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Transcriptional and Posttranscriptional Regulation of Interstitial Collagenase by Platelet-Derived Growth Factor BB in Bone Cell Cultures*

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ABSTRACT

Platelet-derived growth factor (PDGF), a bone cell mitogen, stimulates bone collagen degradation and does not enhance bone matrix apposition rates. The mechanism of the effect on collagen degradation is unknown, and it could involve changes in interstitial collagenase synthesis. We tested the effects of PDGF on interstitial collagenase expression in cultures of osteoblast-enriched cells from fetal rat calvariae (Ob cells). After 4–8 h of treatment, PDGF BB at 0.3 nM increased steady state collagenase messenger RNA (mRNA), whereas PDGF AA had no effect. The effect of PDGF BB on collagenase transcripts was dose dependent. PDGF BB increased the levels of immunoreactive collagenase after 6 h, whereas the levels were decreased after 16 h. Stimulation of collagenase mRNA by PDGF BB was dependent on *de novo* protein synthesis and activation of protein kinase C. PDGF BB prolonged the half-life of collagenase mRNA in transcriptionally arrested cells. PDGF BB initially increased and subsequently decreased the rate of collagenase gene transcription and the levels of collagenase heterogeneous nuclear RNA. In conclusion, PDGF BB regulates interstitial collagenase in Ob cells by transcriptional and posttranscriptional mechanisms, and this effect may contribute to its stimulatory actions on bone collagen degradation. (*Endocrinology* **137:** 431–437, 1996)

PLATELET-DERIVED growth factor (PDGF), a polypeptide with a molecular mass of 30,000, is a homo or heterodimer product of the PDGF A and B genes (1, 2). PDGF is present in the systemic circulation and is expressed by normal and malignant skeletal cells; consequently, it is considered to act as a systemic and local regulator of bone cell function (3–6). PDGF BB and, to a lesser extent, PDGF AA has mitogenic properties for bone cells, but it does not enhance the differentiated function of the osteoblast and does not stimulate matrix apposition rates (7). In addition, PDGF BB was recently shown to increase bone collagen degradation and to increase the number of osteoclasts in cultured rat calvariae (7, 8). This would suggest a role for PDGF BB in bone collagen degradation and bone resorption, but the mechanisms for these possibly related effects have not been explored.

Matrix metalloproteinases and their inhibitors are considered active participants in the degradation of osteoid and are synthesized by osteoblasts and osteoclasts, although their expression varies with the cell line and culture conditions used (9–12). Interstitial collagenase (matrix metalloproteinase 1 or type I collagenase) is the only protease, except neutrophil collagenase, known to degrade native interstitial collagens at neutral pH (9). Although selected growth factors, such as PDGF, are known to modulate the synthesis of collagenase in nonskeletal fibroblasts, the mechanisms involved are not known, and there is no information about the effects of PDGF BB on the synthesis of interstitial collagenase in skeletal cells (9, 13). Furthermore, although bone-resorbing hormones are known to regulate interstitial collagenase in bone, there is limited information about the actions of growth factors on the synthesis of this protease (14). We postulated that the stimulatory actions of PDGF BB on bone collagen degradation may involve an increase in collagenase expression by the osteoblast.

The present study was undertaken to determine whether PDGF AA and BB regulate interstitial collagenase in cultures of osteoblast-enriched cells from fetal rat calvariae (Ob cells) and to examine the possible mechanisms involved in this regulation.

Materials and Methods

Culture technique

The culture method used was described in detail previously (15). Parietal bones were obtained from 22-day-old fetal rats immediately after the mothers were killed by blunt trauma to the nuchal area. This project was approved by the institutional animal care and use committee of Saint Francis Hospital and Medical Center. Cells were obtained by five sequential digestions of the parietal bone using bacterial collagenase (CLS II, Worthington Biochemical Corp., Freehold, NJ). Cell populations harvested from the third to the fifth digestions were cultured as a pool and were previously shown to have osteoblastic characteristics (15). Ob cells were plated at a density of 8,000–12,000 cells/cm^2 and cultured in a humidified 5% CO_2 incubator at 37 C until reaching confluence (~50,000 cells/cm^2). Cells were cultured in DMEM supplemented with nonessential amino acids, 100 μg/ml L-ascorbic acid, penicillin, strep-

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tomycin, and 20 mM HEPES (all from Life Technologies, Grand Island, NY), and 10% fetal bovine serum (Hyclone, Logan, UT). Except for the nuclear run-off experiments, cells were grown to confluence, transferred to serum-free medium for 20–24 h and exposed to test or control medium in the absence of serum for 1–24 h, as indicated in the text and legends. For nuclear run-off experiments, cells were grown to subconfluence, trypsinized, replated, and grown to confluence, at which time they were serum deprived and exposed to DMEM or PDGF BB for 2–24 h. PDGF AA and BB (Austral, San Ramon, CA), sangivamycin (kindly provided by the NCI, Bethesda, MD), and cycloheximide (Sigma Chemical Co., St. Louis, MO) dissolved in water and 5,6-dichlorobenzimidazole riboside (DRB; Sigma) dissolved in ethanol were added to the culture medium to attain the concentrations indicated in the text and legends. At the end of the incubation, medium was stored at −80 C for collagenase determination, RNA was extracted from the cell layer for analysis, or nuclei were obtained by Dounce homogenization for nuclear run-off assays.

Northern blot analysis

Total cellular RNA was isolated with guanidine thiocyanate at acid pH, followed by a phenol-chloroform extraction (16). RNA was precipitated with isopropanol, resuspended, and reprecipitated with ethanol. The RNA recovered was quantitated by spectrometry, and equal amounts of RNA from control or test samples were loaded on a formaldehyde-agarose gel after denaturation. The gel was stained with ethidium bromide to visualize ribosomal RNA and photographed under UV light before and after transfer, documenting equal RNA loading of the various experimental samples. The RNA was blotted onto Nytran 0.2-micron nylon membrane (ICN, Costa Mesa, CA). Restriction fragments containing a 2.6-kilobase (kb) rat interstitial collagenase complementary DNA (cDNA; kindly provided by Dr. Cheryl Quinn, St. Louis, MO) and a 0.75-kb murine 18S cDNA (American Type Culture Collection, Rockville, MD) were labeled with [α-^{32}P]deoxy-CTP ([α-^{32}P]dCTP) and [α-^{32}P]dATP (3,000 Ci/mmol; DuPont, Boston, MA), using the random hexanucleotide primed second strand synthesis method (17, 18). Hybridizations were carried out at 42 C for 16–24 h. Posthybridization washes were performed in 1 × saline sodium citrate (SSC) at 55 C for interstitial collagenase and in 0.1 × SSC at 65 C for 18S. The bound radioactive material was visualized by autoradiography on Kodak X-AR5 film employing intensifying screens. Relative hybridization levels were determined by densitometry. Northern analyses shown are representative of three or more cultures.

RT-PCR

Collagenase heterogeneous nuclear RNA (hnRNA) was analyzed by RT-PCR using a sense primer 5′-CATTCAGCTATTCTGGCCAC-3′, spanning nucleotides 4–23 of exon 1 of the rat collagenase gene, and an antisense primer, 5′-AAAAGACCAGAACAACCAGC-3′, spanning nucleotides 61–80 of intron 1 (17) (Jeffrey, J., and A. Delany, unpublished data) to yield a 186-bp product. RNA samples were treated with amplification grade deoxyribonuclease I according to the manufacturer's instructions (Life Technologies) to remove potentially contaminating DNA. RNA (1 μg) was copied into cDNA using Moloney murine leukemia virus reverse transcriptase (Life Technologies) and antisense primer according to the manufacturer's instructions, except that *Taq* polymerase buffer was used instead of RT buffer (19). A DNA standard was synthesized by PCR amplification of pGL2-Basic plasmid DNA (Promega Corp., Madison, WI) using the rat collagenase hnRNA primer set and low stringency annealing conditions, as described by Forstr (20). The reaction products were fractionated on an agarose gel and visualized by ethidium bromide staining. A 150-bp band was excised and reamplified by PCR using the collagenase hnRNA primer set to be used as DNA standard. The newly synthesized cDNA and 0.05 attomole DNA standard were amplified by PCR using 24 cycles of 94 C for 1 min, 59 C for 1 min, and 72 C for 1 min in the presence of *Taq* polymerase (Life Technologies), 0.15 μmol sense and antisense primers, and 5 μCi [α-^{32}P]dCTP. PCR products were resolved on a 6% polyacrylamide gel containing 100 mM Tris-borate-1 mM EDTA and visualized by autoradiography. The amplification protocol yielded products that were within the linear range for both the collagenase hnRNA and the standard. Data on hnRNA are representative of two cultures.

Nuclear run-off assay

To examine changes in the rate of transcription, nuclei were isolated by Dounce homogenization in a Tris buffer containing 0.5% Nonidet P-40. Nascent transcripts were labeled by incubation of nuclei in a reaction buffer containing 500 μM each of ATP, CTP, and guanidine triphosphate; 150 U RNAsin (Promega); and 250 μCi [α-^{32}P]UTP (3,000 Ci/mmol; DuPont) (21). RNA was isolated by treatment with deoxyribonuclease I and proteinase K, followed by phenol-chloroform extraction and ethanol precipitation. Linearized plasmid DNA containing about 1 μg cDNA was immobilized onto GeneScreen Plus by slot blotting according to the manufacturer's directions (DuPont). The plasmid vector pGL2-Basic (Promega) was used as a control for nonspecific hybridization, and a rat glyceraldehyde-3-phosphate dehydrogenase (GAPD) (22), kindly provided by R. Wu (Ithaca, NY), was used to estimate loading of the gel. Equal counts per min of [^{32}P]RNA from each sample were hybridized to cDNAs at 42 C for 72 h and washed in 1 × SSC at 62 C for 20 min. Hybridized DNAs were visualized by autoradiography.

Western immunoblot analysis

At the completion of the culture, medium was aspirated and stored at −80 C after the addition of polyoxyethylene sorbitan monolaurate (Pierce Chemical Co., Rockford, IL) to a final concentration of 0.1%. Samples were fractionated by PAGE using denaturing and nonreducing conditions and transferred onto Immobilon P membranes (Millipore Corp., Bedford, MA). After blocking with 2% BSA, the membranes were exposed to a 1:1000 dilution of rabbit antiserum raised against rat interstitial collagenase, previously characterized for specificity and immunoreactivity (23), followed by the addition of goat antirabbit IgG conjugated to horseradish peroxidase. The blots were washed and developed with a horseradish peroxidase chemiluminescence detection reagent (DuPont), visualized by autoradiography on DuPont Refection film employing Reflection intensifying screens, and analyzed by densitometry. The blots were stained with colloidal gold reagent (Integrated Separation Systems, Hyde Park, MA) for visualization of protein. The data shown are representative of three cultures.

Statistical methods

Data on collagenase messenger RNA (mRNA) decay were analyzed by linear regression, and the slopes of the regression lines obtained for control and PDGF BB-treated cells were compared for significant differences using the computer program Crunch version 4 (Crunch Software Corp., Oakland, CA), as previously described (24).

Results

Confirming prior studies, Northern blot analysis of total RNA from Ob cells revealed an interstitial collagenase transcript of 2.9 kb (Fig. 1) (11). Continuous treatment of Ob cells with PDGF BB for 1–24 h at 0.3 nM caused a transient increase in collagenase steady state transcripts. A small increase was observed after 2 h of exposure to PDGF BB, whereas treatment for 4–8 h caused a maximal stimulation of collagenase transcripts (Fig. 1 and Table 1). PDGF BB had no effect after 16–24 h. In contrast to the effect of PDGF BB, exposure to PDGF AA at 0.3 nM (Fig. 1 and Table 1) or 3.3 nM (data not shown) did not modify interstitial collagenase expression in Ob cells. Continued exposure of Ob cells to PDGF BB at 0.3 and 3.3 nM for 6 h increased collagenase transcripts by 7- and 13-fold, respectively (Fig. 2). PDGF BB also regulated the levels of immunoreactive interstitial collagenase in the culture medium of Ob cells, as determined by Western blot analysis (Fig. 3). Collagenase was identified by comigration with a purified rat collagenase standard. PDGF BB at 3.3 nM

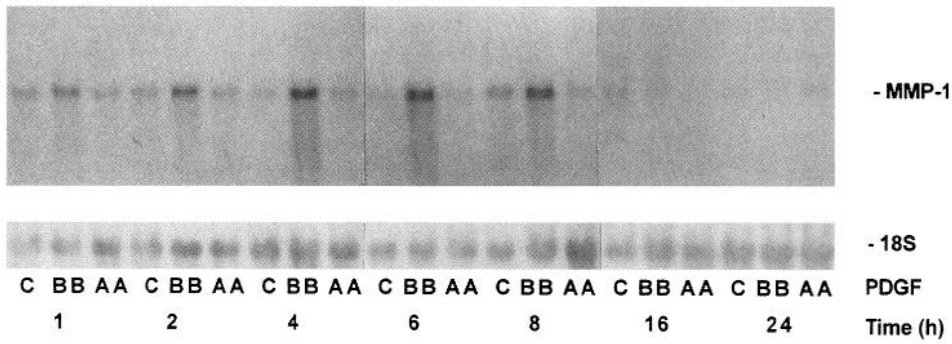

FIG. 1. Effects of PDGF AA and BB at 0.3 nM on interstitial collagenase mRNA levels in cultures of Ob cells treated for 1–24 h. Total RNA (15 μg/lane) from control (C), PDGF AA-treated (AA), or PDGF BB-treated (BB) cultures was subjected to Northern blot analysis and hybridized with ^{32}P-labeled collagenase and 18S cDNAs. The *upper panel* shows collagenase mRNA (MMP-1), and the *lower panel* shows 18S ribosomal RNA (18S) levels from one of three independent cultures.

TABLE 1. Changes in steady state collagenase mRNA in PDGF AA- and BB-treated cells

Treatment time (h)	PDGF AA	PDGF BB
1	0.6 ± 0.2	1.3 ± 0.2
2	0.8 ± 0.2	1.9 ± 0.3
4	1.5 ± 0.2	14.3 ± 7.0
6	1.9 ± 1.7	7.1 ± 1.3
8	1.0 ± 0.3	5.5 ± 3.3
16	0.5 ± 0.6	1.2 ± 0.3
24	0.5 ± 0.1	1.3 ± 0.2

Total RNA from control cultures or cultures treated with PDGF AA or BB at 0.3 nM for 1–24 h were analyzed for collagenase mRNA and 18S ribosomal RNA by Northern hybridization followed by densitometry. The changes in steady state collagenase mRNA in PDGF-treated cells relative to control values, after normalizing for changes in 18S ribosomal RNA, are shown as the mean ± SEM for three independent cultures.

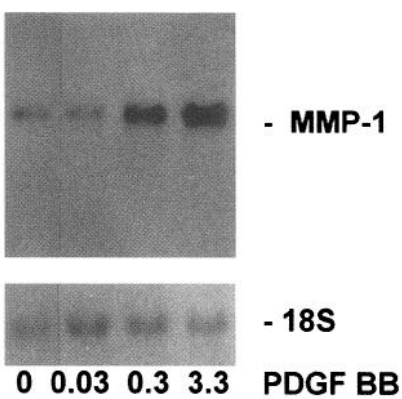

FIG. 2. Effect of PDGF BB at 0.03–3.3 nM on interstitial collagenase mRNA levels in cultures of Ob cells treated for 6 h. Total RNA (15 μg/lane) from control or PDGF-treated cultures was subjected to Northern blot analysis and hybridized with ^{32}P-labeled collagenase and 18S cDNAs. The *upper panel* shows collagenase mRNA (MMP-1), and the *lower panel* shows 18S ribosomal RNA (18S) from one of three independent cultures.

increased immunoreactive collagenase concentrations by 3-fold after 6 h, although PDGF BB was inhibitory after 16 h. Colloidal gold staining of the blot revealed equal amounts of protein in control and treated cultures (data not shown).

To determine whether the effect of PDGF BB on interstitial collagenase mRNA levels was dependent on protein synthesis, confluent cultures of Ob cells were treated with PDGF BB in the presence or absence of cycloheximide at doses known to inhibit protein synthesis (25). Cycloheximide at 3.6 μM superinduced collagenase transcripts, and cotreatment with PDGF BB for 6 h caused no additional increase in collagenase mRNA levels in cells treated with cycloheximide and the growth factor (Fig. 4). To determine whether protein kinase C (PKC)-dependent pathways were involved in the stimulation of interstitial collagenase transcripts by PDGF BB, we examined the effects of PDGF BB in the presence and absence of sangivamycin at 50 μM, a specific inhibitor of PKC (26). Sangivamycin decreased collagenase mRNA levels in control cultures and PDGF BB-treated cultures (Fig. 5).

To test the effect of PDGF BB on interstitial collagenase transcript stability, Ob cells were exposed to control or PDGF BB-containing medium for 4 h before the arrest of transcription with the RNA polymerase II inhibitor DRB at 75 μM (27). Interstitial collagenase mRNA levels were determined 0–24 h after the cells were exposed to DRB or to DRB and PDGF BB and are expressed as a percentage of the collagenase

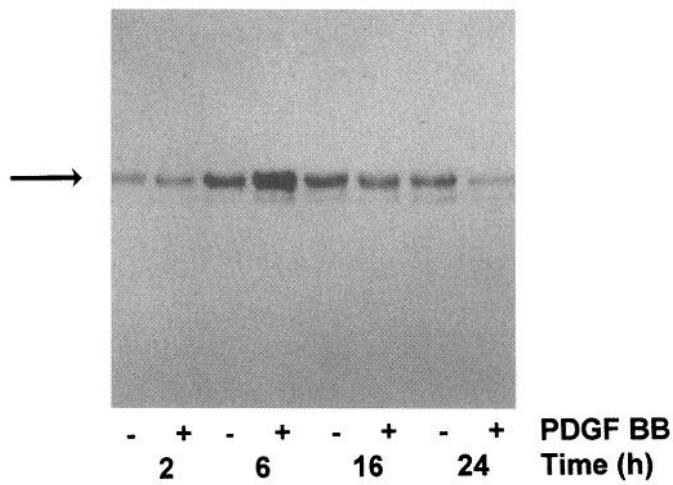

FIG. 3. Effect of PDGF BB at 3.3 nM on procollagenase secretion in Ob cell cultures treated for 2–24 h. Western blot analysis was performed using equal amounts of culture medium from control (−) and PDGF BB-treated (+) cultures. Procollagenase (*arrow*) was detected using rabbit antirat collagenase antibody and the horseradish peroxidase chemiluminescence detection system. Data are representative of one of three independent cultures.

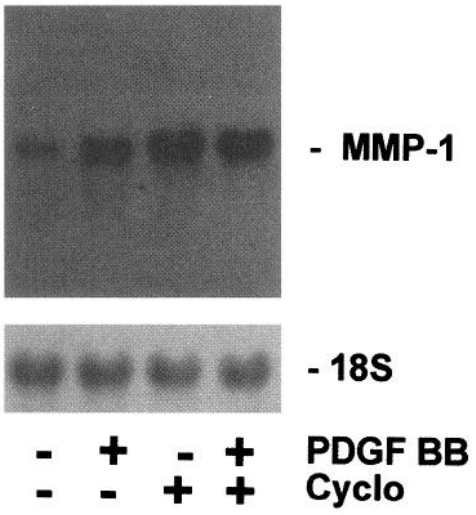

FIG. 4. Effect of PDGF BB at 0.3 nM in the presence or absence of cycloheximide (Cyclo) at 3.6 μM on interstitial collagenase mRNA levels in cultures of Ob cells treated for 6 h. Total RNA (15 μg/lane) from control or treated cultures was subjected to Northern blot analysis and hybridized with ^{32}P-labeled rat collagenase and 18S cDNAs. The *upper panel* shows collagenase mRNA (MMP-1), and the *lower panel* shows 18S ribosomal RNA (18S) from one of three independent cultures.

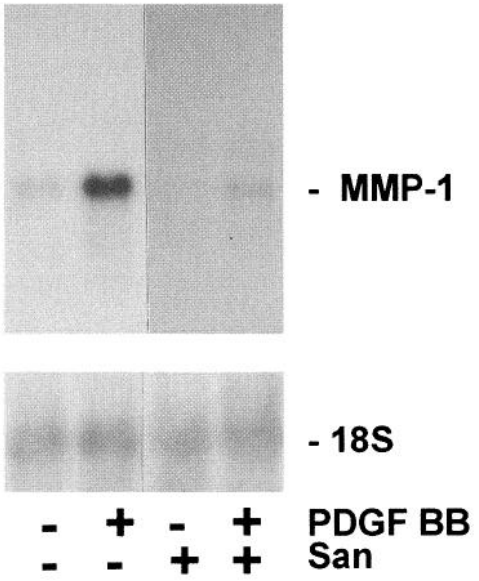

FIG. 5. Effect of PDGF BB at 0.3 nM in the presence or absence of sangivamycin (San) at 50 μM on interstitial collagenase mRNA levels in cultures of Ob cells treated for 6 h. Total RNA (15 μg/lane) from control or treated cultures was subjected to Northern blot analysis and hybridized with ^{32}P-labeled rat collagenase and 18S cDNAs. The *upper panel* shows collagenase mRNA (MMP-1), and the *lower panel* shows 18S ribosomal RNA (18S) levels from one of three independent cultures.

mRNA present at the time of DRB addition (Fig. 6). The interstitial collagenase mRNA half-life was approximately 6 h in control cultures and approximately 24 h in cells exposed to PDGF BB at 0.3 nM, indicating that PDGF BB caused a significant stabilization of collagenase mRNA.

PDGF BB at 0.3 nM for 1–4 h increased interstitial collagenase hnRNA, as determined by RT-PCR (Fig. 7). A maximal increase of approximately 3-fold in hnRNA was observed after 4 h of exposure to PDGF BB, although the growth factor decreased interstitial collagenase hnRNA levels by approximately 30% after 8 h (data not shown). Coamplification of an exogenous DNA standard, designed to use the same set of primers, revealed uniform PCR efficiency, and omission of the reverse transcription step resulted in no signal, proving the lack of DNA contamination. To confirm that PDGF BB modified collagenase RNA transcriptional rates, the rate of gene transcription was measured by a nuclear run-off assay. PDGF BB at 3.3 nM increased the transcriptional rate of the collagenase gene by 2-fold after 2 h, but it decreased the transcriptional rate after 6 h (Fig. 8), and the inhibitory effect persisted up to 24 h (not shown).

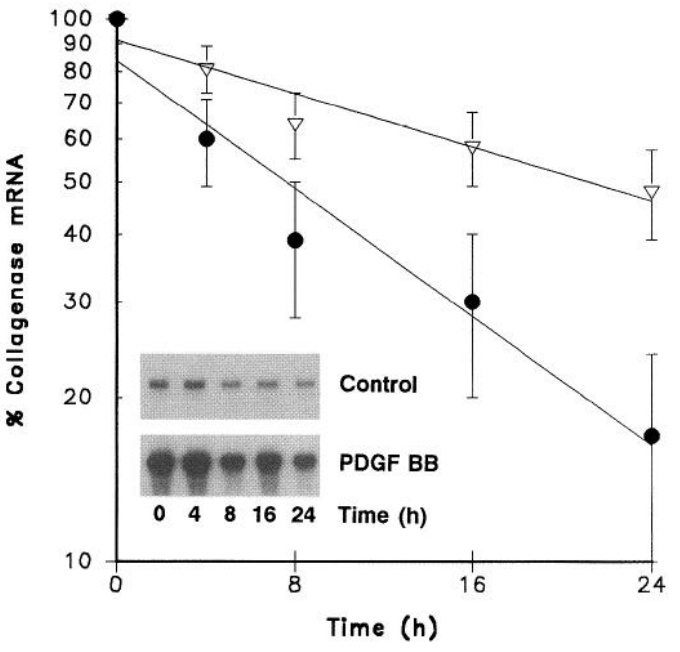

FIG. 6. Effect of PDGF BB at 0.3 nM on interstitial collagenase mRNA decay in Ob cell cultures. Ob cells were exposed to control or PDGF BB-containing medium for 4 h before the addition of DRB at 75 μM. Total RNA (12 μg/lane), obtained 0–24 h after DRB addition, was subjected to Northern blot analysis and hybridized with a ^{32}P-labeled rat collagenase cDNA. Collagenase mRNA was visualized by autoradiography and quantitated by densitometry. Data from control (●) and PDGF BB-treated (△) cultures are expressed as the mean ± SEM for three or more cultures and as a percentage of mRNA levels before the addition of DRB. *Inset*, A representative experiment showing collagenase mRNA after the addition of DRB in control and PDGF BB-treated Ob cell cultures.

Discussion

Recent studies revealed that PDGF BB stimulates bone collagen degradation, suggesting the possibility that PDGF may regulate the expression of interstitial collagenase. The present investigation was undertaken to study the regulation of interstitial collagenase in bone cells. PDGF BB transiently increased collagenase steady state transcripts, whereas PDGF AA had no effect. A modest decrease in steady state collagenase mRNA levels was observed in control cultures after 24 h, possibly due to the secretion of autocrine/paracrine factors, such as insulin-like growth factors that are

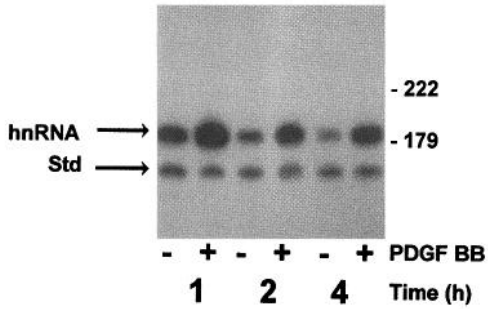

FIG. 7. Effect of PDGF BB at 0.3 nM on collagenase hnRNA levels in cultures of Ob cells treated for 1–4 h. Total RNA (1 μg) from control and treated cultures was reverse transcribed and amplified by PCR in the presence of 5 μCi [α-^{32}P]dCTP using collagenase exon I- and intron I-specific primers to generate a 186-bp product. An exogenous standard DNA (Std), designed to use the same primers, was coamplified with each reaction to assess PCR efficiency. PCR products from one of two independent cultures, visualized by autoradiography, are shown. The molecular size of products in base pairs is indicated on the *right margin*.

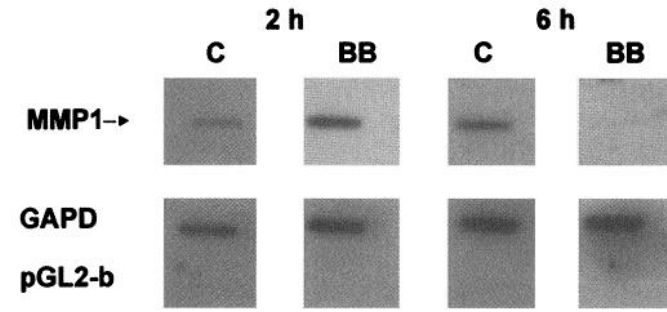

FIG. 8. Effect of PDGF BB (BB) at 3.3 nM on interstitial collagenase transcription rates in cultures of Ob cells treated for 2 or 6 h. Nascent transcripts were labeled *in vitro* with [α-^{32}P]UTP, and the labeled RNA was hybridized to immobilized cDNA for interstitial collagenase. GAPD cDNA was used to demonstrate loading, and pGL2-Basic vector DNA (pGL2-b) was used as a control for nonspecific hybridization.

inhibitors of collagenase (28). Confirming prior observations (11), cycloheximide superinduced interstitial collagenase mRNA, suggesting that it stabilized the transcripts (29, 30). PKC agonists, such as phorbol esters, are potent inducers of interstitial collagenase mRNA in Ob cells (31). Sangivamycin, an agent known to block PKC activity (26), suppressed collagenase expression in control cultures and blunted PDGF-mediated induction of collagenase, suggesting that PKC may play a role in basal and PDGF-stimulated collagenase expression. Alternatively, sangivamycin may affect collagenase gene expression by some unknown mechanism.

PDGF BB increased immunoreactive collagenase in the medium of Ob cells after 6 h, as expected from the increase in collagenase gene expression. After 16–24 h of culture, the levels of immunoreactive collagenase in the medium of control cells were modestly decreased, whereas collagenase protein levels were dramatically decreased in the medium of PDGF BB-treated cells. Hence, it is possible that PDGF BB stimulated the clearance of secreted collagenase in Ob cells by a mechanism similar to that described for osteosarcoma cells. Omura *et al.* (32) characterized a cell surface receptor for collagenase that mediates the uptake and degradation of extracellular collagenase in PTH-treated osteosarcoma UMR 106 cells. In Ob cells, the levels of immunoreactive collagenase in the medium of PDGF BB-treated cells decreased with time after 6 h, suggesting that PDGF BB may enhance the uptake and degradation of collagenase, as demonstrated for PTH.

PDGF BB increased the stability of collagenase mRNA in Ob cells. This effect is similar to that previously reported for retinoic acid (11). The higher levels of mRNA in transcriptionally arrested cells relative to steady state RNA levels may be due to blocking transcriptional suppression and RNA stabilization. In addition to collagenase transcript stabilization, PDGF BB caused an increase in collagenase hnRNA levels and rates of transcription of collagenase gene. However, this increase was transient, and after 6–24 h, PDGF BB inhibited the rates of interstitial collagenase gene transcription. PDGF BB also decreased the levels of collagenase hnRNA after 8 h. These complementary techniques of hnRNA analysis and nuclear run-off assays suggest that PDGF BB initially stimulates and subsequently inhibits collagenase transcription. PDGF BB is known to induce the c-*fos* and c-*jun* protooncogenes whose protein products bind to AP-1 sequences. The interstitial collagenase gene promoter contains AP-1-binding sites (33, 34) (Jeffrey, J., unpublished observations), and the fact that PKC inhibitors may block the effect of PDGF BB on collagenase mRNA levels suggest possible interactions with these sites in the collagenase gene (35, 36). The increase followed by a decrease in collagenase rates of transcription observed with PDGF BB in Ob cells is analogous to that observed with phorbol myristate acetate, which regulates collagenase transcription through AP-1-dependent mechanisms (Delany, A., and E. Canalis, unpublished observations) (33, 34). It is possible that both agents use a similar pathway in the osteoblast regulation of the collagenase gene, and that the down-regulation of transcription is due to depletion of PKC activity after prolonged exposure to PDGF BB or phorbol esters. Our results suggest a complex regulation of the interstitial collagenase gene by PDGF BB in osteoblasts. The PDGF-mediated increase in collagenase mRNA levels due to increased rates of transcription and increased transcript stabilization leads to increased levels of the protease. The subsequent decrease in steady state collagenase mRNA levels lags the decrease in collagenase gene transcription, probably due to the PDGF-mediated increase in collagenase mRNA stability.

The effects described were shown at doses of PDGF BB that enhance bone cell replication and at doses that increase osteoclast number and collagen degradation (7, 8). This suggests that the effects observed are relevant to the stimulation of bone collagen degradation by PDGF BB. In accordance with these results, PDGF AA did not induce collagenase transcripts and does not increase bone collagen degradation or the number of osteoclasts in cultured rat calvariae. This suggests differential actions of PDGF AA or BB on bone collagen degradation or possibly differences in potency between PDGF AA and BB, as PDGF AA is less effective than PDGF BB on multiple aspects of bone cell function (7). Earlier studies with different isoforms of PDGF indicated that the biological effects of PDGF AB on bone cells are intermediate between those of PDGF BB and PDGF AA (37). Hence, we presume that PDGF AB may cause a modest increase in

collagenase expression. The effects of PDGF BB are similar to those of other skeletal growth factors with strong mitogenic properties such as basic fibroblast growth factor, which also increases collagenase synthesis in Ob cells (31). In contrast, insulin-like growth factors, agents with weak mitogenic properties and known to stimulate osteoblastic differentiated function, inhibit collagenase expression (28). Mitogenic factors, such as PDGF BB, are probably important in bone repair and may promote the removal of damaged bone matrix from a fracture site by stimulating collagenase production.

In conclusion, the present studies demonstrate that PDGF BB increases interstitial collagenase transcripts in bone cells by transcriptional as well as posttranscriptional mechanisms. As PDGF BB increases bone collagen degradation, it is probable that the stimulation of collagenase synthesis plays a role in this process.

Acknowledgments

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