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Transcriptional Regulation of Collagenase-3 by Interleukin-1 Alpha in Osteoblasts

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Abstract Interleukin-1 (IL-1)α is an autocrine/paracrine agent of the skeletal tissue and it regulates bone remodeling. Collagenase-3 or matrix metalloproteinase (MMP)-13 is expressed in osteoblasts and its expression is modulated by several cytokines including IL-1α. Because the molecular mechanism of increased synthesis of collagenase-3 in bone cells by IL-1α is not known, we investigated if collagenase-3 expression by IL-1α in osteoblasts is mediated by transcriptional or post-transcriptional mechanisms. Exposure of rat osteoblastic cultures (Ob cells) to IL-1α at concentrations higher than 0.5 nM increased the synthesis of collagenase-3 mRNA up to eightfold and the secretion of immunoreactive protein up to 21-fold. The effects of IL-1α on collagenase-3 were time- and dose-dependent. Although prostaglandins stimulate collagenase-3 expression, stimulation of collagenase-3 in Ob cells by IL-1α was not mediated through increased biosynthesis of prostaglandins. The half-life of collagenase-3 mRNA from control and IL-1α-treated Ob cells was similar suggesting that the stabilization of collagenase-3 mRNA did not contribute to the increase in collagenase-3. However, IL-1α stimulated the rate of transcription of the collagenase-3 gene by twofold to fourfold indicating regulation of collagenase-3 expression in Ob cells at the transcriptional level. Stimulation of collagenase-3 by IL-1α in osteoblasts may in part mediate the effects of IL-1α in bone metabolism. J. Cell. Biochem. 90: 1007–1014, 2003.

Key words: cytokines; matrix metalloproteinase-13; RNA stabilization; procollagenase; prostaglandins

Cytokines, such as interleukin-1 (IL-1)α and β, are present in the bone microenvironment and are found to be important regulators of skeletal tissue [Pacifici, 1996]. The stimulation of bone resorption and inhibition of bone formation by IL-1 isoforms have been demonstrated by various investigators [Gowen et al., 1983; Lorenzo et al., 1987; Stashenko et al., 1987; Nguyen et al., 1991]. Earlier studies have provided evidence for the increase in IL-1 in the bone microenvironment after the menopause, suggesting that IL-1 may play a role in the matrix degradation and bone loss associated with postmenopausal osteoporosis [Pacifici et al., 1987, 1989; Ralston, 1994]. Cells of the hematopoietic lineage are possibly the primary source of IL-1 acting on skeletal tissue [Dinarello, 1991]. However, IL-1α and -β are also expressed in osteoblastic cells, indicating an autocrine/paracrine role of IL-1 in bone [Hanazawa et al., 1985; Keeting et al., 1987; Lorenzo et al., 1990]. In osteoblasts, IL-1 isoforms regulate cell proliferation, differentiation, and expression of a variety of genes important for bone cell function, such as collagen, alkaline phosphatase, and osteocalcin [Tatakis, 1993].

Matrix metalloproteinases (MMPs) consist of a family of proteinases that include collagenases, gelatinases, and stromelysins, and they can collectively degrade the components of the extracellular matrix [Woessner, 1991; Murphy, 1995]. Collagenases cleave intact collagen fibrils at physiological pH. Therefore, collagenase activity can be critical in regulating collagen degradation in the bone matrix. Three collagenases, collagenase-1, -2, and -3, are known and these proteases can degrade collagens type I, II,

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and III. Collagenase-1 (also known as interstitial collagenase, fibroblast collagenase, or MMP-1) is present in cells of mesenchymal origin and its expression has been shown in stimulated human osteoblasts [Meikle et al., 1992; Rifas et al., 1994]. Collagenase-2 or MMP-8 is expressed primarily in neutrophils and it is, therefore, also known as neutrophil collagenase. Collagenase-3 or MMP-13 is expressed in normal human cells including chondrocytes and fibroblasts [Freije et al., 1994; Mitchel et al., 1996]. Rodent skeletal collagenase is a homologue of human collagenase-3 [Quinn et al., 1990; Freije et al., 1994]. Studies from our laboratory and those from others demonstrated that bone resorbing agents, such as parathyroid hormone (PTH), prostaglandin E_2 (PGE_2), retinoids, and glucocorticoids stimulate collagenase-3 expression in rodent osteoblastic cells [Heath et al., 1984; Sakamoto and Sakamoto, 1984; Partridge et al., 1987; Delaisse et al., 1988; Shen et al., 1988; Varghese et al., 1994; Delany et al., 1995]. We also observed that skeletal growth factors that enhance bone resorption, such as fibroblast growth factor-2 (FGF-2) and platelet-derived growth factor BB (PDGF BB) stimulate collagenase-3 production [Varghese et al., 1995, 1996]. In contrast, stimulators of bone formation such as insulin-like growth factor I and II, transforming growth factor-β and bone morphogenetic protein-2 inhibit collagenase-3 synthesis [Canalis et al., 1995; Rydziel et al., 1997; Varghese and Canalis, 1997].

Previous studies have indicated that IL-1 isoforms stimulate collagenase-1 and -3 synthesis in osteoblasts, fibroblasts, and chondrocytes [Dayer et al., 1986; Kusano et al., 1988; Conca et al., 1989; Borden et al., 1996]. However, the mechanism of collagenase-3 gene expression by IL-1α or IL-1β in bone cells has not been examined. In this study, we have analyzed the regulation of the collagenase-3 gene by IL-1α in cultures of osteoblastic cells isolated from fetal rat calvariae (Ob cells).

MATERIALS AND METHODS

Culture Technique

The culture method used was previously described in detail [McCarthy et al., 1988]. Briefly, parietal bones were obtained from 22-day-old fetal rats immediately after the mothers were sacrificed 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 bone using bacterial collagenase (CLS II, Worthington Biochemical, Lakewood, NJ). Cell populations harvested from the third to the fifth digestion, previously shown to have osteoblastic characteristics, were pooled and plated at a density of approximately 10,000 cells/cm^2, and cultured until reaching confluence in Dulbecco's modified Eagle's medium (DMEM) (Summit Biotechnology, Fort Collins, CO) supplemented with nonessential amino acids, 100 μg/ml L-ascorbic acid, penicillin, streptomycin, and 20 mM HEPES (all from Life Technologies, Grand Island, NY), and 10% fetal bovine serum (Summit Biotechnology). Except the nuclear run-off experiments, cells were grown to confluence, switched to serum-free medium for 16–24 h, and exposed to human recombinant IL-1α (Hoffmann-LaRoche, Nutley, NJ), PGE_2 (Calbiochem, La Jolla, CA), or control medium as indicated in the text and legends. For nuclear run-off experiments, cells were grown to subconfluence, trypsinized, replated, and grown to confluence when they were serum-deprived and exposed to IL-1α or control medium for 2 and 6 h. PGE_2 and 5,6-dichlorobenzimidazole riboside (DRB; Sigma Chemical Co., St. Louis, MO) were dissolved in ethanol, and the control cultures contained equal amounts of ethanol. At the completion of the culture, medium was aspirated and stored at −80°C after the addition of polyoxyethylene sorbitan monolaurate (Pierce, Rockford, IL) to a final concentration of 0.1% for Western immunoblot analysis. 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 by the acid–guanidium thiocyanate–phenol–chloroform method [Chomczynski and Sacchi, 1987]. Total RNA recovered was quantitated by spectrometry, and equal amounts of total RNA from control or test samples were loaded on a formaldehyde-agarose gel following denaturation [Sambrook et al., 1989]. The gel was stained with ethidium bromide to visualize rRNA and photographed under UV light before and after transfer to assess equal RNA loading of the various experimental samples. The RNA was blotted onto Nytran 0.2 micron nylon memb-

rane (ICN, Costa Mesa, CA). A restriction fragment containing a rat collagenase-3 cDNA (kindly provided by Dr. Cheryl Quinn, St. Louis, MO) [Quinn et al., 1990] or rat glyceraldehyde-3-phosphate dehydrogenase (GAPD) (kindly provided by Dr. Ray Wu, Ithaca, NY) [Tso et al., 1985] was radiolabeled with [α-^{32}P]deoxycytidine triphosphate (dCTP) and [α-^{32}P] deoxyadenosine triphosphate (dATP) (specific activity of 3,000 Ci/mmol; DuPont, Boston, MA), using the random hexanucleotide primed second strand synthesis method [Feinberg and Vogelstein, 1983]. Hybridizations were carried out at 42°C for 16–24 h as described. Post-hybridization wash was performed in 0.15 M sodium chloride/0.015 M sodium citrate, pH 7 (SSC) at 55°C. The bound radioactive material was visualized by autoradiography on Kodak X-AR5 film employing intensifying screens. Relative hybridization levels were determined by densitometry.

Nuclear Run-Off Assay

To determine the changes in the rate of transcription, nuclei were isolated by Dounce homogenization in a Tris-buffer containing 0.5% Nonidet P-40 [Ausubel et al., 1992]. Nascent transcripts were radiolabeled by incubation of nuclei in a reaction buffer containing 500 μM each of ATP, CTP, and guanidine triphosphate (all from Life Technologies), 150 U of RNasin (Promega Corp., Madison, WI), and 250 μCi of [α-^{32}P]uridine triphosphate (800 Ci/mmol, DuPont) [Ausubel et al., 1992]. RNA was isolated by treatment with DNase I (Life Technologies) and proteinase K (Boehringer Mannheim, Indianapolis, IN), followed by phenol–chloroform extraction and ethanol precipitation. Linearized plasmid DNA containing 1 μg of cDNA for collagenase-3 mRNA or 18S rRNA (American Type Culture Collection, Manassas, VA), or pUC 18 vector DNA (Life Technologies) was immobilized onto Nytran nylon membrane by using Hybri-Slot apparatus (Life Technologies). Equal counts/min of [^{32}P]RNA from each sample were hybridized to DNA at 42°C for 48 h [Ausubel et al., 1992], and washed in SSC at 45°C. Hybridization of nascent transcripts to DNA was visualized by autoradiography on Kodak Biomax MS films using Biomax intensifying screens.

Western Immunoblot Analysis

Samples of control and test culture medium were fractionated by polyacrylamide gel electrophoresis using denaturing and nonreducing conditions, and transferred onto Immobilon P membranes (Millipore, Bedford, MA). After blocking with 2% bovine serum albumin, the membranes were exposed to a 1:1,000 dilution of rabbit antiserum raised against rat collagenase-3, previously characterized for specificity and immunoreactivity [Jeffrey et al., 1990], 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.

Statistical Methods

Data on collagenase-3 mRNA decay were compared for significant differences by the method of Sokal and Rohlf [1981] using the computer program Crunch version 4.

RESULTS

The effect of IL-1α on collagenase-3 mRNA levels was examined by Northern blot analysis. Following treatment of confluent Ob cells with 0.5 nM IL-1α for 2–24 h, collagenase-3 mRNA levels increased after 2 h, and the effect was sustained for 24 h (Fig. 1). IL-1α caused a maximal increase in collagenase-3 mRNA of approximately eightfold after 8 h. The effect of IL-1α on collagenase-3 mRNA was dose-dependent and IL-1α increased collagenase-3 mRNA levels at concentrations 0.5–5 nM (Fig. 2). IL-1α also regulated the levels of procollagenase-3 in the culture medium, as determined by Western blot analysis. Authentic procollagenase-3 was identified by co-migration with purified rat procollagenase-3. The secretion of procollagenase-3 into the culture medium was dose-dependent and observed at IL-1α concentrations of 0.5–5 nM which increased procollagenase-3 from sixfold to tenfold after 8 h (Fig. 3). The effect was sustained and IL-1α at 0.5 nM increased immunoreactive procollagenase-3 concentrations in the culture medium by 6- to 21-fold after 8–24 h (data not shown).

Since IL-1α induces PGE_2 biosynthesis in osteoblastic cultures [Sato et al., 1986], we tested whether the effect of IL-1α on collagenase-3 expression was mediated by prostaglandins examining the actions of IL-1α in the

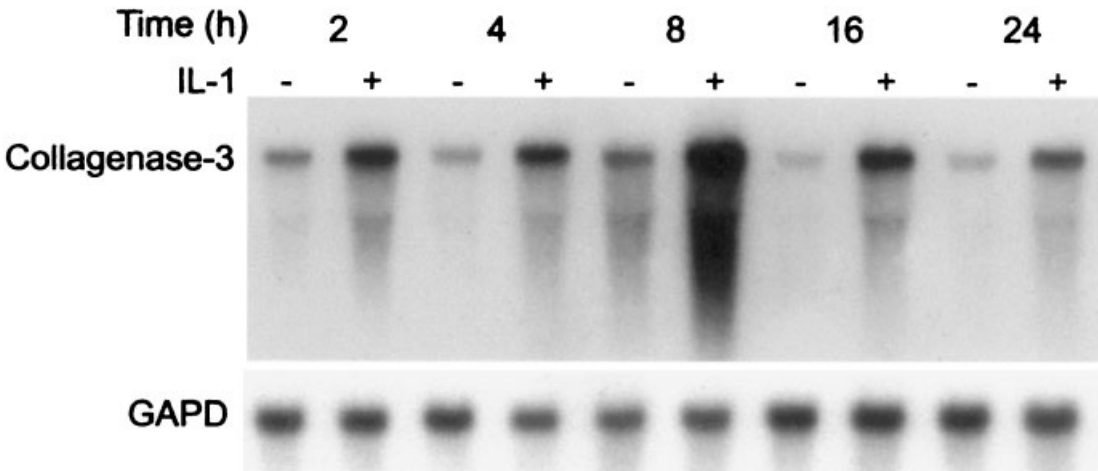

Fig. 1. Time-dependent regulation of collagenase-3 mRNA by IL-1α. Ob cells were cultured in the presence (+) or absence (−) of 0.5 nM IL-1α for 2–24 h. Total RNA (6 μg/lane) was analyzed by Northern hybridization analysis using ^{32}P-labeled cDNAs and mRNA changes were visualized after autoradiography. Collagenase-3 (collagenase-3) and GAPD (GAPD) mRNA bands are shown in the **upper** and **lower panels**, respectively. The data shown are representative of three independent cultures.

presence and absence of indomethacin, an inhibitor of prostaglandin synthesis. PGE_2 at 1 μM increased collagenase-3 mRNA by 16-fold after 8 h in Ob cells (Fig. 4), confirming previous observation in UMR106 cells [Clohisy et al., 1994]. Indomethacin at 10 μM did not affect basal collagenase-3 mRNA levels and it did not block the effect of IL-1α at 0.5 nM on collagenase-3 mRNA stimulation suggesting that the effect of IL-1α on collagenase-3 is independent of prostaglandin biosynthesis. Also, indomethacin at 10 μM did not affect the IL-1-mediated secretion of procollagenase-3 into the culture medium (data not shown).

We examined whether the increase in collagenase-3 mRNA levels by IL-1α is caused by RNA stabilization or by transcriptional mechanisms. To examine the effect of IL-1α on collagenase-3 mRNA stability, Ob cells were exposed to control or 0.5 nM IL-1α-containing medium for 2 h before the arrest of transcription with the RNA polymerase inhibitor DRB at 75 μM [Zandomeni et al., 1983]. Collagenase-3 mRNA levels were determined 0 to 24 h after the cells were exposed to DRB and expressed as a percentage of RNA present at the time of DRB addition. The collagenase-3 mRNA half-life in control and IL-1α-treated cultures was similar,

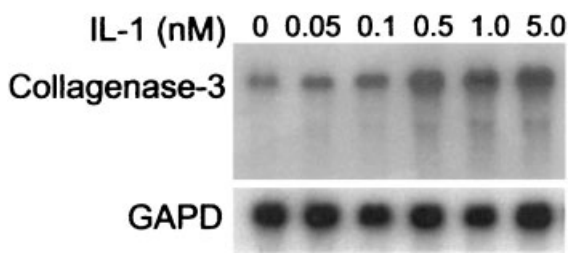

Fig. 2. Dose-dependent changes in collagenase-3 mRNA by IL-1α. Ob cells were cultured in the presence of 0–5 nM IL-1α for 8 h. Total RNA (8 μg/lane) was analyzed by Northern hybridization analysis using ^{32}P-labeled cDNAs and mRNA changes were visualized after autoradiography. Collagenase-3 (collagenase-3) and GAPD (GAPD) mRNA bands are shown in the **upper** and **lower panels**, respectively. The data shown are representative of three independent cultures.

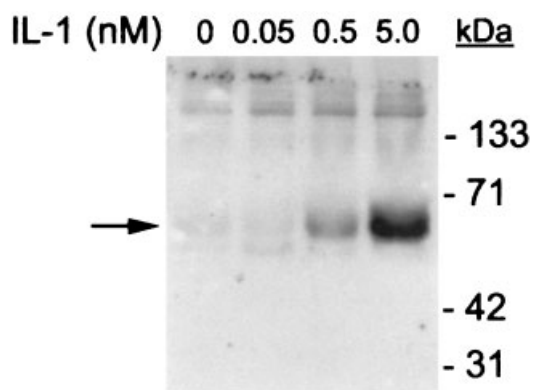

Fig. 3. Dose-dependent effect of IL-1α on procollagenase secretion in Ob cell cultures treated for 8 h. Western blot analysis was performed using equal amounts of culture medium from Ob cell cultures exposed to 0–5 nM IL-1α for 8 h. Procollagenase (arrow), detected using rabbit anti-rat collagenase antibody and a horseradish peroxidase chemiluminescence detection system, and positions of different molecular weight markers, detected by staining with coomassie blue, are shown. The data shown are representative of two independent cultures.

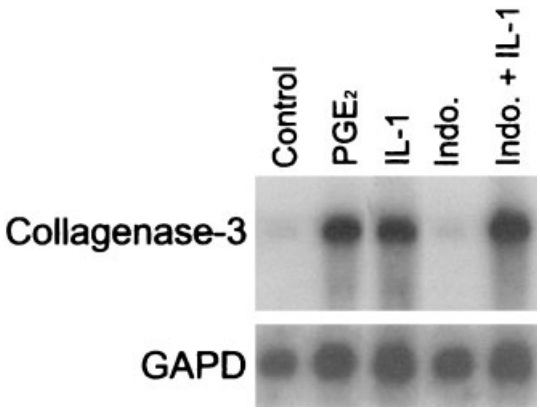

Fig. 4. Effect of indomethacin on collagenase-3 mRNA induction by IL-1α. Ob cells were cultured in medium containing vehicle (control), PGE_2 at 1 μM (PGE_2), indomethacin at 10 μM (Indo.), or IL-1α at 0.5 nM in the absence (IL-1) or presence of indomethacin (Indo. + IL-1) for 8 h. Indomethacin was added 1 h before and during the treatment with IL-1α. Total RNA (8 μg/lane) was analyzed by Northern hybridization analysis using ^{32}P-labeled cDNAs and mRNA changes were visualized after autoradiography. Collagenase-3 (collagenase-3) and GAPD (GAPD) mRNA bands are shown in the **upper** and **lower panels**, respectively. The data shown are representative of two independent cultures.

indicating that collagenase-3 mRNA stability is not affected by IL-1α (Fig. 5). To test if the rate of transcription of the collagenase-3 gene is affected by IL-1α, a nuclear run-off assay was performed (Fig. 6). Exposure of Ob cells to IL-1α at 0.5 nM for 2 and 6 h caused an increase in the rate of transcription of the collagenase-3 gene by fourfold and twofold, respectively. The rate of transcription of the 18S rRNA gene was not affected by IL-1α.

DISCUSSION

This study demonstrates that IL-1α stimulates the synthesis of collagenase-3 in bone cell cultures in a time- and dose-dependent manner. Similar to the effects of the growth factors FGF-2 and PDGF BB in Ob cells, IL-1α caused an increase in collagenase-3 mRNA levels after 2 h [Varghese et al., 1995, 1996]. IL-1α also increases the secretion of immunoreactive procollagenase-3 into the culture medium after 8 h, as determined by Western blot analysis using a sensitive chemiluminescent detection system.

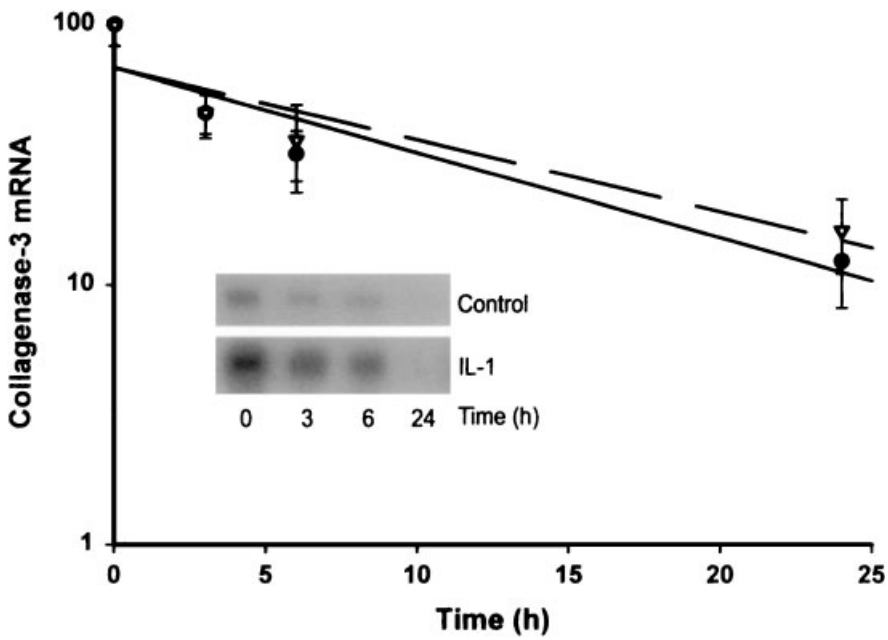

Fig. 5. Effect of IL-1α on collagenase-3 mRNA stability. Ob cells were exposed to control or 0.5 nM IL-1-containing medium for 2 h before the addition of DRB at 75 μM. Total RNA, obtained 0–24 h after DRB addition, was analyzed by Northern hybridization analysis with ^{32}P-labeled collagenase-3 cDNA. Collagenase-3 mRNA was visualized by autoradiography and quantitated by densitometry. Data from control (circles) and IL-1α-treated (triangles) cells are means ± SEM for five or more independent cultures and are expressed as a percentage of collagenase-3 mRNA (% collagenase-3 mRNA) levels at the time of the addition of DRB. The linear regression curves indicate the kinetics of collagenase-3 mRNA degradation in control (solid line) and IL-1α-treated (broken line) cultures. Inset shows a representative experiment showing collagenase-3 mRNA after the addition of DRB in control and IL-1α-treated cultures.

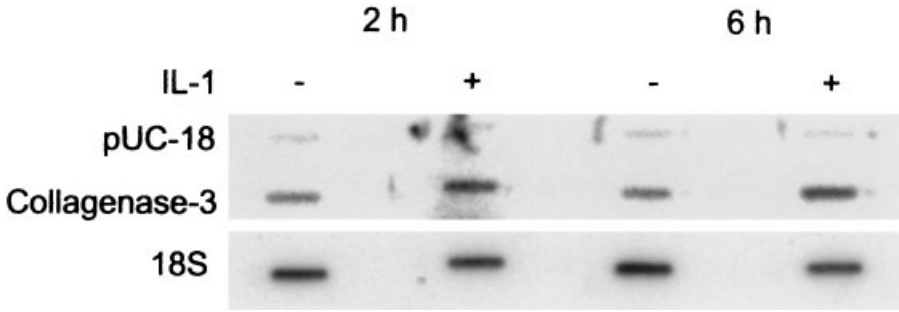

Fig. 6. Effect of IL-1α on collagenase-3 gene transcription. Confluent Ob cells were cultured in the absence (−) or presence (+) of IL-1α at 0.5 nM for 2 and 6 h, and nuclei were isolated. Nascent transcripts were radiolabeled by incubating with [^{32}P]UTP, and total RNA was isolated. Nylon membranes with cDNAs for collagenase-3 (collagenase-3) and 18S rRNA (18S), and pUC18 vector DNA (pUC−18) were hybridized with equal counts per min of [^{32}P]RNA from control and IL-1α-treated cultures, and the levels of different nascent transcripts were visualized by autoradiography.

The bone resorptive action of IL-1α is partly mediated through its ability to stimulate prostaglandin biosynthesis [Lorenzo et al., 1988; Boyce et al., 1989]. Prostaglandins increase the synthesis of collagenase-3 mRNA and secretion of procollagenase-3 in osteosarcoma cell line UMR106 and Ob cells [Clohisy et al., 1994; Varghese and Canalis, unpublished results]. Indomethacin, an inhibitor of prostaglandin biosynthesis, failed to block the effect of IL-1α on collagenase-3 synthesis, suggesting that the effect of IL-1α on the collagenase-3 gene is not mediated through prostaglandin biosynthesis. In contrast, the induction of collagenase-3 expression by FGF-2 was found to be mediated in part through the stimulation of prostaglandin production [Varghese et al., 1995].

Regulation of the collagenase-3 gene by IL-1α in osteoblasts is governed by transcriptional mechanisms. The proximal promoter of the rat collagenase-3 gene contains binding sites for core binding factor-1, activating protein-1, and polyomavirus enhancer activator-3 [Rajkumar and Quinn, 1996; Selvamurugan et al., 1998]. It remains to be determined whether any of the above transcription factor binding sites on the collagenase-3 promoter mediates the transcriptional regulation of the gene by IL-1α in osteoblasts. IL-1α does not affect the stability of collagenase-3 mRNA in osteoblasts, although the stabilization of collagenase-3 transcripts has been shown to be a mechanism by which a variety of bone remodeling agents regulate collagenase-3 expression in osteoblasts [Varghese et al., 1994, 1996; Delany et al., 1995; Rydziel et al., 1997].

Since active collagenase-3 can initiate degradation of intact collagen fibrils in the bone matrix, the effect of IL-1α on collagenase-3 may be important in mediating collagen degradation. Most bone-resorbing agents stimulate collagenase-3 synthesis in osteoblastic cells, suggesting a central role of collagenase-3 in bone resorption. The involvement of collagenase-3 in bone resorption is implicated by studies in which PTH-induced bone resorption was suppressed by MMP inhibitors [Hill et al., 1993; Witty et al., 1996]. Furthermore, PTH-stimulated bone resorption is diminished and bone formation is augmented in collagenase-resistant mutant mice indicating that collagenase activity is critical for bone remodeling in vivo [Zhao et al., 1999, 2000]. Further studies on mechanisms of procollagenase-3 activation and interaction of collagenase-3 with other MMPs and their inhibitors are necessary to obtain a complete picture of the role of collagenase-3 in matrix degradation stimulated by bone resorbing agents, such as IL-1α.

In conclusion, IL-1α stimulates the expression of the collagenase-3 gene in osteoblastic cells in a time- and dose-dependent manner. The effect of IL-1α on collagenase-3 expression is not mediated through prostaglandin biosynthesis. IL-1α increases the rate of transcription of the collagenase-3 gene, but it does not affect the stability of collagenase-3 mRNA. Stimulation of collagenase-3 expression by IL-1α may play a role in the degradation of collagenous bone matrix and bone resorption caused by IL-1α.

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