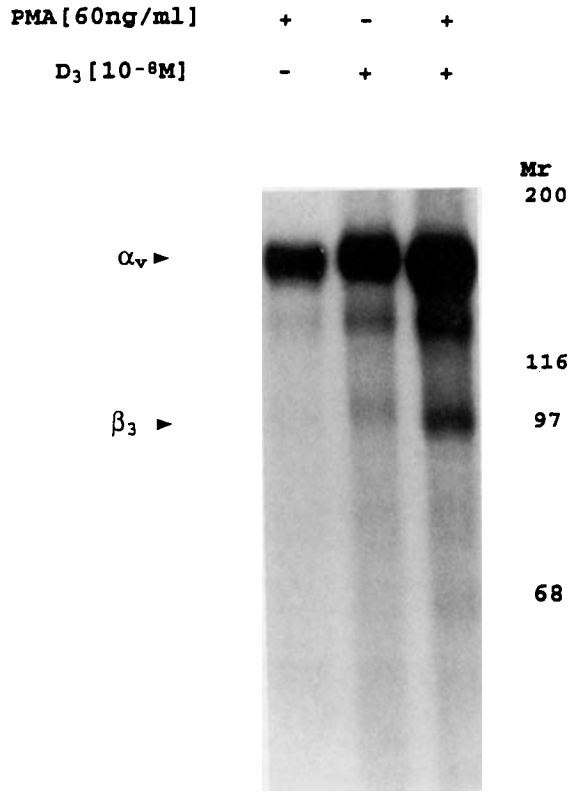

Fig. 9. PMA and $1,25(OH)_2D_3$ induction of $\alpha_v\beta_3$ are additive. HD11 cells were incubated with PMA or $1,25(OH)_2D_3$, or a combination of both agents for 48 h. The cells were ^{125}I surfaced-labeled and lysed and the lysate immunoprecipitated with an anti-$\alpha_v\beta_3$ antibody. The combined effect of the agents is greater than either alone.

lar to selected integrin agonists [Kitazawa et al., 1995; Sheppard et al., 1992], PMA does not transactivate the α_v gene (not shown). As β_3, in cells other than platelets, associates only with α_v while α_v partners with a number of β subunits [Ruoslahti, 1991], our observation suggests that in the circumstance of phorbol ester-mediated induction, it is the β_3, and not α_v, subunit that regulates the integrin appearance on the plasma membrane.

$\alpha_v\beta_3$ is an integrin localizing to focal adhesions, wherein it interacts with cytoskeletal proteins altering cell morphology [Wayner et al., 1991]. Thus, we find that PMA, in minimal β_3-inductive concentrations, profoundly enhances cell spreading. Furthermore, an antibody recognizing the external domain of the avian α_v subunit [Neugebauer et al., 1991] and an RGD peptide mimetic, which blocks α_v-mediated attachment of melanoma cells to vitronectin [Nickols et al., 1995], virtually eliminate PMA-stimulated spreading. These data suggest the PMA-induced morphological change reflects emergence of a functional α_v-containing integrin. Thus, it appears that PMA-mediated enhancement of $\alpha_v\beta_3$ expression is reflected by stimulated interaction of target cells with appropriate ligand.

The complex relationship enjoyed by phorbol esters and steroid hormones [Miner and Yamamoto, 1991] prompted us to explore their associated effect on $\alpha b_v\beta_3$ expression, which we find additive at the protein, mRNA and transcriptional levels. On the other hand, the impact of PMA in combination with $1,25(OH)_2D_3$ on HD11 spreading is no greater than PMA alone (not shown), suggesting the magnitude of $\alpha_v\beta_3$ induction by the phorbol ester is sufficient to invoke the cell's maximum response.

While the mechanism underlying PMA/$1,25(OH)_2D_3$-simulated $\alpha_v\beta_3$ expression remains

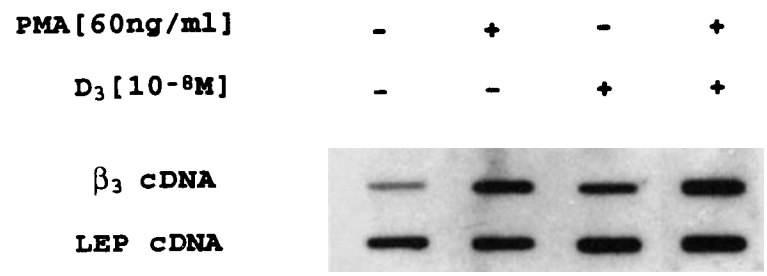

Fig. 10. PMA and $1,25(OH)_2D_3$ alone, and in combination, transactivate the β_3 gene. Nuclei from HDll cells, treated for 48 h with various combinations of PMA and $1,25(OH)_2D_3$, were isolated and subjected to run-on assay using avian β_3 and LEP (control) cDNAs. The β_3 gene is transactivated by both agents, and their effects are additive.

to be determined, phorbol esters and steroid hormones each, and in combination, influence fos/jun. For example, $1,25(OH)_2D_3$ enhances the *fos/jun* inductive properties of phorbol esters [Pan et al., 1991] and the steroid alone may modulate c-*fos* expression [Brelvi et al., 1986]. *fos/jun* proteins may also interact with the vitamin D receptor and the complex can alter transcription [Hyder et al., 1994]. In addition, AP-1 and steroid response elements may merge, recognizing both steroid receptors and *fos/jun* [Miner and Yamamoto, 1991]. In this circumstance, *fos/jun*-steroid receptor interactions may either enhance or suppress transactivation of the composite response element-bearing gene [Diamond et al., 1990]. In fact, such appears to be the case in the osteocalcin promoter in which AP-1 and vitamin D response elements overlap [Schüle et al., 1990]. The regulatory component of the β_3 integrin gene thus presents itself as a member of a family of promoters in which the vitamin D receptor and PMA-induced transcription factors may interact.

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