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Uncoordinate Regulation of Collagenase, Stromelysin, and Tissue Inhibitor of Metalloproteinases Genes by Prostaglandin E_2: Selective Enhancement of Collagenase Gene Expression in Human Dermal Fibroblasts in Culture

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Abstract The degradative effects of interleukin-1 (IL-1) on the extracellular matrix of connective tissue are mediated primarily by metalloproteinases and prostaglandins. Clinical observations suggest that these effects can be prevented, to some extent, by the use of non-steroidal anti-inflammatory drugs. We have examined the role of prostaglandin E_2 (PGE_2) in IL-1-induced gene expression by human skin fibroblasts in culture. Incubation of confluent fibroblast cultures with varying concentrations (0.01–1.0 μg/ml) of PGE_2 led to a dose-dependent elevation of collagenase mRNA steady-state levels, the promoter activity, and the secretion of the protein, whereas relatively little effect was observed on stromelysin and TIMP gene expression. Exogenous PGE_2 had no additive or synergistic effect with IL-1 on collagenase gene expression. Furthermore, commonly used non-steroidal anti-inflammatory drugs (indomethacin, acetyl salicylic acid and ibuprofen), at doses which block prostaglandin synthesis in cultured fibroblasts, failed to counteract IL-1-induced collagenase and stromelysin gene expression, nor did they affect TIMP expression. Although the effects of PGE_2 did not potentiate those of IL-1 on collagenase gene expression in vitro, one could speculate that massive production of PGE_2 by connective tissue cells in vivo in response to inflammatory mediators such as IL-1 or tumor necrosis factor-α, could lead to sustained expression of collagenase in connective tissue cells after clearance of the growth factors.

Key words: interleukin-1, prostaglandin E_2, matrix metalloproteinases, dermal fibroblasts

INTRODUCTION

Matrix metalloproteinases (MMPs) comprise a family of proteolytic enzymes involved in the degradation of the extracellular matrix of connective tissue. These enzymes play a critical role in a number of physiological and pathological processes involving connective tissue remodeling, such as embryonic development, wound repair, tumor metastasis, and certain inflammatory diseases characterized by destruction of the extracellular matrix, as in rheumatoid arthritis [Woessner, 1991]. Breakdown of the fibrillar collagen network is initiated by interstitial collagenase, whereas the other components of the matrix are degraded by stromelysin and gelatinase. Furthermore, stromelysin is required for maximal activation of collagenase. Both enzymes can be inhibited by the tissue inhibitor of metalloproteinases (TIMP), a ~25 kDa peptide which forms stoichiometric 1:1 complexes with MMPs and inactivates them, by as yet unknown mechanism [Woessner, 1991].

Abbreviations FCS, fetal calf serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL, interleukin; MMP, matrix metalloproteinase; NSAID, nonsteroidal anti-inflammatory drug; PGE, prostaglandin E; TIMP, tissue inhibitor of metalloproteinases; TNF-α, tumor necrosis factor-α

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The expression of MMPs by connective tissue cells is modulated by a variety of cytokines, including interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), which are primarily produced by macrophages within the inflammatory cell infiltrates [reviewed in Krane et al., 1990; Mauviel, 1993]. IL-1 and TNF-α can, therefore, modulate connective tissue destruction mediated by collagenase and stromelysin. Also, upon stimulation by either IL-1 or TNF-α, activation of the arachidonate cascade occurs, leading to production of a variety of metabolites, such as prostaglandins and leukotrienes [Dayer et al., 1985, 1986] which play an essential role in the development of the inflammatory reaction through activation of neutrophils, macrophages, and platelets. We have previously shown that IL-1 induces high levels of PGE_2 in dermal fibroblasts, which reach up to 0.3 μg/ml over a 24 hour period [Mauviel et al., 1988a]. These prostaglandins mediate some of the effects of IL-1 in certain cell types. For example, PGE_2 mediates IL-1-induced monocyte cytotoxicity [Onozaki et al., 1985] and IL-1-induced IL-1 receptor expression in fibroblasts [Akahoshi et al., 1988]. Similarly, IL-1 stimulation of synovial fibroblast plasminogen activator production is mediated by endogenous PGE_2 [Mochan et al., 1986]. In fact, patients with inflammatory diseases are often treated with nonsteroidal anti-inflammatory drugs (NSAIDs), which interfere with the cyclooxygenase pathway of arachidonate metabolism, thus blocking the formation of prostaglandins [Vane, 1971].

This study was undertaken to determine whether prostaglandin E_2 (PGE_2), the predominant form of prostaglandin in dermal fibroblasts, could account for some of the effects of IL-1 on fibroblast gene expression. Our results demonstrate that PGE_2 selectively enhances collagenase gene expression, whereas NSAIDs do not counteract any of the effects of IL-1 that were monitored in this study.

MATERIALS AND METHODS

Cell Cultures

Adult human dermal fibroblast cultures, established from explanted tissue specimens obtained during surgical procedures, and neonatal foreskin fibroblast cultures, were utilized in passages 3 to 8. The cells were maintained in Dulbecco's Modified Eagle's (DME) medium supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, and antibiotics.

Cytokines/reagents

Prostaglandin E_2 was purchased from Sigma Chemical Co. (St. Louis, MO). Nonsteroidal anti-inflammatory drugs were purchased either from Sigma (St. Louis, MO) or ICN Biochemicals (Cleveland, OH). Human recombinant IL-1β was purchased from Boehringer Mannheim (Indianapolis, IN).

Northern Analyses

Adult skin fibroblasts in confluent monolayer cultures were fed with fresh medium containing 10% FCS 4 h prior to experiments. At the end of incubation, total RNA was isolated as previously described [Chirgwin et al., 1979] and analyzed by Northern hybridization with ^{32}P-labeled cDNA probes. The [^{32}P]cDNA-mRNA hybrids were visualized by autoradiography, and the steady-state levels of mRNA were quantitated by scanning densitometry using a He-Ne laser scanner at 633 nm (LKB Produkter, Bromma, Sweden).

Western Blotting

Human dermal fibroblasts were incubated for 24 h with various concentrations of exogenous PGE_2. At the end of incubation, the supernatants were concentrated 20 times with Centricon 10 concentrating units (Amicon Corp., Beverly, MA), separated on a 10% SDS-polyacrylamide gel, and transferred to a nitrocellulose membrane (MSI, Westboro, MA). Interstitial collagenase was detected using a rabbit anti-human collagenase antibody, affiny purified on collagenase-CNBr-sepharose 4B column [Saarialho-Kere et al., 1993], and an affinity purified alkaline phosphatase-conjugated goat anti-rabbit secondary antibody (Promega Corp., Madison, WI), revealed with Western Blue™ stabilized substrate (Promega).

Transient Transfections of Cultures Cells

Human neonatal foreskin fibroblasts in late logarithmic growth phase were transfected with 5–20 μg of plasmid constructs, together with a RSV-promoter/β-galactosidase construct, which allowed determination of the transfection efficiency [Sambrook et al., 1989]. The transfections were performed with the calcium-phosphate/DNA co-precipitation method [Graham and van der Eb, 1973], followed by a 1–1.5 min (15%) glycerol shock. After the glycerol shock, the cells were placed in fresh medium supplemented with 10% FCS. At the end of the experi-

ments, the cells were harvested and lysed by three cycles of freeze-thawing in 100 μl of 0.25 M Tris-HCl, pH 7.8. β-Galactosidase activity was determined in each sample, and CAT assay was performed using [^{14}C]chloramphenicol as a substrate [Gorman et al., 1982], with aliquots containing the same amount of β-galactosidase activity.

cDNAs/Plasmid Constructs

The following cDNAs were used for Northern hybridizations: for collagenase mRNA, a 2.0 kb human cDNA [Goldberg et al., 1986]; for stromelysin mRNA, a human 1.5 kb cDNA [Saus et al., 1988]; and for the tissue inhibitor of metalloproteinases (TIMP), a 0.7 kb human cDNA, pUC9-F5 (kindly provided by Dr. D. Carmichael, Synergen, Boulder, CO). A rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA [Fort et al., 1985] was used in control hybridizations to normalize for differences in the loading and transfer of RNA.

To study the regulation of collagenase and stromelysin gene expression at the level of their respective promoters, the following plasmid constructs were used in transient transfection experiments: pCLCAT3, which contains ~3.8 kb of 5′-flanking DNA of human collagenase gene linked to the CAT reporter gene [Frisch et al., 1990]; and 4^{+}CAT, which contains 1.3 kb of 5′-flanking DNA of human stromelysin gene [Buttice et al., 1991].

RESULTS

Prostaglandin E_2 Elevates Fibroblast Collagenase mRNA Steady-State Levels in a Dose-Dependent Manner

In order to establish the role of PGE_2 in mediating some of the degradative effects of IL-1 on the extracellular matrix, human dermal fibroblasts in culture were incubated with varying doses of PGE_2. Total RNA was extracted after 24 h of incubation and analyzed by Northern blotting. As shown in Figure 1A, control cultures exhibited relatively low levels of collagenase and stromelysin mRNAs. Addition of PGE_2 resulted in increased collagenase mRNA steady-state levels, whereas stromelysin and TIMP mRNA levels remained essentially unchanged. Densitometric analysis of the blots, after normalization by the GAPDH mRNA levels (Fig. 1B), indicated a dose-dependent elevation of collagenase mRNA steady-state levels in response to PGE_2 (3.8- and 8.0-fold in the presence of 0.1 and 1 μg/ml of PGE_2, respectively). In contrast, maximal stimulation of stromelysin and TIMP mRNA was 2.1- and 2.4-fold, respectively. As expected, parallel incubations of dermal fibroblasts with IL-1β resulted in a marked elevation of collagenase and stromelysin gene expression (10.5-fold and 20.9-fold, respectively). Also IL-1β increased TIMP expression to levels ~2.3-fold above the control levels, similar to the levels reached following PGE_2 stimulation (Fig. 1B).

Exogenous PGE_2 Stimulates Collagenase Secretion by Human Dermal Fibroblasts

In order to determine whether PGE_2-mediated elevation of collagenase mRNA levels was accompanied by a corresponding enhancement of collagenase secretion, confluent fibroblast cultures were incubated for 24 h with various concentrations of PGE_2 and the amounts of secreted collagenase were determined by Western blot analysis. A major band with a MW of ~57 kDa, corresponding to the major form of latent collagenase, was detected in the supernatants from control cultures (Fig. 2, left lane), together with a minor band of slightly higher MW corresponding to glycosylated latent collagenase [Murphy and Reynolds, 1993]. The amount of collagenase was strongly elevated in supernatants from PGE_2-treated fibroblast cultures (Fig. 2), which correlates with the elevation of the corresponding mRNA levels (Fig. 1).

The Effects of IL-1 and PGE_2 on Collagenase Gene Expression Are Neither Additive nor Synergistic

In order to determine whether the effects of IL-1 and PGE_2 on collagenase gene expression could be additive or synergistic, fibroblast cultures were incubated with IL-1β (1 U/ml) and PGE_2 (0.1 μg/ml) either alone or in combination, and total RNA from the cells was analyzed by Northern hybridizations after 24 h of incubation (Fig. 3A). As expected from our previous observations (see above), both IL-1β and PGE_2 elevated collagenase mRNA steady-state levels, ~13-fold and ~5-fold, respectively, after correction for the GAPDH mRNA levels. Stromelysin gene expression was enhanced by IL-1 (~10-fold), whereas PGE_2 had little, if any, effect (Fig. 3B). Interestingly, concomitant addition of IL-1

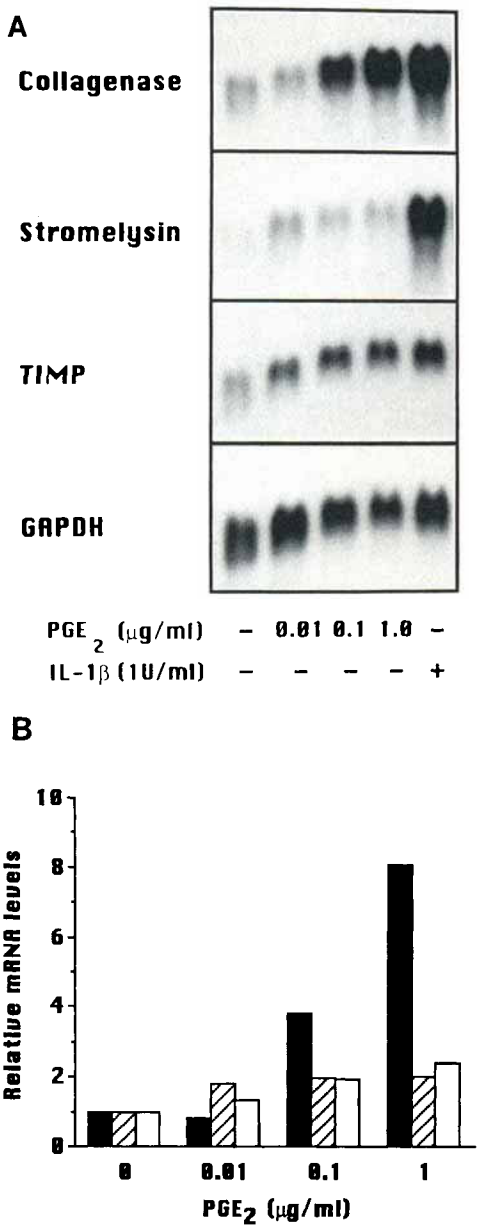

Fig. 1. Effects of PGE_2 on collagenase, stromelysin, and TIMP mRNA steady-state levels in fibroblasts. Confluent skin fibroblast cultures in DMEM containing 10% FCS were incubated for 24 h with varying concentrations of PGE_2 (0.01–1 μg/ml), or with recombinant human IL-1β (1 U/ml). Total RNA (20 μg/lane), extracted from the cells, was analyzed by Northern hybridization with ^{32}P-labeled cDNAs for collagenase, stromelysin, TIMP, or GAPDH (**A**). The results were quantitated by densitometric analysis, corrected for GAPDH mRNA in each RNA preparation, and presented as relative mRNA levels, setting the control value as 1.0 (**B**). Solid bars, collagenase; hatched bars, stromelysin; open bars, TIMP.

and PGE_2 to the cultures did not result in further elevation of collagenase mRNA steady-state levels (~15-fold), as compared to IL-1 alone (~13-fold), suggesting the involvement of shared mechanisms by IL-1 and PGE_2 in stimulating fibroblast collagenase gene expression. Also, PGE_2 did not alter the upregulation of stromelysin or TIMP mRNA levels, as elicited by IL-1β.

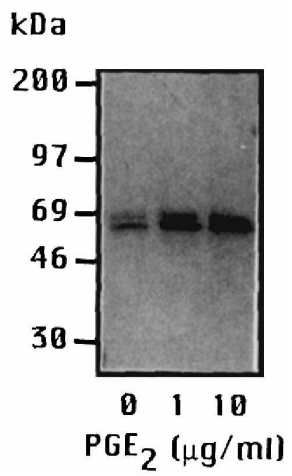

Fig. 2. Western blot analysis of collagenase production by PGE_2-stimulated fibroblasts. Confluent fibroblast cultures were incubated for 24 h with various concentrations of prostaglandin E_2 (0, 1, and 10 μg/ml). At the end of incubation, media were collected, concentrated, and analyzed by Western blotting with a rabbit anti-human interstitial collagenase antibody. A doublet band with a MW characteristic of human interstitial procollagenase was detected using an alkaline phosphatase-conjugated goat anti-rabbit secondary antibody.

Prostaglandin E_2 Modulates Collagenase Gene Expression at the Transcriptional Level

Transient cell transfections with a collagenase promoter/CAT reporter gene construct, pCLCAT3, were performed to examine whether PGE_2 enhances collagenase mRNA steady-state levels through activation of transcription at the promoter level. Human neonatal fibroblasts were transfected with the promoter/CAT construct and the cultures were then treated with PGE_2 (1 μg/ml). Assay of CAT activity after 40 h of incubation indicated that PGE_2 enhanced the promoter activity by ~3-fold, as compared to that of control cultures (Table I), whereas an SV_2 promoter/CAT gene construct, as well as the 4^+CAT construct, which contains 1.3 kb of the stromelysin promoter linked to the CAT gene, were not responsive to PGE_2, attesting for the specificity of the effect of PGE_2 on the collagenase promoter activity. Thus, the enhancement of collagenase gene expression, as detected at the mRNA level, is mediated, at least in part, by enhancement of the transcriptional activity of the corresponding promoter.

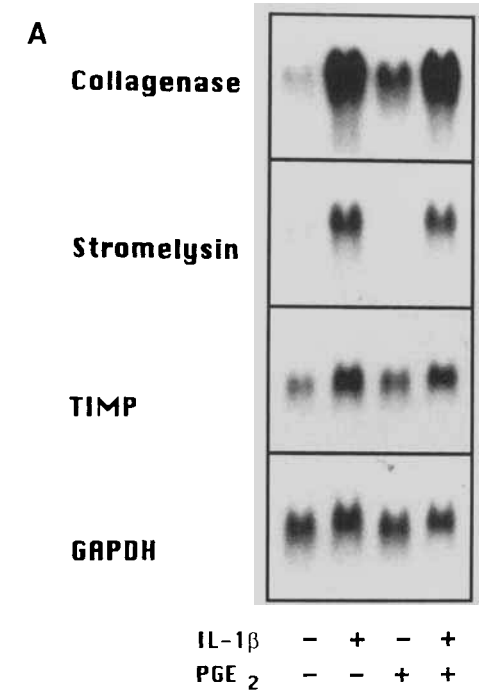

Fig. 3. Effects of PGE_2 in combination with IL-1 on collagenase, stromelysin, and TIMP mRNA steady-state levels in fibroblasts. Confluent fibroblast cultures were incubated for 24 h with (+) or without (−) 0.1 μg/ml PGE_2, in the presence or absence of IL-1β (1 U/ml). Total RNA (20 μg/lane) was analyzed by Northern hybridization with ^{32}P-labeled cDNA probes for collagenase, stromelysin, TIMP, or GAPDH (**A**). The results were quantitated by densitometric analysis, corrected for GAPDH mRNA in each RNA preparation, and presented as relative mRNA levels, setting the control value as 1.0 (**B**). Solid bars, collagenase; hatched bars, stromelysin; open bars, TIMP.

Inhibition of Endogenous PGE_2 Synthesis by Nonsteroidal Anti-Inflammatory Drugs Does Not Affect IL-1-Induced Collagenase, Stromelysin, and TIMP Gene Expression in Fibroblasts

In order to evaluate the role of endogeneous PGE_2 on IL-1-induced MMP and TIMP gene expression, we tested three different NSAIDs commonly utilized in human therapy, namely, indomethacin, acetyl salicylic acid, and ibuprofen. One hour prior to the addition of IL-1, NSAIDs were added to the culture medium at a 10^{-5} M concentration, which has previously been shown to block endogenous PGE_2 synthesis by more than 90% in dermal fibroblasts and rheumatoid synovial cells, even after IL-1 stimulation [Dayer et al., 1984; Mauviel et al., 1988b,c, 1991a]. As shown in Figure 4, IL-1β alone (lane 2) strongly elevated collagenase and stromelysin mRNA steady-state levels, as well as those for TIMP, although the latter ones were elevated to a lesser extent. None of the NSAIDs used in this study altered the basal expression of the genes tested (Fig. 3, lanes 3, 5, 7), nor did they modify their response to IL-1 (lanes 4, 6, 8). Similar results were obtained when the incubations were carried out in medium containing either 10% or 1% FCS (not shown). Also in transient transfection experiments with a collagenase promoter/CAT construct, indomethacin did not block IL-1-induced elevation of the promoter activity (not shown). In contrast, proα1(I) collagen mRNA levels were elevated ~1.5-2-fold after NSAID treatment (not shown), which correlates with the inhibition of prostaglandin synthesis [Varga et al., 1987; Mauviel et al., 1988b].

Therefore, our data suggest that the effects of IL-1 on collagenase, stromelysin, and TIMP gene expression are not dependent on IL-1-induced PGE_2 production by fibroblasts, contrasting with the effects of IL-1 on collagen gene expression, which can be modulated by endogenous produc-

TABLE I. Effects of Prostaglandin E_2 on Collagenase and Stromelysin Promoter Activities

	Promoter activity (cpm)[a]	
	Control	PGE_2
pCLCAT3	1,225 ± 146[b] (100)	3,452 ± 235 (282)
4⁺CAT	238 ± 35 (100)	275 ± 42 (116)
pSV_2CAT	202 ± 26 (100)	219 ± 30 (108)

[a]Fibroblasts in late logarithmic growth phase were transfected with various plasmid constructs, as described in Material and Methods. Three hours after the glycerol shock, cells were treated without (Control) or with prostaglandin E_2 (1 μg/ml) in medium containing 10% fetal calf serum. Incubations were continued for 40 h and CAT activity, representing the respective promoter activities, was determined.
[b]Quantitation of CAT activity, expressed as cpm of acetylated [^{14}C]chloramphenicol, is the mean ± S.D. of three independent determinations. The numbers in parentheses indicate the activation relative to the control cultures.

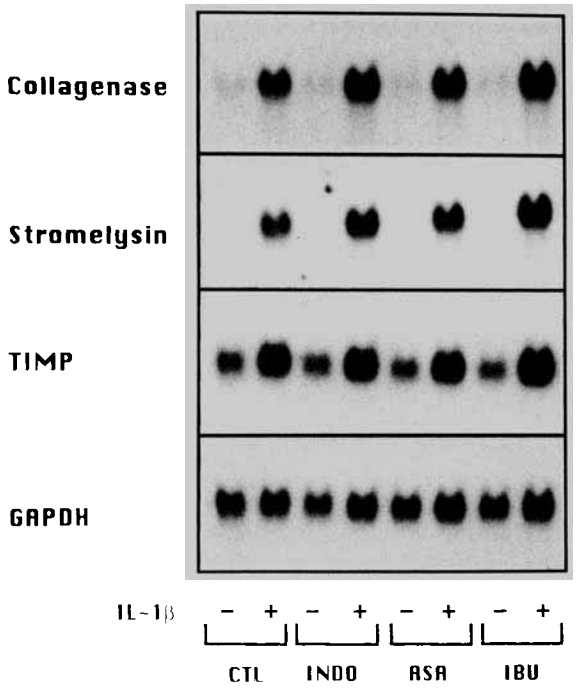

Fig. 4. Effects of various nonsteroidal anti-inflammatory drugs on IL-1-induced collagenase, stromelysin, and TIMP gene expression. Confluent fibroblast cultures were incubated for 24 h with (+) or without (−) IL-1β (1 U/ml), in the absence (control, CTL) or the presence of indomethacin (INDO), acetyl salicylic acid (ASA), and ibuprofen (IBU), at the concentration of 10^{-5} M. The NSAIDs were added 1 h prior to the addition of IL-1.

tion of PGE_2 [Varga et al., 1987; Goldring et al., 1987; Mauviel et al., 1988c, 1991a].

DISCUSSION

The role of PGE_2 as an endogenous modulator of fibroblast gene expression has been studied in a variety of experimental systems. For example, PGE_2 is a well known inhibitor of fibroblast collagen and fibronectin synthesis, acting both at the pretranslational level by reducing the corresponding mRNA steady-state levels [Varga et al., 1987], as well as at the post-translational level by increasing degradation of the newly synthesized procollagen molecules, possibly by elevating intracellular levels of cyclic AMP [Baum et al., 1978, 1980; Clark et al., 1982]. Conversely, the inhibition of IL-1-induced PGE_2 synthesis with NSAIDs reverses the inhibitory effect of IL-1 on the collagen synthesis by synovial cells [Mauviel et al., 1988c]. Also PGE_2 has been shown to downregulate the expression of TNF-α induced by cytokines and lipopolysaccharide, and this effect takes place at the level of transcription [Knudsen et al., 1986; Kunkel et al., 1988]. PGE_2 also inhibits cytokine-induced proIL-1β production by fibroblasts [Mauviel et al., 1991b], suggesting autoregulation of IL-1 and TNF-α production through the induction of PGE_2. This possibility is further supported by the fact that NSAIDs potentiate cytokine-induced proIL-1β production by fibroblasts [Mauviel et al., 1991b].

NSAIDs are widely used in the treatment of human inflammatory diseases characterized by excessive degradation of connective tissue, such as rheumatoid arthritis. These drugs are believed to act through the inhibition of prostaglandin synthesis and have been shown to reduce the activities of matrix degrading enzymes in a variety of experimental models. For example, certain NSAIDs can block IL-1-induced cartilage resorption in vitro [Shinmei et al., 1988] and they reduce IL-1-induced collagenase activity in rheumatoid synovial cells in culture [Dayer et al., 1984].

In this study, we have demonstrated that addition of PGE_2 to fibroblast cultures results in the specific enhancement of collagenase gene expression, whereas the expression of stromelysin and TIMP genes is only slightly affected. The effect of PGE_2 on collagenase gene expression is, at least in part, due to activation of the corresponding promoter, as demonstrated by transient cell transfection experiments. However, the extent of stimulation of the collagenase promoter activity by PGE_2 is lower than that of the corresponding mRNA steady-state levels, which implies participation of post-transcriptional mechanisms, such as stabilization of collagenase mRNA transcripts. The latter pathway has been shown to play an important role in the elevation of collagenase mRNA levels by phorbol esters and epidermal growth factor [Brinckerhoff et al., 1986; Delany and Brinckerhoff, 1992]. Another explanation for the smaller stimulation of collagenase promoter/CAT construct as compared to the elevation of collagenase mRNA steady-state levels by PGE_2 is that the 3.8 kb of collagenase 5′-flanking sequences of the construct may not contain all the PGE_2-response elements of the collagenase gene.

We have also shown that IL-1 effect on fibroblast collagenase gene expression is not dependent on enhanced PGE_2 production. This conclusion is based on several lines of evidence: first, NSAIDs, which inhibit endogenous PGE_2 production, do not reduce collagenase gene expression induced by IL-1; and second, exogenous addition of PGE_2, together with IL-1, does not alter the IL-1 response of fibroblasts in terms of collagenase gene expression. The latter observation suggests that PGE_2 and IL-1 enhance colla-

genase gene expression by shared regulatory mechanisms. Interestingly, we observed an uncoordinate regulation of the genes encoding collagenase and stromelysin by PGE_2, whereas IL-1 stimulation resulted in upregulation of the expression of both genes (see Fig. 1 and Fig. 3). The genes encoding these two MMPs are structurally very similar [Woessner, 1991], and their regulation is often coordinate [Frisch et al., 1987]. However, even if the genes encoding collagenase and stromelysin share common structural elements, their regulation is apparently independent through different mechanisms in various systems. For example, cycloheximide, and other protein synthesis inhibitors, preferentially up-regulate stromelysin gene expression [Otani et al., 1990]. Uncoordinate expression of collagenase and stromelysin genes has also been observed in cytokine-stimulated rheumatoid synovial cells [McNaul et al., 1990].

Recently, it was reported that addition of PGE_1 to dermal fibroblasts and rabbit synoviocytes inhibits collagenase gene expression [Salvatori et al., 1992]. These findings may provide new therapeutic interest for PGE_1 analogs such as misoprostol [Akdamar et al., 1982], but it is unlikely that natural PGE_1 could downregulate cytokine effects on collagenase gene expression in connective tissue cells in vivo, since PGE_1 represents a minor fraction of the prostaglandins produced [Dayer et al., 1976, 1979]. Also, the concentrations of exogenous PGE_1 used in the previous study [Salvatori et al., 1992] (0.01–1 μM) are far in excess of the endogenous production by connective tissue cells in culture. However, our data and those reported previously [Salvatori et al., 1992] suggest that two closely related products of the arachidonate cascade may compete in regulating collagenase gene expression, even though it is likely that PGE_2, which represents about 80% of the total prostaglandin pool [Dayer et al., 1976, 1979], will be the principal active compound. This conclusion is further supported by the fact that NSAIDs, which block PGE synthesis, do not enhance IL-1 effect on collagenase gene expression (see Results), as would be expected if PGE_1 played an active role in collagenase gene expression in this experimental system.

In summary, we have demonstrated that PGE_2 is a potent inducer of fibroblast collagenase mRNA levels, promoter activity, and secretion. Also, we have shown that NSAIDs, which inhibit endogenous prostaglandin synthesis by fibroblasts, do not counteract IL-1-induced collagenase gene expression. A possible explanation for the beneficial effects of NSAIDs in human pathology, and for their ability to reduce collagenase activity in vivo, is that they may reduce the pool of stable metabolites, such as PGE_2, capable of stimulating collagenase gene expression in a sustained manner after natural clearance or degradation of the short-lived pro-inflammatory cytokines (e.g., IL-1 or TNF-α).

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